Biological Resources Laboratory: Faculty of Science

A Profile

1986-2011

Nepal Academy of Science and Technology

Khumaltar, Lalitpur

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Editor
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Foreword

Biological Resources Laboratory has a brief history, established recently to accomplish objectives of conservation, sustainable harvesting and research on valuable biological resources effectively. Nepal is endowed with plenty of biological resources, however, poor management and inferior technology has not accorded genuine advantage yet, with a proper perspective plan, this sector would transform into a reliable industrial infrastructure and generate bulk employment opportunity, and leverage national growth targets. Category of biological resources is so vast that its conservation, sustainable use and value addition for commercial exploitation can be only achieved by a comprehensive strategy and big investment. Since biotechnological advancements are applied to increase the volume and also to upgrade the quality of the biological resources of all stock, i.e. animal and plant origin, Molecular Biotechnology Laboratory will be playing a complementary role in the conservation and modification of valuable biological stocks.

The ever increasing market of biological or genetic resources due to their demand in industries, has led to a rise in bio- prospecting activities in developing countries, such activities led to the formulation of variety of consumer goods. Therefore, to develop infrastructure for research and resource mobilization for the development of quality products will pay rich dividend in the long run. Mechanism of the development of a product from the biological raw material, passes through many stages, and depends on the skill of professionals of many disciplines, and quality of the products depend on commitment level of every individual involved in the process.

In the context of Nepal, medicinal and aromatic plants figure most prominently in the category of biological resources, distribution of valuable medicinal plants in Nepal's flora is well documented. Though large number of people are involved in this sector, annual turnover in this trade is not up to the expectations. This sector is unorganized and encounter many problems, however, there is a scope for the improvement in the present state. Research on various aspects of medicinal plants started in the NAST laboratories from the very beginning, and still continues to develop processes to enhance quality of plant material and optimize yield of valuable medicinal constituents. Activities have also been extended to suitable agro climatic zone of the country for conservation and cultivation of medicinal plants applying appropriate harvest and postharvest technology.

Biological resources are immensely important for the survival of human beings and other organisms, and proper management of available stock may completely change the national economic status. In addition to the major items like food grains, cereals and vegetables, other biological resources viz. tea, coffee, spices, flowers,NTFPs including medicinal plants, livestock, poultry, horticulture products etc. are remarkably potential resources that need to be harnessed for the economic growth and the prosperity of the country. The importance of materials derived from biological resources to develop bio energy as renewable energy by using variety of biomasses, such as forest and mill residue, agricultural crops and wastes, fast growing trees and plants etc. are organic matter abundantly available on a renewable basis. Biodiesel can be derived from seeds of J. curcas and other potential plant species. Thus, biological resources can play an important role in bio energy sector.

This compilation would provide information on research pattern on biological resources in NAST since very beginning, and be useful in many ways.

Dinesh R Bhuju, PhD
Chief
Faculty of Science

Acknowledgement

NAST laboratories has completed two and half decades of existence incorporating mandated research and development activities. Many research papers and other relevant documents have been published in this time period in science journals, proceedings of national and international conferences and seminars.

Research component was added in Nepal Academy of Science and Technology in 1986 - 1987 with the establishment of multidisciplinary Central Research Laboratory. The structure, however, transformed with time and at present four laboratories exists in the academy with well defined research modules. A.D. 1986 is therefore, projected as the base year for each laboratory.

Biological resources laboratory has a brief history, formed by reorganizing Biotechnology Laboratory. Papers compiled in this profile have been selected categorically from the overall research papers published so far, remaining papers included in the profile of Molecular Biotechnology Laboratory. Biological Resources Laboratory has proved its worth in a short period of existence by conducting in-house research, field survey and sampling in remote areas of Manaslu and Chure hills to incorporate essentials of thematic projects.

I would like to express my sincere thanks to Dr. Dinesh Raj Bhuju, Chief, Faculty of Science for his valuable suggestions to bring out profile of Biological Resources Laboratory. I would also like to acknowledge and thank colleagues, Mr. Mahendra Kapali and Mr. Anandi R. Yadav for their contribution to complete this Profile.

Vijay Singh
Editor
Faculty of Science

Biological Resource Laboratory in a Nutshell :

*Approved Annual Program(F.Y. 2067/68)

1. Study on Biodiversity of Manaslu Area: Medicinal Plants, insects and microorganism prevailing in the soils
2. Study on diversity of Rhododendron and its endophytic fungi in Manaslu area + Climate change study
3. Control of microbes borne diseases by the use of green manures, in house and field evaluation of activity of green manures
4. Flora of Nepal
5. Chure Area: Study on land use pattern, plant biodiversity + living standard and knowledge level of the local people
6. Analytical Survey

Budget(F.Y.2067/68)

Internal	: NRs 500 (x 000)
Others	: NRs 150 (x 000)
Total	: NRs 650 (x000)

Manpower:

Senior Scientific Officer	6
Scientific Officer	2
Ph.D.	3
M.Sc.	5

Important Instruments/Equipments

Laminar air flow hood

Lyophilizer

Fermenter

Many research papers have been published over the years covering every aspect of biological resources like distribution, conservation and utilization of natural resources. Most of the research papers have been published in journals and proceedings of scientific events are compiled to provide a complete information regarding past research work and current research initiatives, the list of papers are arranged in chronological order.

Apart from the publication of research papers in journals and proceedings of the conferences and seminars, various reports has been prepared and submitted to the academy and collaborating agencies .

Reports Submitted to the NAST/ Collaborating Agency:

1. Final Report on Study of Mushrooms Diversity and its Economic Value in Sagarmatha National Park Area(www.evk2cnr.org):2006
2. Local Knowledge on Plant Uses in Khumbu Region Sagarmatha National Park(www.evk2cnr.org):2009
3. FinalReport on Assessment of Nutritional Value of Some Wild edible Mushrooms of Sagarmatha National Park(SNP)(www.evk2cnr.org):2008
4. Efficacy of Micro-Flora from Compost and Vermicompost on Different Plant Pathogens and Analysis of their Chemical Constituents:2010
5. Efficacy of Invasive Green Manures and Mycorrhiza onmgrowth and Yield of Different Legume Crops and Study their Antimicrobial Properties:2010
6. Isolation of Rhizobium, Bradyrhizobium and Mycorrhiza Sp., Development of Inoculum and its Effect on Yield of Lentil(Lens culanaris L.) and Pigeon Pea(Cajanus cajan L.):2010
7. Selection of Ectomycorrhizal(EM) Fungi for the Rehabilitationof Mid Hill Pine Forest of Nepal:1994
8. Influence of Green Manure in Ipil Ipil in Chalnakhel Field Experiment:2009
9. Influence of Mycorrhizal Fungi for stabilizing the Eroded Site:2009
10. Establishment of a BioVillage for Increasing Productivity of Rural Communities in Nepal(Biannual Project Report):2004and 2005
11. Effect of Micorrhiza Innoculum in Maize-Millet relay Cropping Pattern in the Farmers Field(Fulbari V.D.C.) Kavre District:
12. Study the Mycorrhizal Fungi such as EM and VAM of Eroded Sites as well as New Planted in the Eroded Sites:2009
13. Study the Ectomycorrhizal Fungi as a biocontrolling Agent:2002
14. Conservation and Management of Selected Medicinal and Aromatic Plants (MAPs) in Dang Valley:2007
15. Mycroflora Compost and Vermicompost and its Antimicrobial Activity Towards Pathogenic Bacteria:2008
16. Antimicrobial Assay and Phytochemical Screening of Water Hyacinth(Eichhornia crassipes; an Invasive Aquatic Weed of Fewa Lake:2011
17. Microflora Analysis from Compost and Vermicompost and their Influence on Different Plant Pathogens and Analysis of theirChemical Constituents:2011
18. Biological and chemical Potentials of Mikania micrantha Kunth ex H.B.K.:2011
19. Task Force Report on National Organism Bank:1991
20. Innoculum Production of Vam Fungi in Mass Scale(Glomus microcarpum) as a Pure Culture:

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Ectomycorrhizal Effect on Mushrooms and Pine Forest of Nepal

Geeta Shrestha

Abstract

Pinus roxburghii is an important indigenous forest tree species. About 40% of the existing nursery seedlings are of pine species. The survival or success status of plantations has been low due to the lack of proper ectomycorrhiza inoculation previous studies have been conducted mainly in subtropical to temperate midlands ecosystem which provide a natural habitat for a large number of fleshy fungi, many of them comprising ectomycorrhizal mushrooms. Forty species of Mushrooms were collected from different pine forests of Nepal and sixteen types of ectomycorrhiza were isolated from sporocarp and used to obtain pure syntheses. *Scleroderma verrucossum* produced more EM fungi than other fungi inoculated plants have higher ectomycorrhizal roots as compared to uninoculated control plants. This was particularly true in case of *Scleroderma verrucossum,* while a lower survival rate was observed for *Rusula* sp. inoculated pines.

Introduction

Ectomycorrhiza research in Nepal was started in 1991 at Royal Nepal Academy of Science and Technology (RONAST). Ectomycorrhizae represents a case of symbiosis where tree growth is enhanced by way of an increased uptake of nutrients from the soil and exposing a greater absorbing surface. It also makes plants drought and frost resistant in addition of protecting of root ends from attack by parasitic fungi with reference to the symbiotic relationship between fungi and roots of pine trees (Bakshi and Kumar 1968). Symbiotic benefits of ectomycorrhiza has long been recognised by Hatch 1937, Harley and Smith 1983. Trees of many species includings pines will not grow and develop normally without fungi (Marx and Kenny 1991). Reforestation efforts with species that require ecotmycorrhiza inevitably fail in the absence of suitable ectomycorrhiza.

Nepal is facing a serious problem of deforestation. There are 2.3 million hectares of potential land for development into Chir Pine *(P. roxburghii)* forest. About 40 % of the existing nursery seedlings belongs to pine species. However, the survival or success of plantation is low. One of the suspected reason was the lack of propet ectomycorrhiza (EM) inoculation. Traditionally, soil inocula are collected from nearby pine forest for using as inoculum source for mycorrhizae. But some of the nursery plants are found to be unhealthy and poor in growth. This traditional method may also carry diseases and pests. Mostly, the soil inocula may not have desirable and suitable EM species

The study has been carried out in subtropical, temperate forest in the midlands of Nepal. These ecological regions provide a natural habitat for a large number of fleshy fungi, many of them comprising ectomycorrhizal mushrooms, at which a few of them are also used for food and medicine.

Methodology

Collection of sporocarps. Forty species of mushrooms were collected from different Pine forest of Nepal for the most suitable time for collection of sporocarps is from July to October. Therefore, sporocarps of various developmental stages were collected during that season. Young sporocarps were used for direct isolations. Fully matured specimens were also collected for identification (Mirko Svcele). Identification have done according to Largent, Walling and Reinhard Agerer.

Isolation Method

Isolation from sporocarps. Young sporocarps that are free of rot and insect (Larvae) damage were selected. A shallow (1- 2 mm) slit across the middle of the cap surface were cut and along the length of one side of the stipe. The interior tissue were gently pulled apart the sporocarps and the interim surface were exposed for areas of sound tissue. Small pieces (2-5 mm cubes) of sound tissues were cut with a fine tipped scalpel (flame sterilized). The tissue were transferred with the sterile scalpel or needle directly on

Modified Melin Norkans (MMN) media in tubes or plates and were incubated at room temperature (26-28 °C) Molina and Palmer 1991).

Isolation from sexual spores. In this cases, sterile needle or forceps was used for taking the small quantity of spores from the sexual spores such as *Scleroderma verrucossum* (Puff ball fungi). *Boletus* sps. etc. and were inoculated in test tube slants and then were incubated at room temperature (Fries 1978).

For avoiding the bacterial contaminants, stereptomycin sulphate at 80 ppm was used in the nutrient solutions according to Zak and Bryan (1963). The isolation work was done on laminar flow.

In all above cases, ectomycorrhiza fungi were isolated from sporocarps and from sexual sproes. The mycorrhizal growth usually occurred 2 to 4 weeks after inoculation.

These fungi were maintained on slant of MMN nutrient agar media in test tube or Mackenny bottle under refrigerator at 3 - 5 °C and stored in sterile cold water (Marx and Daniel 1991).

Preparation of fungi for inoculation. Fungi were introduced into the synthesis vessel either as agar culture transfers or from liquid cultures. Agar cultures were first grown on nutrient agar in petridishes. Two to four plugs of 5-8 mm diameter were then aseptically removed and placed 1 to 2 cm deep around the seedlings in the synthesis vessels. Liquid cultures were prepared by growing agar cultures in the flasks containing 150 mL of sterile nutrient solution (MMN) with small bits of broken glass in the bottom. Liquid cultures were grown three to six weeks at 25 °C mycelium 5 to 10 mL of the liquid culture were then aseptically transferred into the sterile synthesis vessel.

For pure synthesis the mixtures of 50% sand and 50% soil was used. Since the formation of mycorrhiza was not successful. Vermiculite was imported from IFS (Sweden) and used according to the method given by Zak and Marx (1964).

One litre conical flasks taken and filled with 420 mL, vermiculite, 20 mL peatmoss and 275 MMN nutrient solutions and then were capped with an inverted 50 mL glass beakers and were autoclaved for 1 hour at 20 °C on three days interval.

Seeds were aseptically planted into plastic tray containing mixture of 50% soil and 50% sand which was made sterile by autoclaving for one hour at 120 °C for three consecutive days. Planting was done in a laminar flow hood. Then, these were kept in incubation room having artificial light.

Germinants (germinanted seeds) were ready when radicles were approximately 1 to 2 cm long and cotyledons were still within the seed coat. Germinants were aseptically transferred into the synthesis flasks in such a way that the entire radicle was inserted in the substrate.

Five to ten mL of the liquid cultures were transferred aseptically into the synthesis flask with a sterile pipette and then seedlings were kept in the growth chamber.

For the pot experiment, the seeds were aseptically germinated as in case of pure synthesis. Germinants were aseptically transferred into the pot in such a way that radicle was inserted in the substrate. Pots were filled with substrate containing 3000 mL vermiculite, 210 mL peatmoss and 1910 mL liquid MMN media. Ten mL of the liquid cultures were transferred into the pots with a sterile pipette aseptically and then seedlings were kept in the growth chamber for few days and was kept in the plastic house instead of glass house. Control were kept in all cases. Ten pots were kept for one fungus and ten pots were kept for control.

Typical ectomycorrhiza synthesis was completed after four to six months, depending upon the growth rates of fungus and hosts (Molina and Palmer 1991).

(a) A small amount of substrate was removed aseptically from the synthesis flasks or from the pots as the case may be and was transferred into nutrient agar inoculated, and reisolated the original fungus to see whether there was no contamination.

(b) The seedlings were removed from the synthesis flask and from the pot. The roots were washed gently with tap water to remove the vermiculite.

(c) The root systems sere placed entirely under water in a tray or petridish and was observed under a stereo microscope for ectomycorrhiza formation and its characters.

(d) The healthy mycorrhizal roots were selected, sectioned by hand to characterise the mantle (Molina and Palmer 1991).

Results

The different ectomycorrhial fungi isolated from different species of sporocarps found in pine forest and were inoculated in different species of pines for the pure culture synthesis (Hacskaylo 1953).

The positive results of pure culture synthesis are given below

Table 1. Pine root infection by different ectomycorrhiza inoculated in pure synthesis

S.N.	Name of fungi inoculated	Name of Pine sp	Mtp
1.	*Boletus* sp	*P. roxburghii*	55
	Stipe	*P. Patula*	20
2.	*Cystoderma amianthinum*	*P. roxburghii*	67
	(Pileus)	*P. wallichiana*	25
		P. roxburghii	40
3.	*Hypholoma fasciculare*	*P. Patula*	23
4.	*Lacaria lacata*	*P. roxburghii*	20
5.	*Lactarius volemus*	*P. roxburghii*	50
6.	*Rhizopogon* sp.	*P. roxburghii*	27
		P. roxburghii	47
7.	*Russula mustelina*	*P. patula*	40
		P. roxburghii	10
8.	*Russula sanguinea*	*P. patula*	55
		P. wallichiana	15
9.	*Scleroderma verocossum*	*P. roxburghii*	30
		P. roxburghii	150
10.	*Calocybe gambosa*	*P. patula*	135
		P. roxburghii	50

Mtp = Mycorrhzal tips present

In case of pure synthesis more mycorrhizal tips were present in *P. roxburghii* in both isolated from stipe and pileus of *Cystoderma amianthium* incoculate plant but least number in *P. patula* in both stipe and pileus part.

Among these ten speices more mycorrhizae were present in *P. roxburghii* and *P. patula* inoculated *Scleroderma verocossum* and least number of mycorrhizae were present in *P. roxburghii* inoculated with *Russula mustelina* fungi.

Table 2. Pine root infection by different types of ectomycorrhizae inoculated in pot

S.N.	Name of the fungi inoculated	Name of pine sp.	% of M.r.	% of M.r.I
1.	*Boletus* sp. (stipe)	*P. roxburghii*	6	35
		P. patula	2	10
2.	*Cystoderma amianthinum*	*P. roxburghii*	4	60
	(Pileus)	*P. patula*	3	23
		P. wallichiana	2	20
3.	*Lacaria lacata*	*P. roxburghii*	4	35
		P. patula	3	15
4.	*Russula mustelina*	*P. roxburghii*	3	45
	(Stipe)	*P. patula*	3	15
5.	*Russula sanguinea*	*P. roxburghii*	6	32
6.	*Sclereoderma*	*P. roxburghii*	5	105
	Verucossum	*P. patula*	2	70

M.r. = Mycorrhizal root in control

M.r. I = Mycorrhizal root in mycorrhiza inoculated plants

In this case, the mycorrhizae were much more higher in an inoculated pot compared to the control pot. The mycorrhizae in *P. roxburghii* and *P. patula* in which *Scleroderma verrucossum* were inoculated than other plant with others mycorrhizal fungi were inoculated. In same way, least number of mycorrhiza were present in *Boletus* sp. inoculated in *P. Patula.*

In this case, mostly check plants died. In all cases, ten pots were taken for control plant and ten pots were taken for mycorrhizal inoculated plants.

Table 3. The number of survive plants in pine root infection by different types of Ectomycorrhiza in inoculated plants in pot experiment

S.N.	Name of fungi inoculated	Name of plants	Tcp	Tsp	Timp	Tsp
1	*Boletus* sp.	*P. roxburghii*	10	4	10	7
	(Stipe)	*P. patula*	10	1	10	2
2	*Cystoderma*	*P. roxburghii*	10	3	10	6
	Amianthinum	*P. patula*	10	2	10	4
	(Pileus)	*P. wallichiana*	10	0	10	2
3	*Lacaria lacata*	*P. roxburghii*	10	4	10	7
		P. patula	10	2	10	3
4	*Russula mustelina*	*P. roxburghii*	10	2	10	4
	(stipe)	*P. patula*	10	0	10	1
5	*Russula sanguinea*	*P. roxburghii*	10	1	10	2
6	*Sclereoderma*	*P. roxburghii*	10	3	10	8
	verucossum	*P. patula*	10	0	10	5

Tcp = Total control plant

Tsp = Total survival plant

Timp = Total inoculated mycorrhizal pots

In this case, the survival rate of mycorrhizal inoculated plants were higher than uninoculated plants. The survival rate of *Scleroderma verrucossum* inoculated plants were more than other five mycorrhizal fungi inoculated in *P. patula* and *Russula sanguinea* inoculated in *P. roxburghii.*

Discussion

In case of ectomycorrhizal fungi isolated from different species of sporocarps found in pine forest, more mycorrhizal roots were present in *Scleroderma verrucossum* in *P. roxburghii* and least number of mycorrhizal roots were present in case of *Russula* mustelina inoculated in *P. roxburghii.*

In case of *P. patula* plant *Scleroderma verrucossum* fungi produced more mycorrhizal roots and least number of mycorrhizal roots were produced by *Boletus* sp. which was isolated from stipe.

In case of *P. wallichiana* plant, cystoderma amianthium (isolated from stipe) fungus was produced more and *Russula mustelina* (isolated from stipe fungus produced least number of mycorrhizal root. But the mycorrhizal root produced in *P. wallichiana* plant was not satisfactory.

In case of pot experiment, more percentage of mycorrhizae were present in *Scleroderma verrucossum* inoculated plants in *P. roxburghii* and *P. patula.* Least number of mycorrhizal roots were produced by *Boletus* sp. The survival rate of inoculated plants with mycorrhizal fungi was higher compare to uninoculated check. The survival rate was higher in case of *Scleroderma verrucossum* inoculated in *P. roxburghii* pot and less number of survival rates were in case of *Russula sanguinea* inoculated in *P. roxburghii* pot. The survival rate was higher in the *Scleroderma verucossum* inoculated *P. patula* pot and least number of survival rate was in the Russula mustelina inoculated *P. patula* pot. *Scleroderma verrucossum* is recorded as a mycorrhizal fungus of many conifers tree species including *Pinus* sps. (Trappe 1962b).

Acknowledgements

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An Appraisal of Human Impact on Vegetation in High Altitudes (Khumbu Region) of Nepal

Dinesh R. Bhuju and Prabina Rana

Abstract

Impact of anthropogenic disturbances on vegetation through growing trekking in the Khumbu region encompassing Sagarmatha National Park in north-eastern Nepal Himalaya is reported. Five major sites at an interval of 500 m covering the altitudes from 3000 m (near Lukla) – 5000 m (near Lobuche) were examined where species richness decreased with increasing altitude. Littering was found in every 27.6 human steps (ca 14 m) and the tree wounds were rampant on the major trek route. Effects of growing tourism on the alpine vegetation of the region was vividly noticeable. Development of confined trail for the visitors to close superfluous paths and monitoring change in resources are suggested.

Introduction

Tourism has grown considerably in the Khumbu region (Sagarmatha National Park) since 1964 when only 21) tourists visited the region. Between July 1996 and July 1997, 17412 tourists entered the Park (SPCC 1997). The first lodges were built in 1970s which grew from 7 to 81 between 1973 and 1991 (Lachapelle 1995). Bjonness (1983) feared that tourism has caused extensive deforestation. At higher altitudes, Juniper scrub, which requires 60 or more years to mature to the size of 35 cm above the ground, are cut and their roots extracted. At lower altitudes, booming trade in firewood has resulted in tree felling and cutting with little regard to species, age or site (Jefferies 1982). Some have concluded that the Khumbu's watershed has been nearly destroyed by tourists-induced deforestation (Hinrichsen *et al.* 1983).

Despite the work of previous researches and reports of damage to the resource-base through tourism related pressures (such as over grazing and the disposal of expedition waste), no detailed work has been published to specify the extent of any damage or threat to the continuity of plant species in the Park. This study presents (1) anthropogenic disturbances such as littering and damage to the vegetation along Lukla-Lobuche, a major trek route, and (2) an appraisal of human impact on vegetation patterns such as plant species richness and floristic composition.

Methodology

Study Site

The study was carried out in the Khumbu region north-eastern Nepal (Fig. 1). The upper Khumbu region was designated as a National Park by the Nepal Government in 1976 and as a World Heritage Site by the United Nations Educational Scientific and Cultural Organization (UNESCO) in 1979. The Park is 1,113 sq. km in size made up of three vegetation zones; lower zones comprising forests of oak, pine, birch and rhododendron; alpine mid zones where dwarf rhododendrons and juniper scrub dominate and upper alpine zone made up of moss and lichens (Lachapelle 1995).

Fig. 1. Map of Nepal showing Sagarmatha National Park, inset major glaciers and the trail from Lukla to Lobuche

The climate is cool with wet summers and cold-dry winters. Average precipitation in the region is 1078 mm, 845 mm most of which falls in the monsoon that lasts from June to September. Average mean minimum and maximum temperature at Tengboche in the coldest month of January is - 9°C and 3°C and in the warmest month of July is 4°C and 14°C. Some snow falls during the winter months, November to February, during which time, day-time temperature remains close to 0°C (SNP Visitor Center Data 1998).

Sampling

The study conducted in September 1998, included five major sites representing different attitudes viz. 3000 m (near Lukla), 3500 m (near Namche), 4000 m (near Tengboche), 4500 m (near Periche) and 5000 m (near Lobuche) in the Khumbu region, East Nepal (Figure 1). Littering of all types viz. discarded bottles, cans, plastic bags/wrappers etc. were recorded in the sectors between major rest-sites which included Lukla-Phakdin, Phakdin-Monju, Monju-Namche, Namche-Tengboche, Tengboche-Periche, and Periche-Lobuche. The recordings were made while walking in normal pace and totaling about two km (over 30% of the total distance) for each sector in the route. Similarly, wounded trees along the path were counted in the routes to estimate the damage by cutting.

At each site, ten quadrates (size: 1 m x 1 m) were laid nearby the trail and the coverage and number of the vascular plant species were measured. Species richness was calculated as an average of the total number of species counted in those ten quadrates. Height and frequency of occurrence of *Juniperus recurva* were measured in degraded and protected sites in Namche, the most densely populated village in the route.

Results and discussion

Littering and tree damage

Table 1 presents the littering and damage of the trees in various routes from Lukla to Lobuche (ca. 34 km). In average, in every 27.6 human steps (ca. 14 m) there was a litter. Except in the routes between Namche-Tengboche and Periche-Lobuche, the littering occurred in less than 20 human steps, i.e. 10 m. In the former routes, the littering interval was above 40 human steps. The litters were primarily non-degradable plastic wrappers of instant noodles, biscuits, chewing gums and others left by the trekkers. Glass bottles and metal cans were few. Glass bottles are banned by the Park.

Table 1. Littering and damaged tree in trekking route from Lukla to Lobuche, Sagarmatha National Park, Nepal

Route	Distance (km)	Littering*	Damaged tree (no.)	No. of lodges
Lukula-Phakdin	7.0	18.7	6	29
Phakdin-Monju	4.0	17.3	58	14
Monju-Namche	4.0	18.5	6	10
Namche-Tengboche	7.0	46.1	53	36
Tengboche-Periche	7.5	20.5	5	19
Periche-Lobuche	5.0	44.7	No tree	16

* Normal human step (Ca 50 cm)

During the fiscal year 1996-97, the Sagarmatha Pollution Control Committee (SPCC), a local non-government organization, disposed a total of 24,3091 kg of garbage from Khumbu region of which 28% included non-disposable items (SPCC 1998). A majority of such garbage was generated from Namche and Lukla with 60% and 18.65% of the total respectively. Both Namche and Lukla are based with the highest concentration of the lodges (Table 1) indicating a link between garbage generation and number of visitors in the region.

A lot of lopped/wounded trees were also noted on the way (Table 1). The number of such trees were high (above 50) in the routes of Phakdin-Monju and Namche-Tengboche. The former is outside the Park while the latter is inside. The wounds were mostly by the *Khukri*, a typical knife carried by the porters and fuelwood collectors. In the routes of Lukla-Phakdin, Monju-Namche, and Tengboche-Periche, only a few trees (less than six) were found wounded. Whilst the Monju-Lukla was at the entrance of the national park, the forest in Lukla-Phakdin was mainly a secondary growth and it was a naturally sparse type in Tengboche-Periche route. The tree line ended at near Periche. It was reported that the passers by specially the less paid porters cuts the stems that would finally collapse or kill the tree to give them fuel wood (Personal communication M.N. Sherpa, SPCC 1998).

Species Richness

The species richness (per square meter) in the Khumbu region was seven on average, of which 85% was herbaceous species (Figure 2). In general, the species number tended to decline with the increase of the altitude which can be attributed to the severe environment such as cold and short growing season. The species richness was recorded highest in Namche (nine species) and the lowest in Lobuche (below five species). At Lobuche, woody species were absent. Namche (human pop. 1328, livestock pop. 1828; source: SPCC 1999) was found to be the most disturbed site of the all.

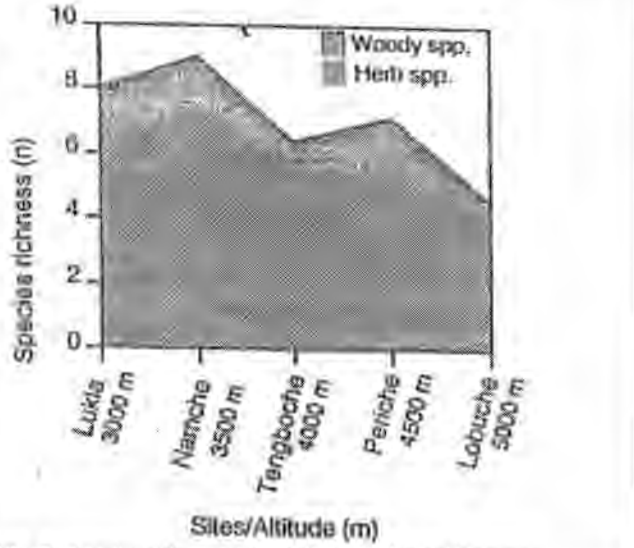

Fig. 2. Species richness (n) at different sites on the way from Lukla to Lobuche, Sagarmatha National Park, Nepal

The increase in the richness in relatively highly disturbed site (Namche) is in consistent with the model of species density under several levels of environmental stress (Connell 1978, Grime 1979). Similarly, species richness increased at the intermediate level of trampling disturbance in arm urban forest in Central Japan (Bhuju & Ohsawa, in press).

Foristic Patterns

In the present study, major trail species recorded from the sites included *Plantago* sp., *Trisetum spicata, Agrostis piosula, Poa* sp., *Danthonia* sp., *Helictotrichon virescens, Calamagrostis* sp. (Table 2). *Plantago* is a common trail perennial of rosette growth form (Mucina *et al.* 1991, Ikeda & Okutomi 1990) which can thrive well in the wear sites. The *Plantago* was recorded throughout the trek route till Periche (4500 m) which could be the limiting altitude for the species. *Agrostis* is an ectozoochrous species, the seeds of which are disseminated by attaching the hairy skin of the cattle and trousers of the passers by. They are also easily transported by the wind.

Table 2. Major species in five different, sites/altitudes nearby the trails en-route from Lukla to Periche, Sagarmatha National Park, Nepal

Near Site	Alt. (m)	Major trail/herb species nearby the trail	Major tree/woody spp. in nearby forest	Remarks
Lukla	3000	*Plantago* sp., *Aster* sp. *Anaphalis contorta*	*Rhododendron arborium, Eurya* sp., *Quercus semicarpifolia, Berberis anglusa, Gaultheria fragranstissima*	Secondary growth of Rhododendron forest maximum Tree height 2.5 m
Namche	3500	Plantago *sp.*, Helictotrichon virescens, Poa sp., *Calamagrostis* sp., *A. contorta, Sedum* sp., *Suphorbia* sp., *Leontopodium* sp.	*Abies spectabilis, Pinus wallichiana, Juniperus recurva, B. anglusa, Cotoneaster microphyllous*	Highly disturbed
Tengboche	4000	*Plantago* sp., *Poa* sp., *Trisetum spicatum, Agrostis* sp, *Gentiana prolata, Persicaria polystachya, Potentila* sp.	*Betula utilis, A. spectabilis, R. arborium, R. campanulatum, B. anglusa*	Intact forest Low light on ground
Periche	4500	*Plantago* sp., *T. spicatum, A. pilosula, Poa* sp., *Danthonia* sp., *G. prolata, Leontopodium* sp., *Ephedra himalensis*	*J. recurva, Rhododendron lepidotum, R. setosum*	Lettuce cultivation
Lobuche	5000	*Potentilla* sp., *Juncus* sp., *Phlomis rotata*	No woody species	No tree

Several natural forest patches of *Rhododendron arboreum, Abies spectabilis* and *Betula utilis* in Namche and Tengboche specifically on the sunny slopes were found replaced/colonized by *Pinus wallichiana*. Sherpa (1983) reported that the blue pine seeds germinated faster than any other species in the Park.

In degraded lands near villages the original bushes of *Juniperus recurva* were short and scanty which were replaced by semi-natural vegetation of *Euphorbia prolifera, Thermopsis inflata, Cotoneaster microphyllus* and *Berberis anglusa. Euphorbia* is a non-edible poisonous shrub while *Cotoneaster and Berberis* are thorny shrubs. In Namche, the frequency of occurrence of *J. recurva* in degraded habitats was only 20% with compared to 100% in protected sites. Similarly, the plants were short (<30 cm) in the disturbed sites with compared to average 4 m in the protected ones (Fig. 3)

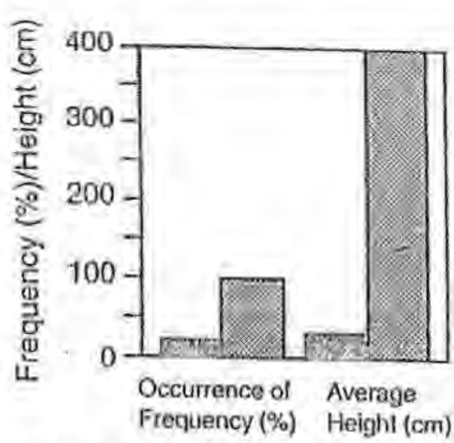

Fig. 3. Occurrence of frequency (%) and average height (cm) of *Juniperus recurva* in degraded (dark) and protected (light) sites.

In a study conducted in Khumbu region, Buffa *et al.* (1998) reported that the over grazed areas are colonized by unpalatable low plants like *Cotoneaster* and *Berberis.* A relationship has been worked out between the degradation of natural forests/grass lands and increased livestock specially the yaks (Rogers 1997, Buffa *et al.* 1998) and this increase in the yak population stems from the demand for cheese and meat by flocking number of tourists (Yonzon & Hunter 1991).

Conclusions

Reports have shown that the alpine vegetation is very sensitive to trampling (Bell & Bliss 1973) and the probability of vegetation damage is greater in high altitudes. Bare ground and trail will proliferate with increased use by the flocking trekkers and high altitude climbers in the Khumbu region. Human trampling may contribute in species richness and diversity but it also affects the succession through soil hardness (de Gouvenain 1996, Bhuju & Ohsawa 1998). Development of asphalt or other types of trails that may confine walking will be effective in closing the superfluous paths and thereby diminishing trampling hazards (Willard & Marr 1971). Monitoring the change in resource and use characteristics should be carried out to assess and manage the effects of increasing trekking in the region.

Acknowledgments

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Role of Fungal Endophytes on Dieback of *Dalbergia sissoo*

B.P. Bashyal, S. Joshi and S. Shrestha

Abstract

Seventy one endophytic fungi were isolated from the dying-back sissoo *trees (Dalbergia sissoo)* collected from various plant parts of different regions of Nepal. Their bioactivity against some common pathogenic microorganisms such as *Pythium* sp. and *Geotrichium* sp. were studied. The isolates were inoculated into the branches of fresh sissoo trees as well as healthy saplings of sissoo to evaluate their pathogenic effect. The isolates were not found pathogenic to sissoo trees.

Introduction

Dalbergia Sissoo is a large deciduous tree growing upto 8 ft in girth and 100 ft in height. It produced very heavy strong and tough wood. It is one of the most important timber trees used in carving, furniture, door and window frames, etc (Limaye 1957, Kayastha 1985, Streets 1962). Sissoo is grown naturally and is also cultivated in community or private land. It is widely distributed in riverain beds, sandy and alluvial soil in sub-Himalayan tract up to 1300 m (Manandhar 1989). It is also found growing throughout the Terai plain (100 m) to inner valley. For the last 30 years or so this species has been the most widely planted tree in Nepal. Now, it has been started dying since last few years. Trees of varying ages right from saplings to mature trees are affected by different diseases (Parajuli *et al.* 1999, FORESC 2000). The characteristic symptoms are yellowing and death of leaves in acropetal succession to the trees. The whole tree appears yellow, leaves shed rendering the branches bare. The tree shows sign of wilting and dying within a few months. Sissoo mortality is one of the major national problems affecting afforestation in the country.

Actual causal agents of sissoo dieback is yet to be identified. However, some linkages have been established between the physiological process of the plant and soil conditions such as pH and nature of soil (sandy-alluvial or clayey), irrigation, water lable of land. Therefore, these are reported to be the primary factors affecting physiological process leading to dying-back of plants. The fungal infection and the insects invasion are secondary factors which affect on the physiological process leading to die-back of sissoo. The fungal infection and insect invasions occur after the plant being weakened by environmental conditions (Negi *et al.* 1999).

It has been observed that wilt and other root diseases are absent in the riverbeds where sissoo grows naturally (Bakshi 1957). Some natural forests situated away from the river suffer from fungal infection. Common fungal infections are wilt *(Fusarium solani),* root, butt rot *(Ganoderma lucidum)* and *Polyporus gilvus,* in varying degrees. These fungi are found in association with sissoo mortality (Sheikh 1989, Bakshi 1954). Root rot is also caused by *Phytopthora* sp. and *P. vascular* wilt by *Fusarium* sp. Fungi grow usually from the exposed damaged parts of a trees, e.g. pinholes, cut bark, branch, damaged root etc. The mycelia of these fungi grow into the xylem and phloem system and block the flow of water and sap. This causes the dieback symptoms. Trees can still live even if the heart-wood and sap-wood are destroyed.

In wilt disease the pathogens are mostly restricted to roots. The fungal hyphae and jelly like substance plug the vessels which may result wilt symptoms. The toxin produced by fungi may probably be responsible for causing wilt (Bakshi & Singh 1959). In *Ganoderma* root rot disease, pathogens are spread rapidly on roots and are root inhabitants.The trees are killed in a short period.

The ultimate cause of dieback disease in sissoo is, probably, due to root decay which was found in every dying tree (FORESC 1997). Nematodes and insect borers were observed both in roots and stems of diseased trees (FORESC 1997, Parajuli *et al.* 1999). Trees showed various nature of damage such as defoliated leaves, stem exuding reddish brown sap, stem with small holes of insect borers and stem covered with termites up to a considerable height. Decaying roots, rhizospheric soil leaves and woods were heavily infested by nematodes and other microorganisms. Decaying of root with blackish brown color sometimes gave offensive odour which indicates towards root infection (PORESC 1997, Parajuli *et al.* 1999).

Four major types of insects are responsible for dieback disease of sissoo trees; pinhole sawdust and oozing red sal) are caused by borer larvae and adults of the order *coleoptera* (beetle), *Perissus dalbergia* and *Agilus dalbergia*, bark and wood borers, attack weakened tress. The heavy infestation by these borers destroy the phloem and cambium.

Pathogenic test of sissoo seedlings inoculating with *F. solani* showed no disease symptoms (Manandhar 2000). However, *F. solani* was recorded in wilted sissoo. It was also considered as facultative parasite associated more with the wounds and on the hosts weakened by unfavourable conditions (CMI 1964). Infection by *F. solani* probably requires stress condition of sapling.

Presumably, there is no single region that may cause the dieback of sissoo. For example, the dying back of sissoo in Kanchanpur is due to land on unsuitability, in Chitwan due to insect attack, in Sunsari due to seedlings grown from immature seeds or low quality seeds (FORESC 1997, Parajuli *et al.* 999).

Sissoo is light demanding and deep rooting plant which grows best on deep, bouldery alluvial soil in the river side, silty clay and silty loams are also normally good. In water depth of 7 m or adjacent to continuously flowing river sites achieve good growth (Japing 1982).

The site such as rice field having compact soil during dry season and water logged during rainy season is unfavourable for sissoo growth and showed dieback disease. The unfavourable site may be one of the factors involved in dieback of sissoo. The long peripheral and tap roots structures in natural condition is changed to less developed root system which effect water absorption capacity. Lack of proper condition for the root growth may be one of the reasons for the dieback (FORESC 1997, Parajuli *et al.* 1999).

No disease appears in plants growing on sandy and sandy loam soil but stiff and clay soil lead to asphyxiation of the feeding roots and are subsequently colonized by wilt fungus, *F. solani*. Similarly, the plant growing in a site having water table 2-3 m, mortality starts at 10-12 years, if it is slightly lower; mortality starts 12-14 years and where it is within 2 m the mortality starts at an early age of 5-6 years. It appears that the root comes in contact with the water table, and the plants become more susceptible to *F. solani* infection and ultimately die (Bakshi 1955, 1976).

It has been shown that pH range of soil in the affected area lies between 7.5 to 9.7 as compared to near neutral pH in healthy locality. The plant systems performs well at pH below neutral (5-6) (Negi *et al.* 1999).

The chemical analysis of the plant from sick localities showed lower concentration of K and P in the leaves as compared to healthy sites. It is due to poor uptake of nutrients by the root in sick localities, Thus, stress site exhibits shortage of P and K in foliage. In addition the high pH (7.4-8.4) observed at these sites may be contributing to the higher mortality. The higher pH hinders P & K uptake. Potassium is known to be responsible for controlling stomatal transpiration and its deficiency disturbs the water balance of the plant thereby causing stress. The other factors affecting dieback of sissoo are soil texture, high water table, poor irrigation which are responsible to the water stress on the plants.

In Bhabhar region with good rains and adequate drainage, sissoo grow healthy. In Terai where water table is high and come to surface during rain, sissoo suffers from wilting (Neil 1988). High moisture content and water logging create favourable condition for pathogen activity resulting in root mortality, affecting nutrient uptake which finally creates stress condition for sissoo (Bakshi 1976). Optimum silviculture practices, such as mixed culture, sufficient spacing, matured and healthy seeds are ideal to grow sissoo free from diseases (Sheikh 1989).

Methodology

The present work involved the collection of roots, stems and leaf samples of dying sissoo from different regions. Those regions include Bhairahawa, Dhankutta, Dharan, Morang, Makawanpur, Saptari and Taulihawa. Branches and root pieces (15-20 cm) long with about 1 cm diameter were collected from dying sissoo, and both the ends of these pieces were sealed with parafilm and kept in sealed plastic bags. Some leaves also collected and packed in sealed plastic bags. All the samples were stored in freeze before the analysis was carried out. The surface of the plant parts were treated with 70% ethanol and flamed to remove it. Bark, phloem, xylem and cambium of root as well as stem were removed with sharp sterilized blade carefully and placed on water agar plate at the rate 3-4 pieces per plate. The water agar plates were made by using 1.5% water agar in de-ionized water and autoclaved for one hour. The solution was then poured in hot sterilized petri plates. The fungal tips grown in petri plates of different cultures were carefully transferred to PDA media for further purification of culture. Fungal identification was carried out based on fruiting structure which is the characteristic of the spores and fungal morphology. The fruiting structures and the spores were produced for three to four weeks on water agar containing several pieces of sterilized carnation leaf.

The effect of these isolates on fresh sissoo trees was studied. For that, about 5 cm of a sampled branch of the fresh tree was sterilized with 70% ethanol and scraped with sharp knife. The isolates in PDA that cut in a rectangular portion was applied over the scraped region and covered with sterilized cotton and tape. It was tagged with the specification of isolates and date of inoculation. Besides tree branches, similar experiment was carried out on healthy saplings to observe their effects.

To observe the effects of these isolates on fresh sissoo, they were inoculated into the branches of fresh sissoo plants and also into the saplings and their effects on plants were monitored regularly. The inoculation was done during the month of December. They were also studied to evaluate the bioactivity against some common fungal pathogens (Table 1). To study the bioactivity of the collected isolates against pathogenic fungi like *Pythium* sp. and *Geotrichium* sp., they were grown for 3-5 days in PDA plates. The pathogenic fungi were then placed at four places around each isolate. The effects of the isolates on the growth of the pathogenic fungi were observed.

Table 1. Endophytes recorded from diseased sissoo trees in Nepal

Culture S.N.	Culture I.D. cells	Identification	Bioactivity	
			Pythium	*Geotrichium*
1.	Bh.S.B.B1	*Fusarium* sp.	+++	-
2.	Bh.S.B.B2	*Fusarium* sp.	++	-
3.	Bh.S.B.B3	*Fusarium* sp.	+	-
4.	Bh.S.B.B4	*Fusarium* sp.		
5.	Bh.s.B.B5	*Fusarium* sp.		
6.	Bh.S.B.B6	Non fruiting structure	-	-
7.	Bh.S.B.B7			
8.	Bh.S.B.Ph1	*Fusarium* sp.	++	-
9.	Bh.S.B.Ph2	Non fruiting structure	+	-
10.	Bh.S.B.Ph3	Non fruiting structure	+	-
11.	Bh.S.B.Ph4	Non fruiting structure	+	-
12.	Bh.S.B.Ph5	Non fruiting structure	+	-
13.	Bh.S.B.XY1			
14.	Bh.S.B.XY2			
15.	Bh.S.B.XY3	*Fusarium* sp.	++	-
16.	Bh.S.B.XY4	Non fruiting structure	+	-
17.	Bh.S.RB1	*Curvularia* sp.	++	-
18.	Bh.S.RB2	Non fruiting structure	-	+
19.	Bh.S.RB3	*Fusarium* sp.		
20.	Bh.S.RB4	*Phoma* sp.	++	
21.	Bh.S.RPH1	*Curvularia* sp.	++	-
22.	Bh.S.RPH2	Non fruiting structure	-	-
23.	Bh.S.R.Xy1	*Fusarium* sp.		
24.	Bh.S.R.Xy2			
25.	Bh.S.R.Xy3	Non fruiting structure	+	-
26.	Bh.S.R.Xy4		-	
27.	Bh.S.R.Xy5	*Fusarium* sp.	+	-
28.	Bh.S.R.Xy6	*Curvularia* sp.	++	
29.	Ta.S.B.B1	*Fusarium* sp.		

30.	Ta.S.B.B2			
31.	Ta.S.B.B3	*Fusarium* sp.	++	-
32.	Ta.S.B.B4	*Phoma* sp.	-	-
33.	Ta.S.B.B5	*Phoma* sp.		-
34.	Ta.S.B.B6	*Fusarium* sp.	++	-
35.	Ta.S.B.Ph1	*Fusarium* sp.	+	-
36.	Ta.S.B.Ph2	*Fusarium* sp.		
37.	Ta.S.B.Ph3	*Fusarium* sp.	+++	-
38.	Ta.S.B.Ph4			
39.	Ta.S.B.Xy1	*Fusarium* sp.	+	-
40.	Ta.S.B.Xy2	*Fusarium* sp.	+	-
41.	Ta.S.B.Xy3	*Phoma* sp.	++	-
42.	Ta.S.B.Xy4	*Phoma* sp.		
43.	Ta.S.B.Xy5	*Fusarium* sp.	++	-
44.	Ta.S.B.Xy6			
45.	Ta.S.B.Xy7	*Fusarium* sp.	+	-
46.	Ta.S.R.Xy			
47.	He.Ma.B.B1		+	-
48.	He.Ma.B.B2	*Trichothecium* sp.	+	+++
49.	He.Ma.B.B3	*Trichothecium* sp.	+	+++
50.	He.Ma.B.B4	Non fruiting structure	+	-
51.	He.Ma.B.Ph_1	*Phoma* sp.	++	-
	He.Ma.B.Xy1		++	+
52.	He.Ma.B.Xy2			
53.	He.B.B.B_1	*Phoma* sp.		
	He.B.B.Ph_1	*Phoma* sp.		
	He.B.B.Ph_2	*Phoma* sp.		
	He.B.B.Xy_1	*Phoma* sp.		
54.	Sa.M.B.Ph_1	*Phoma* sp.		
55.	Sa.M.B.Ph_2	*Phoma* sp.		
	Sa.M.B.B1	*Curcularia* sp.		
56.	Dha. Ti.B.B_1	*Fusarium* sp.	+++	+
57.	Dha.Ti.B.B2	*Fusarium* sp.	+	-
58.	Dha.Ti.B.B3		+	
59.	Dha.Ti.Su.BXy1			
60.	Ba.Sa.B.Ph_1	*Phoma* sp.	+++	-
61.	Ba.Sa.B.Ph2	*Phoma* sp.		
	La.Dha.B.B_1	*Fusarium* sp.		
	La.Dha.B.B_2	*Fusarium* sp.		
62.	La.Dha.B.Ph_1			
63.	Dha.B.P.B.B_1			
64.	Dha.B.P.B.B_2			

Results and Discussion

The present work involved the study of the endophytes isolated from the dieback sissoo from different regions of the country. Seventy one fungal endophytes were isolated from different parts such as xylem, phloem, cambium, bark of the branch, and the root samples and also from leaf sample (Table 1).

It was observed after six months that none of the fungal inoculated branches or saplings was dead. They were healthier during monsoon. This experiment leads us to believe that the plant endophytes were not the primary causal agents for dying back of sissoo.

During monsoon period, the plants were healthy and showed no symptom of disease. Probably the proper irrigation received during monsoon period show the plants healthier. So, it seems more likely that the physiological factors rather than the fungal infections play major role in dieback of sissoo. Thus, the fungal pathogens are playing the secondary role in that process.

Acknowledgements

This research work was undertaken to investigate whether the fungal endophytes were the causal agents for the dieback of sissoo or not. Many endophytes have symbiotic relationship with the host plants and protect the plants from microorganism while the others invaded into the plants, and growing as pathogenic to the host.

Authors are thankful to RONAST for providing its laboratory facilities. Sincere thanks are also to the departments of forests at Taulihawa and Bhairahawa for providing field facilities to carry out experiments on endophytes on sissoo. We are thankful to Dr. C. Regmi and colleagues at Research Department of RONAST for their cooperation and suggestions during this research work. Two of the authors, S. Joshi and S. Shrestha were recipients of RONAST Junior Research Fellowship.

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Antimicrobial Activities of Essential Oils of Some Common Spieces

S. Sharma, A. Singh and M.P. Baral

Abstract

Eight common spieces of Nepal were selected for this research on the basis of their use as household medicines. The essential oils of those spices were assayed for antibacterial and antifungal activities against eight different genera of test bacteria and three fungi which included pathogenic, spoilage and toxigenic strains. The essential oils of *Cinnamomum zeylanicum*, *Syzygium aromaticum* and *Zanthoxylum alatum* showed high degree of inhibition whereas that of *Piper nigrum* and *Coriandrum sativum* showed comparatively less inhibition. Minimum inhibitory concentration (MIC) values of *C. zeylanicum* oil were lowest against the tested microorganisms. The highest and broadest activity was found that of *Cinnamomum zeylanicum*, which was followed in decreasing order by *S. aromaticum, Zanthoxylum alatum, Cuminum cyminum, Myristica fragrans, Amomum subulatum, Coriandrum sativum* and *P. nigrum*. Among the test organisms two Gram positive species *Bacillus subtilis* and *Staphylococcus aureus*, the yeast *Candida albicans* and the fungal strain *Aspergillus niger* were inhibited by all the tested essential oils. The Gram negative species were found more resistant to the inhibitory activity of essential oils.

Introduction

Spices are dried plant products, which are generally used as condiments. Most spices are used as important ingredients of homeopathic medicines (Pandey & Yonjan 1991). Spices have been shown to possess medicinal value, in particular, antimicrobial activity (Arora & Kaur 1999). The plant parts, which constitute the spices contain essential oil and other chemicals which give them fragrant, aromatic, pungent, acrid, bitter or other properties of aroma and taste (Parry 1969). The aroma and flavouring properties of spices are attributed mainly to their essential oil content and composition (Bandyopadhyay 1991).

Synthetic medicines though used largely in modern world, have been found to be associated with different side effects. Faced with the mounting adverse drug reactions of synthesized chemical medications, efforts are currently being made to look for the products of natural origin (Agnihotri & Vaidya 1995). The frequency of the resistant bacteria and the number of drugs to which they are resistant is increasing at an unprecedented rate and has begun to compromise the efficient treatment of the patients (Farah 1998). In such cases the green medicines or the herbal medicines may be proved to be better alternatives.

Spices have different medicinal roles in households. Internally, some spices are beneficial in cases of flatulence and colic, in lozenges, for the alleviation of coughs and pharynx complaints; as expectorants in chest trouble; as stimulant to excite languid stomachs; and as emetics. They are also helpful in case of gastritis and dyspepsia. Externally, some spices are of value in treating rheumatism, lumbago, neuralgia, bronchitis, and similar obdurate complaints. The antiseptic properties of clove and thyme have a place in mouth washes and throat sprays (Parry 1969).

Though the use of spices as medicines has a long history, the wild claims of ancient and medieval physicians and herbalists find no place in modern medical world. Modern pharmacopoeias are cautious in attributing medicinal effects to the spices. Many researchers in different countries have done experiments, the spices and their essential oils have been found bactericidal and fungicidal. The potency of their antimicrobial activity has been found variable. However, such studies are limited in context of Nepal. Nepalese people also use different types of spices and their uses for medicinal purposes are also common. Their activity against infective microorganisms if can be proved, then those essential oils can be used in treatment effectively. Detail study is required to know the composition and active compounds of the oil and in *vivo* study should be done to know their pharmacokinetics. Present study is aimed to screen the antimicrobials among eight very commonly used spices which are cumin, coriander, black pepper, nutmeg, cinnamon, cardamom, clove and Nepal pepper 'Timur' and to determine the minimum inhibitory concentrations of the essential oils required to kill the test bacteria and fungi. The test organisms include pathogenic, spoilage and toxigenic species of bacteria and fungi.

Methodology

The spices used for the experiment were clove, cinnamon, cumin, coriander, nutmeg, black pepper, cardamom and 'Timur'. Best quality spice samples, in packaged forms were bought in market. The samples were ground and passed through No. 20 sieve. Volatile oil was extracted from the spices by water distillation method. The apparatus used was called Clevenger type volatile oil trap. Extraction procedure is AOAC official method 962.17.

Eight different types of bacterial and three different types of fungi were selected for the study. The organisms were chosen to represent several major groups. These bacterial and fungal strains were obtained from the Department of Microbiology, Tribhuvan University Teaching Hospital, Central Public Health laboratory, Teku laboratories of National Institute of Science and Technology and Royal Nepal Academy of Science and Technology. The organisms were tested for their purity and confirmed by testing their morphological, cultural and biochemical characteristics. The bacterial cultures were maintained on Nutrient agar slants and subcultured every two weeks. Fungi were maintained on potato dextrose agar slopes and subcultured every month.

Evaluation of Antimicrobial Activity

Inoculum of bacteria was prepared of concentration comparable to McFarland standard 0.5. Fungal spores were shaken in sterile saline water to make fungal suspension.

Sterile Mueller Hinton agar plates were prepared of thickness of 4 mm. The inoculums was shabbed on the agar surface with the help of sterile cotton swabs. Wells of 4 mm diameter were made in the inoculated media plates. 50µL of half diluted essential oil of spice was kept in the well made in agar media. Diluent used was 2% tween 80 in physiologic solution. Diffusion of the oil occurred within 30 minutes after addition, after that the plates were inverted and incubated. The bacterial cultures were incubatged at 37 ^{o}C covernight and fungi at 28 ^{o}C for 3 days.

The plates were then viewed for the zones of inhibition. In case of presence of zone of inhibition the test was performed in triplicate. Diameters of zones of inhibition were measured using a scale and mean was recorded.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The bacterial and fungal isolates to which the essential oil showed antimicrobial activity were subjected to broth dilution methods to determine MICs. Media used for bacteria was nutrient broth and for fungi was 2.5% malt extract.

In the first test tube concentration of oil taken was 25.6 mg/mL. Dilutions of the oil were prepared by two fold dilution method. The tubes were then inoculated with a loopful of the standard inoculums-bacteria or fungi. The tubes with bacteria were incubated at 37 ^{0}C for two days and the tubes with fungi were incubated at 28 ^{0}C for a week or more. Positive and negative controls were kept for the test. After proper incubation, the tubes were looked for turbidity or pellicle formation and the lowest concentration of the agent that inhibits growth of the organism as detected by lack of visual turbidity is designated the minimum inhibitory concentration (MIC). After obtaining MICs of oils against bacteria, they were subcultured in essential oil free media to determine minimum bactericidal concentrations (MBCs).

Results

The antibacterial and antifungal activities of essential oils highly varied. Some oils were found highly active in inhibiting the tested bacteria and fungi while others showed low or no activity. Means of the diameters of zones of inhibition are tabulated in the result Table 1. No zone of inhibition or very small zones smaller than 8mm were conferred as negative (-) in the table.

Essential oils gave bigger zones of inhibition against fungi than bacterial species showing their relatively higher activities against the fungi which included pathogenic, toxigenic and spoilage types. Clove inhibited all the tested bacteria and fungi with its highest zone of inhibition against *Vibrio cholera* and smallest zone of inhibition against *Escherichia coli*. Cinamon oil also inhibited all the test organisms and zones of inhibited were comparatively bigger than those of other spice oils. Cumin gave no zones of inhibition against *V. cholera* and *E. coli* and inhibited other bacterial and fungal species.

Table 1. Mean Diameter of Zone of Inhibition

S. N.	Organisms	Diameter of Zone of Inhibition (mm)							
		Colve	Cinnamon	Cumin	Coriander	Nutmeg	Cardamom	Black pepper	Timur
1.	*Bacillus subtilis* (ATCC 6633)	14	28	13	9	14	12	10	17
2.	*Escherichia coli* (ATCC 25922)	11	19	-	-	11	-	-	14
3.	*Klebsiella pneumonia*	14	17	12	-	-	-	-	14
4.	*Pseudomonas aeruginosa*	12	16	11	-	-	-	-	-
5.	*Salmonella typhi*	15	24	13	-	-	-	-	15
6.	*Shigella dysenteriae*	16	34	18	-	10	13	-	15
7.	*Staphylococcus aureus* (ATCC 29737)	18	27	18	11	15	11	13	19
8.	*Vibrio cholera*	28	31	-	-	12	11	-	18
9.	*Candida albicans*	16	36	15	12	12	14	9	14
10.	*Aspergillus flavus*	25	38	24	16	26	-	-	20
11.	*Aspergillus niger*	20	48	31	20	12	16	13	40

Coriander oil gave no zones of inhibition against six bacterial species which were *Klebsiella pneumonia, Salmonella typhi, Shigella dysenteriae, Pseudomonas aerugenosa, E. coli* and *V. cholera* and gave zones of inhibition against *Bacillus subtilis* and *Staphylococcus aureus* and the three species of fungi. Nutmeg oil gave no or negligible zones of inhibition against three bacterial species, *K. pneumonia, P. aeruginosa* and *S. typhi* at the tested concentration and showed activity against the remaining bacterial and fungal species. Cardamom oil gave no or negligible zones of inhibition against *E. coli, K. pneumonia, P. aeruginosa, S. typhi* and *Aspergillus flavus*, at the tested concentration, and showed activity against the remaining four bacterial and two fungal species. Balck pepper oil gave no zones of inhibition against all the Gram negative species and showed inhibition against two Gram positive species and two fungal species. The zones of inhibition were comparatively smaller. 'Timur' oil was highly inhibitory to *A. flavus* and *A. niger*. It was ineffective to kill *P. aeruginosa* but inhibitory to other bacterial species and *C. albicans* also.

MIC and MBC values of oils against tested bacteria are tabulated in Table 2. The oils which revealed no lethal activity in the preliminary tests were not processed further. MIC values of oils against tested fungi are tabulated in Table 3.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of oils for the tested bacteria in mg/mL.

S. N	Name of Organism	Clove		Cinnamon		Cumin		Coriander		Nutmeg		Cardamom		Timur		Black pepper	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1.	*B. subtilis* (ATCC 6633)	0.8	1.6	0.2	0.4	12.8	25.6	>25.6	>25.6	1.6	3.2	12.8	25.6	0.8	1.6	>25.6	>25.6
2.	*E. coli* (ATCC 25922)	0.8	1.6	0.1	0.1	ND	ND	ND	ND	1.6	3.2	ND	ND	0.8	1.6	ND	ND
3.	*K. pneumoniae*	1.6	3.2	0.1	0.2	0.8	1.6	ND	ND	ND	ND	ND	ND	1.6	3.2	ND	ND
4.	*P. aeruginosa*	3.2	6.4	0.2	0.4	12.8	25.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5.	*S. typhi*	0.2	0.4	0.1	0.2	1.6	3.2	ND	ND	ND	ND	ND	ND	0.8	1.6	ND	ND
6.	*S. dysenteriae*	0.4	0.8	0.05	0.1	0.8	1.6	ND	ND	1.6	3.2	1.6	3.2	0.2	0.4	ND	ND
7.	*S. aureus* (ATCC 29737)	3.2	6.4	0.2	0.4	1.6	3.2	1.6	3.2	12.8	25.6	3.2	6.4	1.6	3.2	3.2	6.4
8.	*V. cholerae*	0.4	0.8	0.05	0.1	ND	ND	ND	ND	0.8	1.6	1.6	3.2	0.4	0.8	ND	ND

ND = Not done

Table 3. Minimum inhibitory concentration (MIC) of oils for *C. albicans, A. flavus* and *A. niger*

S.N.	Spices	*C. albicans*	*A. flavus*	*A. niger*
1.	Clove	1.6	0.4	3.2
2.	Cinnamon	0.1	0.07	0.009
3.	Cumin	1.6	3.2	0.4
4.	Coriander	12.8	>25.6	12.8
5.	Nutmeg	3.2	12.8	6.4
6.	Cardamom	1.6	ND	25.6
7.	Timur	1.6	6.3	3.15
8.	Black pepper	12.8	ND	6.4

ND = Not Done

Discussion

This study was undertaken with an aim to collect the proofs for the wild claims about the spices and their essential oils that they possess medicinal values, with special reference to their inhibitory effect against the organisms. Spices for the study were selected on the basis of their user as household remedies in

Nepal. The selected spices are commonly used in the chest troubles, in coughs and colds, in cases of gastritis and digestive problems and also as antiseptics. These include clove, cinnamon, cumin, coriander, cardamom, nutmeg, black pepper and 'Timur'. These plants are included in Medicinal Plants of Nepal for Ayurvedic Drugs (Rajbhandari *et al.* 1995) and in the Indian Material Medica (Nadkarni 1976) also. These spices were processed for extraction of essential oils. The essential oils were tested to screen if they had inhibitory activities or not towards the bacteria and fungi. Then they were quantitatively evaluated which determined the minimum amount of oil required to kill the organisms.

The organisms for the study included 8 bacteria, 1 yeast and 2 fungi. Among bacteria, three were ATCC strains and others were isolated from patients in hospital laboratories. Most were potent pathogens found in clinical specimens. These bacteria are also common in environment, water and food products. The yeast used for the test was pathogenic *Candida albicans*. Among the fungi, *Aspergillus flavus* is toxigenic which can produce the potent aflatoxin in food and *A. niger* is very common spoilage fungi. Our results have been found comparable to the results of many former researches; however, some differences have also been observed. It may be because of variation in the test conditions and methods or in the quality, age, etc. of the spice-oil.

In present study fungicidal activities of clove oil are more prominent than bactericidal activity, which is similar to the result obtained by Dean *et al.* (1995). The spoilage type and mycotoxigenic *Aspergillus* spp. were the most inhibited which tallies with our result. However, the result differs in case of bacteria, as they found *P. aeruginosa*, the least affected whereas in our study, *E. coli* was the least affected.

Essential oil of cinnamon is found highly inhibitory to both bacteria and fungi. In fact, it strongly inhibited (inhibition zone > 20 mm) 5 of the 8 bacterial strains under investigation, showed a good activity (inhibition zone > 10 mm < 20 mm) against the remaining three bacteria and showed highest activity (inhibition zone > 30 mm) against the yeast and filamentous fungal strains. Rao and Nigam (1978) also found the oil of *Cinnamomum zeylanicum* with the highest average antibacterial activity compared to the essential oils of *Cymbopogon flexuosus, Eucalyptus citriodira, Scimmia laureola*, etc. Many researchers have proved cinnamon as absolute antimycotic substance, such as by Lewellyn *et al.* (1981) and Maruzella *et al.* (1959).

Cumin oil also showed good antibacterial and antifungal activities in our research. Syed *et al.* (1986) demonstrated remarkable activities of cumin essential oil against *S. aureus, E. coli, S. typhi, S. dysentery* and *V. cholera* at low concentration whereas no activity was observed against *E. coli* and *V. cholera* in our study. Our results are also comparable to those of Shetty *et al.* (1994).

Coriander and black pepper oils showed very weak antimicrobial activities. All gram negative species tested were resistant to these oils. The zones of inhibition given by coriander in our study are comparable to zones of inhibition given by coriander oil in similar study done by Baratta *et al.* (1998). In a study done by Jain and Kar (1971) and Jain and Jain (1972), black pepper oil produced very small inhibition zones against bacteria and fungi, which are also similar to the zones of inhibition given by black pepper oil in the present research.

Zones of inhibition produced by nutmeg essential oil were somewhat irregular and not smooth. It may be because of its low diffusion in the media. However, it showed good antimicrobial activity against both bacteria and fungi. Our result is comparable to that of Miranda *et al.* (1993) who found nutmeg as effective antibacterial agent against four species of *Shigella* and *E. coli*. Like that of nutmeg essential oil, the diffusion of essential oil of cardamom in media was also found low and it produced irregular zones of inhibition. Antimicrobial activity of cardamom was also lesser comparable to other oils tested. Our result is found comparable to that of Sinha *et al.* (1976) who had found cardamom oil as slightly effective against *S. aureus* and *S. sonnei* but not effective against *E. coli, S. typhi* and *P. aeruginosa*. Observing the zones of inhibition produced by 'Timur' oil, it can be said that it possesses significant antibacterial and antifungal activities with antifungal activities against filamentous fungi more prominent.

For MIC determination, the highest concentration of oil taken was 25.6 mg/mL. Then the concentration of oil decreased serially in ½ dilution rate. The essential oil which gave no or negligible zones of inhibition was not processed further to obtain the MIC and MBC values. The minimum concentration that inhibited the bacteria was 0.05 mg/mL which was concentration of the cinnamon essential oil and against the bacteria *S. dysenteriae* and *V. cholerae*. MIC values of cinnamon oil ranged from 0.1 and 0.2 mg/mL and MBC values ranged from 0.1 to 0.4 mg/mL. Thus cinnamon oil was found lethal at very low concentration also due to which it is considered as the best antimicrobial among the oils tested. MIC values of clove ranged from 0.2 mg/mL (against *S.typhi*) to 3.2 mg/mL (against *S. aureus and P. aeruginosa*) and MBC values ranged from 0.4mg/mL to 6.4 mg/mL MIC values of 'Timur' ranged from 0.2 mg/mL (against *S.dysenteriae*) to 1.6 mg/ml (against *S.aureus* and *K. pneumonia*) and MBC values ranged from 0.4 to 3.2 mg/mL. MIC values of cumin ranged from 0.8 (against *S. dysenteriae* and *K. pneumonia*) to 12.8 mg/mL (against *B. subtilis* and *P. aeruginosa*) and MBC values ranged from 1.6 to 25.6 mg/mL. MIC values of nutmet ranged from 0.8 mg/mL (against *V. cholera*) to 12.8 mg/mL (against *S. aureus*) and MBC values ranged from 1.6 mg/mL to 25.6 mg/mL.

MIC values of cardamom ranged from 1.6 mg/mL (against *S. dysenteriae* and *V. cholera*) to 12.8 mg/mL (against *B. subtilis*) and MBC values ranged from 3.2 mg/mL to 25.6 mg/mL. MIC values of coriander ranged from 1.6 mg/mL (against *S. aureus*) to more than 25.8 mg/mL (against *B. subtilis*) and MBC values from 3.2 mg/mL to more than 25.6 mg/mL. MIC values of black pepper ranged from 3.2 mg/mL (against *S. aureus*) to more than 25.6 mg/ml (against *B. subtilis*) and MBC values ranged from 6.4 to more than 25.6 mg/mL. In case of fungi also minimum MIC values were of cinnamon oil. Minimum MIC value was 0.009 mg/mL against *A. niger*. The highest MIC value was more than 25.6 mg/mL which was that of coriander oil (against *A. flavus*). To inhibit *C. albicans* minimum MIC was 0.1 mg/mL that was of cinnamon oil and maximum MIC was 12.8 mg/mL that was of coriander and black pepper oils.

Thus he highest and broadest antimicrobial activity was found that of cinnamon oil among the spices tested. The clove oil came as the second most potent. When ranged with decreasing lethal activity third member was 'Timur' oil, which was followed by cumin, nutmeg, cardamom, coriander and the last one with the least activity was black pepper oil.

Thus from the results obtained we can say that essential oils are good antimicrobials but their activities depend upon their composition, active compounds and also on types of microorganisms. Studying different works on aromatic chemicals it can be generalized that groups of aromatic chemicals possess the following order of decreasing antibacterial activity; aldehydes, alcohols, acids, lactones, ethers, ketones, esters and acetals. So, detail study about chemical composition of the volatile oils of plant is very important to determine the active principal against microorganisms. The actual mode of inhibition of the essential oil against microorganisms has not been studied in detail. The growth of any microorganism is affected by the various physical, chemical, and biotic factors associated with the micro-environment, including storage temperature, water activity, solute concentration and type, pH nature and concentration of atmospheric gas (Davies *et al.* 1976). The essential oil may change any of the physical or physiological condition of the organism, which kill them or inhibit their growth. Guenther (1948) reported that, because of the complexity of essential oils, no general statement could be made as to their antimicrobial properties. Helander *et al.* (1998) from the components of essential oil, carvacrol and thymol decreased the intracellular ATP pool of *E. coli* and increased extracellular ATP, indicating disruptive action on the cytoplasmic membrane.

The essential oils, which were found inhibitory to certain bacteria, could be tried as effective remedies against them. Essential oils were found inhibitory to spoilage type bacteria and fungi, so they could also have preservative action. Essential oils are used for flavouring food and are generally accepted by the Food and Drug Administrations as additives in certain type of foods. Different types of spices are naturally occurring antimicrobial agents which can be used in its natural form or incorporated into health care products including antiseptic creams, shampoos, conditioners, tooth pastes and soaps. Essential oils of spices are used as preservatives in Indian system of medicine. The water distillate and essential oils of cinnamon and clove have been reported to have preservative property without altering the physico-chemical properties of Kwatha by Venkataram *et al.* (1983).

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Organic Tea Farming in Nepalese Mountains

Kayo D. Yami and Ishwor Khanal

Abstract

Tea gardens in Nepal cover a total area over 10,000 ha of this, hill plantation is estimated at 3,609ha. Total export of Nepalese tea in 1998/1999 was 83,793kg equivalent to Rs 3,00,81559. Because of the high value of organic tea in the international market and also because of health hazards created by application of fertilizers and pesticides, there is growing interest of tea growers to produce more organic tea. Organic tea is a low energy input product ideally suited for small farmers along with dairying. Quality and flavour of organic tea is better. Although yield of organic tea is around 5-10% less than the conventional tea, the price realisation is more than it compensates for the loss in yield. In organic farming there is a maximal use and recycling of on farm resources like fodder, livestock manure and organic wastes. There is no problem of pesticide residues, heavy metals, etc. There are few tea gardens in eastern Nepal which are organic since the beginning. Total production of certified organic tea in Nepal is only 35 mt from 7O ha of tea plantation. Traditionally farmers use organic manure in the form of compost which because of low quality becomes only bulky mass of farmyard wastes that do not contribute in tea production. The objective of this study was to improve the fertility status of soil of tea gardens by organic farming by the use of microbial inoculants in composting. The analysis of composts prepared by microbial inoculation on various combinations of agricultural wastes, and that of vermicompost prepared by using earthworm *(Eisenia foetida)* showed that nitrogen, phosphorus and potash contents of the compost was significantly higher in the treatments inoculated with a fungus *(Trichoderma viridae)* and a bacterium *(Azotobacter)* and in vermicompost samples.

Introduction

Tea is a high value, labor intensive perennial crop with a potential for generating foreign exchange, reducing rural poverty, promoting economic growth and improving environment and ecology. Today tea gardens cover a total land of over 10,000h a. Of this, hill plantation is estimated at 3,609 ha. Tea grown in Terai is used for making commonly used cut tea and curl (CTC) black tea whereas hill tea is exclusively processed for making the Orthodox tea. Production of tea has become one of the important economic sources in Nepal. The production of CTC and Orthodox tea in Nepal are around 4.1 and 0.9 million kilograms, respectively (Singh 2001). Table 1 shows total tea production and area of plantation and table 2 shows area and production of CTC and Orthodox tea in Nepal.

Table 1. Total plantation area and its production in Nepal

Fiscal year	Area (ha)				Production (kg)			Total
	Private	NTDC	Small holder	Total	Private	NTDC	Small holders	
1995/96			828		1500000	1112329	125000	2737329
1996/97	1685.2	937.6	879	3501.2	1800000	925942	18000	2905942
1997/98	2192	937.6	1385.4	4515.0	1964555	603136	468980	3018571
1998/99	6073.2	937.6	2050	9060.8	3577857	496881	418242	4492980
1999/2000	6073.2	937.6	3239	10245	3577857	496881	1010499	5085237

Source: National Tea and Coffee Development Board, 2057.

Table 2. Orthodox and CTC tea plantation area and production in 1999/2000.

Particulars	Orthodox		CTC		Total	
	Area (ha)	Productin (kg)	Area (ha)	Productin (kg)	Area (ha)	Productin (kg)
Private	1088.4	244603	4984.6	3333254	6073.0	3577857
Govt.	2320	50738	705.6	446143	937.6	496881
Small holders	2289.0	611646	950.0	398853	3239.0	1010499
Total	3609.4	906987	6640.2	4178250	10249.6	5085237

Source: NTCDB, Tea and Coffee Newsletter, 2057/58.

Himalayan orthodox tea is the brand name of orthodox tea produced in Nepal. It is one of the finest teas in the world which is produced from tea gardens located in the hill regions of eastern Nepal with altitude ranging from 914 to 2134 m. Table 3 shows major hill tea producers and Table 4 shows the list of tea processing factories in Nepal. The demand for the special hill orthodox tea has always been high in the overseas market because it has distinct naturally occurring aroma, delicate flavour and exquisite bouquet. Presently, the major markets for high value orthodox tea are European countries, Japan and USA. Total export of Nepalese tea in 1998/1999 was 83,793 kg equivalent to Rs. 3,00,81,559 (Singh 2001). Tea industry as a whole commands about 1200 manpower and about 3,250 small farmers are actively involved in the hill tea industry (HOTPA 1999).

Table 3. List of hill (Orthodox) tea producer factories registered to date.

S.N.	Company	Location	Farm area (ha)
1.	Keshar Tea Estate	Panchthar	NA
2.	Nulung Chiya Alainchi Udhyog	Bhojpur	NA
3.	Dewan Tea Garden Pvt. Ltd.	Dhankuta	NA
4.	Guransae Tea Estate	Dhankuta	400
5.	Makalu Krishi Farm Pvt. Ltd.	Dhankuta	NA
6.	Alokati Estate	Ilam	40
7.	Anjana	Ilam	40
8.	Baraha Chiya Udhyog	Ilam	37
9.	Deaurali Tea Estate	Ilam	50
10.	Dhahil Tea Estate	Ilam	90
11.	Gorkha Tea Estate	Ilam	50
12.	Ilam Tea Producers Pvt. Ltd.	Ilam	50
13.	Jogmai Tea Estate	Ilam	100
14.	Kaji Koti Tea Estate Pvt. Ltd	Ilam	200
15.	Mahabharat Tea Estate	Ilam	16
16.	Morohang Tea Estate	Ilam	37
17.	Muge Khola Tea Estate	Ilam	20
18.	Puwahill Tea Estate	Ilam	60
19.	Sakti Devi Tea Estate	Ilam	60
20.	Siddi Devi Tea Estate	Ilam	50
21.	Singha Devi Tea Estate	Ilam	60
22.	Faktaklung Chiya Bagan Pvt. Ltd	Panchthar	NA
23.	Janta Tea Estate	Panchthar	15
24.	Kanchanjungha Tea Estate	Panchthar	100
25.	North Nepal Tea Estate	Panchthar	100
26.	Pathivara Tea Estate Pvt. Ltd.	Panchthar	100
27.	Phungsen	Panchthar	125
28.	Ravi Sinchelengma Tea Estate	Panchthar	150
29.	Tinchule Tea Estate	Panchthar	150
30.	Triveni Tea Estate	Panchthar	NA
31.	Ghumane Tea Estate	Solukhumbhu	NA
32.	Piramid Valley Pvt. Ltd.	Solukhumbhu	NA
33.	Everest Tea Estate	Singhuplanchok	200
34.	Langthang Tea Estate	Sindhuplanchok	300
35.	Sagarmatha Tea Estate	Sasnkhuwasabha	(Proposed) 1500

NA = not available

Table 4. List of tea processing factories (orthodox only)

S.No.	Company	Location	Capacity (kg)	1997/98 Production (kg)
1.	Kanchanjungha Tea Estate	Panchthar	100,000	30,000
2.	Himalayan Range Tea Estate	Ilam	150,000	45,000
3.	Nepal Small Tea producers Ltd.	Ilam	200,000	73,000
4.	Ilam Team Producers (P.) Ltd.	Ilam	200,000	70,000
5.	Guranse Tea Estate	Dhankuta	100,000	20,000
6.	Kanyam Tea Estate	Ilam	200,000	70,000
7.	Ilam Tea Estate	Ilam	100,000	40,000

Use of chemical fertilizers in tea cultivation. Most of the tea farmers use inorganic fertilizers for increasing tea production shows commonly used chemical fertilizers and their application rates as per cultural practices in tea cultivation (Table 5). Even though mineral fertilizers increase available nutrients in soil their constant use increases soil acidity, deteriorates soil structure reducing water holding capacity and organic matter content. Nepal imports all these chemical fertilizers from foreign countries. Since these fertilizers have to be imported from abroad they are not available when there is an urgent need. Often the chemical fertilizers used are date expired so tea growers have to bear both financial loss of fertilizer as well as the crop loss.

Table 5. Commonly used chemical fertilizers and their application rate in tea cultivation as per cultural practices followed.

Chemical fertilizers	Time of application	
	Plucking 2.1.2.(NPK)	Cutting 2.1.3(NPK)
Nitrogenous	90kg N/ha	90 kg/ha
Ammounium Sulphate 20% N	437 kg	437 kg
Urea 46% N	196 kg	196 kg
Ammonium Sulphate Nitrate 26% N	346 kg	346 kg
Di-Ammonium Phosphate 18%	500 kg	500 kg
Phosphatic	45kg P_2O_5	45 kg P_2O_5
Single Super Phosphate 16% P_2O_5	281	281
Triple Super Phosphate 46% P_2O_5	98	98
Rock Phosphate 10-40% P_2O_5	450-113	450-113
Dicalcium Phosphate 45% P_2O_5	105	105
Diammonium Phosphate 46% P_2O_5	98	98
Potassic	90 kg K_2O	90 kg K_2O
Muriate of Potash 60% K_2O	150	225
Potassium Sulphate 50% K_2O	180	270

Use of pesticides in tea cultivation. It has been estimated that the loss due to pests and diseases is 250 to 500 million kg of tea annually which in terms of value could be of 500 million to one billion US$ in the Asian region (Agnihothrudu 1999). There are more than 1000 arthropod pests and over 400 taxa of fungi reported in tea. Thus the use of pesticide is very high in tea as compared to other crops. Table 6 shows the commonly used chemical insecticides, fungicides and herbicides in tea plantation in Nepal. There is an inevitable problem of pesticide residues, particularly because the end product used is the leaf which has fairly large surface area exposed to pesticide deposits. Extensive use of plant protective toxic chemicals are posing serious health problems all over the world. Because of expensive labor requirement for weed control in tea gardens, more and more tea growers are attracted towards use of herbicides. However, there are many reports of phytotoxicity in tea plantation such as chlorosis, curling of leaves, stunted growth etc. of tea plants (Singh 1999).

Table 6. Commonly used pesticides in tea cultivation in Nepal

Common name	Dose	
Insecticides/miticides		
Dicotol 5% EC	1:400 (Water)	
Ethion 50 EC	Do	
Dimethoate 30 EC	Do	
Sulfur 80 WP	1:100	
Four star weight sulfur	1:200	
Endosulfan 50 EC	1:400 (Water)	
Fenitrothion 50 EC	Do	
Chloropyrifos 20 EC	DO	
Quinalphos 25 EC	Do	
Monochrotophos 40 EC	DO	
Synthetic pyrethroids	Do	
Cypermethrin 10 EC	Do	
Deltamethrin 2.8 EC	DO	
Fenvalerate 20 EC	Do	
Carbofuran 3 G	5-10 gm/B	
Fungicides		
Cuprousoxide		1:4 (Water)
Sulfur powder		
Bordo mixture		1:100
Copper oxychloride		1:4 (Water)
Herbicides		
2,4-D sodium salt	0.8 kg/ha	
Simazine	2.0 kg/ha	
Paraquat	0.32 kg/ha	
Diuron	4.8 kg/ha	
Glyphosphate	0.82-1.0 kg/ha	
Oxyfluorfen	0.25 kg/ha	

Source : Narabahadur Amgal, 2054

Importance of organic farming in tea cultivation. In recent years, global awareness of health and environmental issues have been growing, and sustainability has become the key word in the discussions of economic development, particularly in relation to developing countries. Government policies in industrialized as well as developing countries increasingly encourage organic and other forms of sustainable agriculture. Farming in Nepal was organic till the end of nineteen sixties. However with the introduction of Green Revolution in India modern farming started in Nepal with intensive use of chemicals as fertilizers as well as pesticides for increasing food production. But there are still many hills and mountainous regions where farming is still organic due to inaccessibility of those regions for the transportation of chemical fertilizers.

Organic tea is a low energy input agriculture. It is ideally suited for small farmers along with dairying. Quality and flavour of organic tea produced is better. Although yields of organic tea are around

5-10% less than the conventional tea, the price realisation is more than it compensates for the loss in yield (Khurana 2001). Organically grown tea basically takes advantage of nature's equilibrium, sustaining itself for optimum productivity without creating any ecological problems. Because of the high value of organic tea in the international market and also because of the health hazards created by the application of chemicals as fertilizers and pesticides there is growing interests by tea growers to produce more organic tea. In organic farming there is a maximal use and recycling of on farm resources like fodder, livestock manure and organic wastes. Its main aim is to support and strengthen biological processes. Table 7 shows approximate nitrogen, phosphorus and potash contents of biological materials used in organic fertilizers. Use of organic fertilizer to maintain organic matter and nutrients in the soil, use of nitrogen fixing plants, pest resistant varieties, soil management techniques such as mulching, and the use of tallow periods, various cropping systems and agroforestry are the key characteristics of organic farming. Virtually all the inputs required for organic tea are produced in the garden, hardly any external chemical outputs are required. Organic farming merges traditional and respectful views on nature with modern scientific insights. In Nepal there are few reports on the use of local plant resources as botanical pesticides against various types of insects of vegetables (Vaidya 2000). However there are no reports of scientific research on the use of pesticidal plants in tea farming. For the control of tea pests the classical approach of biological control with parasites and predators has been successfully used. Commercial formulations of *Bacillus thurengiensis*, a bacterium, has been used in the control of different species of caterpillars, tea leaf roller and looper caterpillar in North East India (Barbora 1995). There is no problem of pesticide residues, heavy metals, etc. in using biopesticides.

Table 7. Approximate NPK contents of common materials used in rural composting

Name	N (%)	P (%)	K (%)
Paddy straw	0.5	0.07	0.6
Wheat straw	0.5-0.6	0.1	1.3
Cattle dung	2.0	0.4	1.7
Cattle urine	9.4	0.05	8.81
Poultry dung	5.0	0.8	1.0
Bone meal (raw)	3-4	9-11	-
Water hyacinth	2.4	0.3	4.4
Grass/weeds	2.03	0.48	1.7
Guatemala grass	1.6	0.6	1.3
Tea pruning	2.5	0.5	1.5
Oilcake (linseed)	7.4	1.7	1.3
Oilcake (sesame)	5.5	2.1	1.3

There are few tea gardens in Ilam and almost all in Panchthar in Eastern Nepal which are organic since the beginning. Total production of certified organic tea in Kanchanjunga Tea Estate alone is 35 mt from 70 ha of land. In Nepal organic tea is certified by National Association for Sustainable Agriculture Australia (NASAA) accredited to international Federation of Organic Agriculture Movement (IFOAM) according to the rule of European Union. Since certification by such international agencies is highly expensive. Only rich tea growers are certifying their products and selling in the international market. Traditionally farmers use organic manure in the form of compost in their tea gardens. Such composts are prepared from organic residues such as crop residues, cattle dung, animal wastes, habitat wastes, night soil and vegetable wastes. Because of the poor knowledge of producing and using a high quality compost such inputs become only bulky mass of farmyard wastes which contribute least in tea production.

Experience or RONAST in soil fertility research. Soil microorganisms play crucial roles in biological convertion of various elements and organic wastes into humic materials in soil. Inoculation of microorganisms including bacteria and fungi has become general practice now-a-days for enriching soil with nitrogen and phosphorus and for expediting process of composting. Microbiological activity in the soil under the organic system is extremely high (Khanna 2001).

RONAST has been doing research on soil fertility since 1985 with the grant assistance from International Foundation for Science (IFS), Sweden and FAO, Rome. With these two major grants RONAST has established a moderate laboratory for research on biofertilizers. More than 100 strains of nitrogen fixing bacteria like *Rhizobium, Azotobacter, Azospirillum,* (Yami 1997, Yami & Khanal 1997), *Frankia* (Yami 1999) and Mycorrhizae (Shrestha 1999) have been isolated and tested in various field trials. One of the organisms popularly used extensively for the biological control of lepidopterian insects is *Bacillus thurengiensis.* Several strains of this bacterium have been isolated from various agroclimatic conditions of Nepal at RONAST (Sijapati *et al.* 1999). RONAST has produced a glossary of pesticidal plants available In Kathmandu valley (Adhikari *et al.* 1991).

Methodology

Rural compost is prepared from organic residues such as crop residues, weeds, cattle dung, urine, and other animal wastes, and habitation wastes such as garbage, night soil and vegetable wastes (Photo l). The organic substances undergo intensive decomposition under thermophillic and mesophillic conditions in heaps or pits with adequate moisture and finally yield a dark colored humified material in three to six months which is more stable in form and valuable for replenishment of plant nutrients, maintenance of soil organic matter and in improving and maintenance of soil fertility. Generally compost is bulky in nature and contains on an average of 0.5-0.8%, 0.3% and 0.4%, thus there is a need to reduce its bulkiness as well as the period of preparation and to improve the quality so as to supply more nitrogen, phosphorus and humic substances per unit weight. Research in this project has been initiated to hasten the process of composting by inoculation with nitrogen fixing and cellulolytic microorganisms as well as to improve nitrogen phosphorus and potash contents, and also to reduce the bulkiness more quickly.

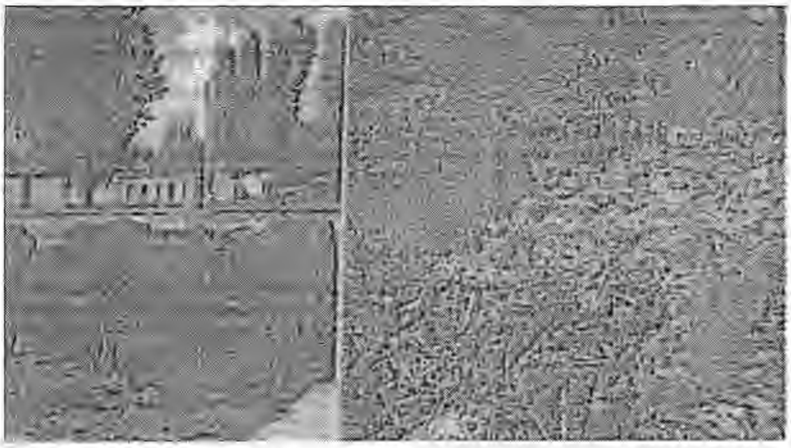

Photo 1. Composts prepared by traditional ways

Isolation, purification and identification of the following microorganisms were carried out at the RONAST laboratory

i. Nitrogen fixing bacteria e.g. *Azotobacter chroococcum*

ii. Cellulose degrading fungi e.g. *Trichoderma viridae*

iii. Phosphate solubilizing bacteria and fungi e.g. *Bacillus* and *Aspergillus.*

Selection of microbial Inoculants. Among the microorganisms isolated at RONAST only broth cultures of *T. viridae* and *A. chroococcum* were used as microbial inoculants for the compost preparation. Since some of the tea growing farmers wanted to know if they could use Japanese effective microorganisms (EM). RONAST also has used them for inoculating the compost materials.

Preparation of compost pits. In the month of February, 2001 five compost pits each with one square meter capacity were dug at RONAST premises to study best method of composting by using various composting materials (Photo 2). Five different treatments were given in five different pits. Following composting materials were collected and used for making compost: a. Rice straw, b. Cauliflower cabbage leaves, c. Mustard oil cake (Pina), d. Cattle manure and e. Chiken manure.

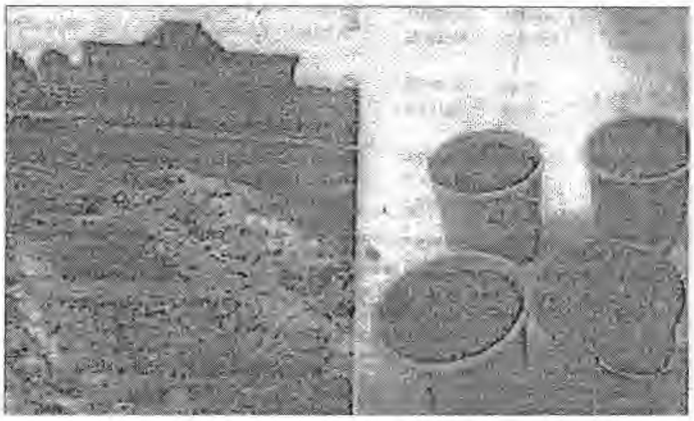

Photo 2. Compost prepared at RONAST for research purposes

All the treatments had rice straw, vegetable leaves and oil cake in common. Quantities of these materials used per pit were 10 kg of rice straw, 174 kg of vegetable leaves and 2 kg of oil cake. Wherever used the quantity of cowdung was 42 kg per pit and that of poultry manure was 12 kg per pit.

Vermicompost was also prepared by using red earthworm in buckets containing kitchen wastes and fruit wastes.

Field training programme. Trainings were given to tea growing farmers of Dhankuta, Terhathum, Panchthar and Ilam about organic farming practices, scientific way of preparing compost for tea organic farming, detrimental effects of chemical fertilizers and pesticides on soil fertility and human health.

Analysis of soil samples from different tea gardens. For understanding the limiting nutrient factors in the soil it is necessary to assess representative soil samples for their nutrient status at periodic intervals. Such an assessment would help to take a suitable and justifiable decision on soil and nutrient management. Fourteen soil samples were also collected from various places of the above tea gardens for analysing nitrogen, phosphorus, potash, pH and organic matter content.

Analysis of composts. After three months of composting all the composts were taken out from the pits and analysed for nitrogen, phosphorus, potash, moisture and pH.

Results and Discussions

From our field visits and training programs in three districts, Dhankuta, Ilam and Panchthar we came to know that farmers are becoming more and more aware of the harmful effects of chemicals used either in the form of fertilizers or pesticides. They are also aware of the cost benefit of producing organic tea. However because of the low production of tea and because of the lack of knowledge of organic farming technologies the farmers are hesitant to go for producing organic tea (Photo. 3). Among the private companies Kanchunjunga Tea Estate and Guranse Tea Estate are producing certified organic tea and exporting to European countries. Because of the high cost of certification and unavailability of factories for organic tea processing production of organic tea from so many organic tea gardens is unaccounted.

Photo 3. Training of tea growing farmers in Ilam

From the soil analysis report (Table 8.) it can be noted that most of the tea growing areas of Dhankuta and Panchthar have very poor soil fertility status indicating the need of applying best quality compost.

Table 8. Analysis of soil samples from different tea gardens.

Location	Sample (No.)	Soil (pH)	Organic matter (%)	Nitrogen (%)	Phosphorus (kg/ha)	Potash (kg/ha)
Dharmasala (Dhankuta)	1	5.8	1.61 L	0.049 VL	97 H	935 VH
,,	2	4.5	0.80 VL	0.003 VL	35 M	229 H
,,	3	4.5	0.80 VL	0.048 VL	33 M	334 H
,,	4	5.0	0.54 VL	0.037 VL	36 M	362 H
Nigalai (Dhankuta)	5	4.1	2.01 L	0.123 M	67 H	372 H
,,	6	4.0	1.74 L	0.017 VL	38 M	467 H
,,	7	4.5	0.94 L	0.048 VL	281 VH	572 VH
Guranse (Dhankuta)	8	4.0	5.69 H	0.160 M	130 VH	639 VH
,,	9	4.0	5.90 H	0.216 M	62 H	457 H
Kanchanjunga (Panchthar)	10	4.9	3.35 M	0.111 M	57 H	763 VH
,,	11	4.1	2.34 L	0.148 M	25 L	620 H
,,	12	4.5	3.68 M	0.105 M	26 L	419 H
,,	13	4.4	5.36 H	0.204 H	27 L	286 H

Note: VH = Very High; H = High; M = Medium;VL =Very Low; L = Low

The analysis of the composts (Table 9) prepared at RONAST with various combinations of organic materials shows that all the samples were better than sample no. 6 collected from Kunchunjunga tea state, Phidim which has very high content of potash. Obviously it may be due to the lack of any scientific input. Among the composts prepared in RONAST, composts prepared by inoculating *A. chroococcum* and *T. viridae* showed higher percentage of nitrogen, phosphorous and potash than those treated with Japanese EM. The two vermicompost samples showed significantly high percentage of nitrogen, phosphorous and potash. From this analysis it could be concluded that composts if prepared in scientific way could be of best quality within a period of three months. Such best quality compost if applied in tea plantation areas, it would contribute to increase N, P and K contents of rhizospheric soil of tea plants. Palaniappan and Annadurai (1999) have reported that microbial inoculation of mesophyllic cellulolytic fungi like *Coprinus ephemerus* to compost pit may reduce the composting period by one month with an improvement in compost quality, and inoculating the compost previously amended with rock phosphate with cultures of *Azotobacter chroococcum* and the phosphate solubilizing strain *Aspergillus arvamori* increases the total nitrogen and humus content.

Table 9. Analysis of compost samples prepared at RONAST.

Sample No.	Soil pH	Moisture (%)	Nitrogen (%)	Phosphorus (%)	Potash (%)
Compost S.No. 1	7.6	43.55	0.68 VH	0.52	3.78
Compost S.No. 2	7.9	44.65	1.35 VH	1.30	3.37
Compost S.No. 3	7.1	44.35	0.95 VH	1.05	2.29
Compost S.No. 4	8.0	63.45	1.29 VH	0.55	2.43
Compost S.No. 5	8.0	45.65	0.77 VH	0.75	2.02
Compost S.No. 6	7.9	48.80	0.45 VH	0.34	6.07
Vermi-compost S.No. 7	7.5	49.00	3.96 VH	2.07	8.28
Vermi-compost S.No. 8	8.1	55.00	1.88 VH	1.75	5.24

Note : VH = Very High, H = High, M = Medium

A commercial preparation called BPPS developed in RRL Jorhat has been prepared from soil bacteria isolated from tea plantations. This preparation has been used to control plant pathogenic fungi that induce termite aggregation on live plants and other serious plant pests of tea. Such bacterial inoculation on tea plant cuttings or seeds or planting pits, induced growth vigour in tea plants. In addition to the strains that produce BPPS, several other plant beneficial strains have been obtained which can be introduced into the root zone at the planting time of young tea (Bezbaruah 1999).

Above all it is absolutely necessary to know the quality of organic ammendments that enter the soil. Organic matter is a growth substrate for soil microorganisms and not for plants. The microorganisms mobilize the nutrients present in the organic matter for plants.

Problems and Future Activities

The main problems faced by the organic tea farmers in Nepal is the lack of technical knowhow about how to produce good quality of organic fertilizers and how to and what to use as biopesticides for controlling insect pests and diseases of tea.

Lack of factory for processing organic tea for small farmers is another problem. Although there is only one tea factory in Panchthar, which does not process tea leaves of any other tea farmers even if the tea produced by them also is of organic origin. For example, Pathibhara Tea garden and many other tea gardens have been producing organic tea leaves since last four years. Because of the lack of processing factory, tea leaves produced at the gardens have to be processed by hand and sold in Kathmandu market.

Third problem faced by organic tea producers is the high cost charged by international certifying agents in certifying tea as organic without which such tea cannot be exported abroad.

Among future activities, plant experiments will be carried out by using improved composts to study the impact of organic fertilizers on growth of tea plants and quality of leaves. In tea, organic farming since there is a high demand for doing research on possible use of plant pesticides study will carried out to do research work on plant botanicals which could be used as biopesticides in tea cultivation.

Proc. Internat. Sem. On Mount.- Kathmandu, 517-577, 2002

Study of Antagonistic Action of Two Ectomycorrhizal Fungi Isolated from Pine Forest of Dadeldhura

Geeta ShresthaVaidya and Sujan Piya

Abstract

Two ecto-mycorrhizal fungi, *Pisolithus arrijus* and *Scleroderma vericossum*, isolated from sporocarps of pine forest of Dadeldhura were evaluated against the following fungi and bacteria: *Pythium sp.*, *Rhizoctonia* sp., *Fusarium sp.*, *Geotrichum* sp., *Curvularia Pallescens*, *Curvularia critici*, *Aspergillus niger*, *A. ochraceous* and bacteria, *Pseudomanas solanacerum*, *Agrobacterium tumefaciens*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Escherichia coli*, *Kleibsella* sp. and *Bacillus* sp. Most of them were isolated from soil of pine forest of Chovar, Kathmandu. The antifungal and antibacterial action of ectomycorrhizae *in vitro* test was carried out by direct cross inoculation and extraction of metabolites from liquid culture and activity by agar well diffusion method. *Pisolithus arrijus* and *Scleroderma varrucossum* had higher activity against *Pythium* sp., *Rhizoctonia* sp., *Fusarium* sp., *A. tumefaciens*, *P.* solanacerum sp. and *Kleibsiella* sp., *Staphylococcus aureus*, *Shigella dysenteriae*, *Escherichia coli*, but no activity was found against *Vibrio cholarae*, *A. clavatus*, *A. granulosis*, *A. niger*, *A. sydowi*, *Aspergillus ochraceous* and *A. ustus*. Mycorrhizal plants seemed to be less affected by plant pathogens.

Introduction

Chir pine *(Pinus roxburghii)* is an important multipurpose species. It establishes well on new clearings, landslides and in eroded areas (Mohan & Pun 1956) and has been successful in checking erosion in areas where grazing is uncontrolled (Shrestha 1975). The study has been carried out in subtropical forest in Dadeldhura, far western midland of Nepal. It lies between 1000 to 2000 m asl. The distribution of chir pine in Nepal is shown in Fig. 1.

Fig. 1. Distribution of Chir pine in Nepal

Nepal is very prone to soil erosion and is susceptible to sediment disasters mainly caused by slope failure, landslides, debris and bank erosion. There is an urgent need to control erosion and prevent potential sediment disasters in Nepal. Therefore, ectotrophic mycorrhizae are essential for the establishment of tree seedlings and for their good growth and development in soils low in nutrients. Because mycorrhiza is beneficial to tree growth as they increase nutrient uptake and expose a greater absorbing surface. The inability of tree seedlings free from mycorrhizal fungi to grow in soils with low nutrients and their successful establishment following inoculation of the soil with suitable mycorrhizal fungi is well documented by reports from various parts of the world.

The mechanism involved in root disease suppression by ectomycorrhizal fungi is poorly understood. Root protection may be the result of synthesis of host plant, a barrier effect caused by the presence of a fungal sheath around roots, or nutrient competition (Zak & Marx 1964). But few attempts have been made to ascertain the role of antibiosis by ectomycorrhizal fungi *in situ*. They also suggested that ectotrophic mycorrhizal roots may be less susceptible than non mycorrhizal roots to infection by root pathogens by secreting antibiotiathogens. Mycorrhizal fungi may also afford protection by stimulating host root cells to elaborate inhibitions that may maintain the symbiotic state, and that also serve to inhibit infection by pathogens. Many ectotrophic mycorrhizal fungi produce antifungal and antibiotics In pure culture, effective against many root pathogenic fungi and various bacteria.

Methodology

Collection and Identification. Sporocarps and sexual spores were collected from pine forest of Dadeldhura district far western Nepal in July. They were identified according to Adhikari (1999) and Brundrett *et al.* (1996).

Some plant pathogens were contributed by Plant Pathology Division at Nepal Agriculture Research Institute (NARI), Khumaltar, Nepal and other were isolated from soil of pine forest of Chovar. Central Nepal at the research laboratory of Royal Nepal Academy of Science & Technology (RONAST).

Isolation of ectomycorrhizae from collected sporocarps and sexual spores were done according to Shrestha (1999). Selection criteria was done according to Shrestha (1999).

Antagonistic Action Study

A. Direct cross inoculation

Mycorrhizal fungi were isolated from known sporocarps and were incubated at 27 °C (Shrestha 1999). After incubation, plant pathogens were inoculated at four different sites on the plate and again incubated for 1-2 weeks at 27 °C and observed for inhibition of growth.

B. Extraction of metabolites

Mycorrhizal fungi was inoculated into liquid modified Melin-Norkan's media and incubated for 30-40 days at 27 °C. After incubation fungal mat were separated by filtering through muslin cheese cloth. The filtrate was subjected to solvent extraction with chloroform. The solvent was recovered and the resulting residue contained metabolites having different concentration.

C. Agar well diffusion

(I) For bacteria. Bacterial broth was prepared to match the turbidity standard and it was seeded on MHA plate and left for few minutes at room temperature. Wells were made on inoculated plate with sterile cork borer and solution was loaded in these wells. It was again left for 30 minutes for diffusion. Then the plates were incubated at 37 °C for 18-24 hours. After incubation the plates were viewed for zone of inhibition.

(II) For fungi. Dry plate of PDA was taken and wells were made on it with sterile cork borer then solution was loaded in these wells. The plates were left for 30 minutes for diffusion. Fungal spores were loaded in centre of wells and incubated at 27 °C for 1-2 weeks. After incubation observed for growth inhibition.

Results

The cross inoculation method show that *Pisolithus arrijus* and *Scleroderma vericossum* were highly active against fungi like *Pythium* sp., *Rhizoctonia* sp., *Fusarium* sp., and less active against *Geotrichum* sp. and *Curvularia* sp (Table 1) but show no active against *Aspergillus* sp.

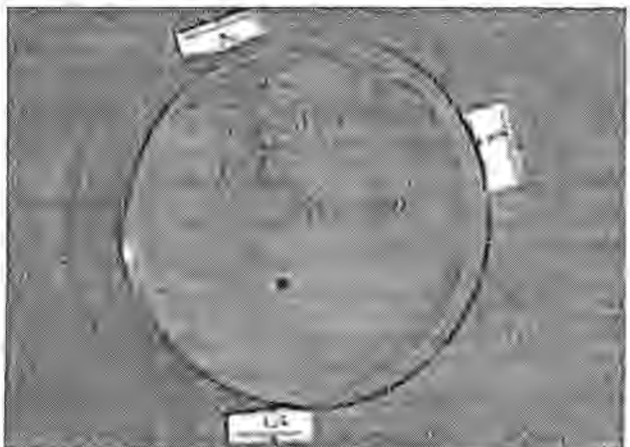

Photo 1 Inhibition shown by *Pisolithus arrijus* against (1) *Fusarium* sp., (2) *Rhizoctonia* sp. and (3) *Pythium* sp.

Table 1. Results of cross inoculation methods

Cross inoculated fungi	Mycorrhizae inoculated	
	Pisolithus arrijus	*Scleroderma verrucossum*
Pythium sp.	+++	+++
Rhizoctonia sp.	+++	+++
Fusarium sp.	+++	+++
Geotrichum sp.	++	++
Curvularia sp.	+	+

+++ = Highly inhibited, + = Moderately inhibited, ++ = slightly inhibited

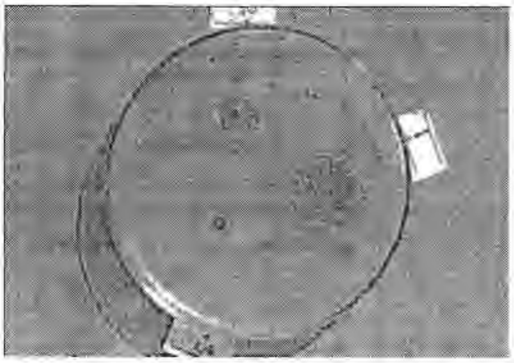

Photo 2. Inhibition shown by extract of (1) *P. arrijus*, (2) *S. verrucossum* and (3) control against *bacillary dysenteriae*

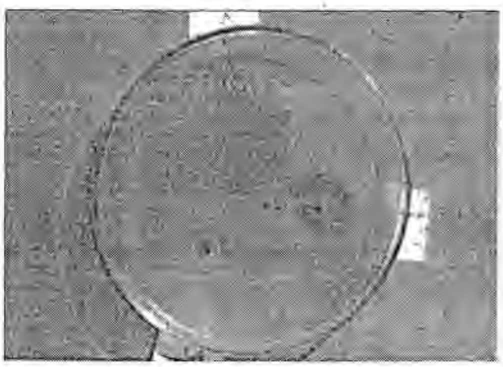

Photo 3. Inhibition shown by extract of (1) *S. verrucossum*, (2) *P. arrijus* and (3) Control against *E.coli*

The liquid culture *of Pisolithus arrijus* yields around 270 mg/litre while *Scleroderma verrucossum* yield 100 mg/litre metabolites in chloroform solvent system. The metabolites of *P. arrijus* and *S. verrucossum* in concentration 10 mg/mL show higher zone of inhibition in bacteria. *Pseudomonas aeruginosa, P. solanacerum, Shigella dysenteriae, Escherichia coli, Kleibsiella* sp., *Bacillus* sp. but in case of *Vibrio cholerae* bacteria did not show any inhibition in agar well diffusion method. These are shown in Fig. 2 *(Pisolithus arrijus* against bacteria) and Fig. 3 *(Scleroderma verrucossum* against bacteria). In case of fungi - *Rhizoctonia* sp., *pythium* sp., *Fusarium* sp., *Geotrichum* sp., *Curvularia pallescens* and *Curvularia critici show* zone of inhibition but *Aspergillus ochraceous, A.sydowi, A. niger, A. ustus, A. clavatus* and *A. granulosis* did not show zone of inhibition (Table 2).

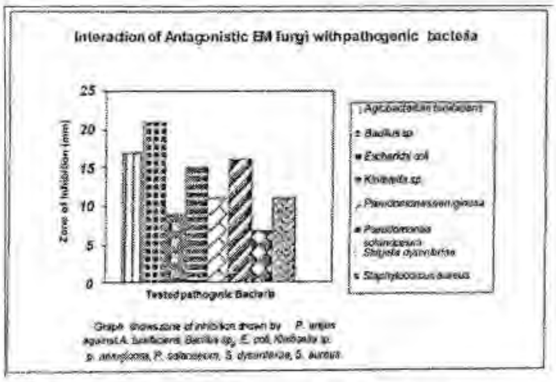

Fig. 2. Zone of inhibition shown by *P. arrijus*

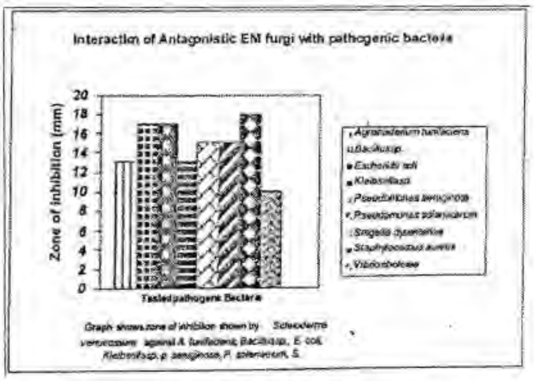

Fig. 3. Zone of inhibition shown by *S. verrucossum*

Table 2. Antifungal activity of metabolites

Test fungi	Zone of inhibition (mm) shown by	
	Pisolithus arrijus	*Sclerodema verrucossum*
Aspergillus clavatus	-	-
Aspergillus granulosis	-	-
Aspergillus niger	-	-
Aspergillus ochraceous	-	-
Aspergillus Sydowi	-	-
Aspergillus ustus	-	-
Curvularia critici	11	9
Curvularia pallescens	10	9
Fusarium sp.	18	15
Geotrichum sp.	12	11
Pythium sp.	14	12
Rhizoctonia sp.	14	14

In this Tabla *Pisolithus arrijus* shows highly antagonistic characters against fungi than *Scleroderma varrucossum.*

Discussion

The metabolites of EM *Pisolithus arrijus* and *Scleroderma verrucossum* were found highly antifungal activity against *Pythium* sp., *Fusarium* sp., *Rhizoctonia* sp., *Curvularia pallescens, C. critici* and antibacterial activity against *Pseudomonas aeruginosa, P. solanacerum, Agrobacterium tumefaciens, Shigella dysenteriae, Bacillus* sp., *Escherichia coli* and *Kleibsiella* sp. Such antifungal and antibacterial also reported by (D.H. Marx 1969 and Perrin & Garbaye 1983). Same way (Duchesne *et al.* 1988 & Buscot *et al.* 1992) also reported on antifungal and antibacterial activity of EM fungi. The inoculation of *Pisolilthus arrijus* and *Scleroderma verrucossum* in pine nursery as well as in out plant was proved better in field trial (Shrestha 1999). (Bakshi & Kumar 1968) also reported that ectomycorrhizae having an antimicrobial activity and makes plants drought and frost resistant and protects root ends from attack by parasite fungi. Ectomycorrhizal fungi isolated from sporocarps of *Pisolithus arrijus* and *Scleroderma verrucossum* show high activity against plant pathogens. Due to antimicrobial activity, mycorrhizal plants seems to be less affected by plant pathogens.

Ectomycorrhizal fungi isolated from sporocarps of *Pisolithus arrijus* and *Scleroderma verrucossum* show high activity against plant pathogens. So utilization of mycorrhizal soil not only promotes the growth of plant it also protects against such plant pathogens. Thus we can conclude that pine trees for mid hills and hilly regions are suitable for reforestations. By exploring such fungi we can substitute chemical fertilizer and make greenery in bare land and maintain biodiversity.

Acknowledgement

We thanks to Prof. D.H. Marx, Director, Institute for Mycorrhizal Research and Development, Athens (USA) and also thanks to Mrs. Gyanu Manandhar, Nepal Agriculture Research Council for providing the plant pathogens. Authors are grateful to RONAST for providing the laboratory facility.

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Study the Ectomycorrhizal Fungi as a Bio-control Agent

Geeta Shrestha Vaidya

Abstract

The ectomycorrhizal fungi were isolated from sexual spores and sporocarps of different places of pine forest of Nepal. Out of the isolated ectomycorrhizal fungi, two ectomycorrhizol fungi namely *Scleroderma citrinum* and *Amanita verna* were studied for their antimicrobial activily against pathogenic fungi like *Pythium* spp, *Rhizoctonia* spp, *Fusarium* spp., *Geotrichum* spp, *Aspergillus niger*, *Pythopthora intestons* and bacteria like *Pseudomonas solanacerum*, *Klebsiella* spp., *Shigella dysenteriae*, *Escherichia coli* and *Bacillus* spp. Such antifungal and antibacterial action of ectomycorrhizal fungi *in vitro* test by direct cross inoculation and extraction of metabolites from liquid culture and activity by agar well diffusion method. Among these two mycorrhizal fungi *Scleroderma citrinum* has shown inhibition zone against *Pythium* spp., *Rhizoctonia* spp., *Fusarium* spp., *Phytopthora infestans*, *Pseudomonas solanacerum*, *Bacillus* spp. But no inhibition were found against *Aspergillus niger*, *Geotrichum* spp., *Shigella dysenteriae*, *Escherichia coli*. In case of *Amanita verna*, it has shown inhibition zone only with *Fusarium moniliforme* and *Fusarium* spp. But in other case it did not show the antimicrobial properties. So, in this paper, *Scleroderma* spp. has shown better atimicrobial properties than *Amanito verna*. Due to these antimicrobial properties, mycorrhizal plants seems to be less effected by plant pathogens. These ectomycorrhizal fungi can be used for biological control of plant, animal and it can use for drugs only.

Introduction

Mycorrhizae is beneficial to tree growth by way of increased nutrient uptake and exposing a greater absorbing surface. It makes plants drought resistant and protects root ends from attack by parasite fungi (Bakshi & Dinesh Kumar 1968).

Ectotrophic mycorrhizae are essential for the establishment of tree seedlings and for their good growth and development in soils low in nutrients.

The root in its soil environment is part of a complex interacting system. The dynamics of rhizosphere organisms exert measurable effect on higher plants by altering the morphology of the root, stem and changing the phase equilibria of the soil and nutrients in such a fashion so that they are more readily available to plants with efficient transport system. No less Important is the significant charge brought about, in the chemical composition of soils involved such symbiotic processes and the influence exerted by soil-borne organisms on root exudation. In turn soil microorganisms bring about measurable shift in the permeability of root cells either by damaging root tissue, by altering root metabolism, selective utilization of certain root exudates components,' or by excreting toxins. The magnitude of such rhizosphere responses, particularly in terms of microbial number and activity is found to be influenced markedly by biological factors.

Mycorrhizal roots are functionally longer than non-mycorrhizal ones and therefore they seem to be less susceptible to certain types of pathogenic attack. Zak and Marx 1964, suggested that ectotrophic mycorrhizal roots may be less susceptible than non mycorrhizal roots to infection by root pathogens by secreting antibiothiagens. Mycorrhizal fungi may afford protection and also by stimulating host root cells to elaborate inhibitions that may maintain the symbiotic state and that also serve to inhibit infection by pathogens. Many ectotrophic mycorrhizal fungi produce antifungal and antibiotics in pure culture, effective against many root pathogenic fungi and various bacteria.

Most of the ectomycorrhizal fungi are fine candidates for biological control of unwanted plants still other fungi attack insect pests and other pathogenic fungi in the soil or on plant surfaces. They are important sources of biological control for insect problem and plant disease in forestry, agricultural and horticultural crops.

Methodology

Collection, identification and isolation have done according to (Shrestha 1999), Most of the plant pathogenic fungi have collected from the Nepal Agriculture Research Centre (NARC), Khumaltar, Lalitpur, Kathmandu, Nepal and some pathogenic fungi and bacteria were isolated in the RONAST laboratory.

Selection criteria

In this case most of the ectomyconhizal fungi isolated from the sporocarps, i.e. Mushrooms. Among them I have selected only two ectomycorrhizal fungi have selected such as *Scleroderma citrinum* which is hypogeous and *Amanita verna* which is epigeous.

Antagonistic action study

a. Direct cross inoculation method

b. Extraction of metabolites

c. Agar well diffusion method

These all methods have done according to Shrestha & Piya 2002.

This work sought to verify the presence of compounds with antimicrobial properties in extracts of ectomycorrhizal fungi. Extracts from *Scleroderma citrinum* and *Amanita verna* grown in liquid culture media were proceed to obtain fractions with chloroform. These fractions were tested for the presence of inhibitory constituents against *Fusarium moniliforme, Fusarium* spp., *Pythium* spp., *Phytopthora infestanse, Geotrichum* spp., *Rhizoctonia* spp., *Bacillus* spp., *Pseudomonas solanaserum, Shigella dysenteriae* and *Escherichia coli.*

Results

The cross inoculation method has shown that *Scleroderma citrinum* was highly active against fungi like *Fusarium* spp., *Pythium* spp. and *Pythopthora infestants* but did not show any microbial activity against *Geotrichum* spp. and *Rhizoctonia* spp. In case of *Amanita verna,* it was less active only in *Fusarium* spp. but in other cases *Amanita verna* has not shown any activity.

Results of cross Inoculation methods

Cross inoculated fungi	Ectomycorrhizae inoculated	
	Scleroderma citrinum	*Amanita verna*
Fusarium	+++	+
Geotrichum spp.	-	-
Pythium spp.	+++	-
Phytopthora infestans	+++	-
Rhizoctonia spp	-	-

Note: +++ = Highly inhibited; ++ = Moderately inhibited; + = Slightly inhibited; - = Not inhibited

Liquid culture

The metabolites of *Scleroderma citrinum* in concentration 10mg/mL show higher zone of inhibition in Fungi *Fusarium moniliforme, Fusarium* spp., *Pythium* spp., *Phytopthora infestans* but it has not shown any activity against *Geotrichum* spp. and *Rhizoctonia* spp. In case of Bacteria *Bacillus* spp and *Pseudomonas solanacerum* have shown the higher zone of inhibition but no inhibition were found against *Aspergillus niger, Geotrichum* spp., *Shigella dysenteriae* and *Escherichia* coli. In case of *Amanita verna,* it has shown inhibition zone only with *Fusarium moniliforme* and *Fusarium* spp. But in other pathogenic bacteria and fungi it did not show an anti-mcrobial properties in agar well diffusion method.

Antibacterial activity of matabolites

Test bacteria	Zone of inhibition shown by	
	Scleroderma citrinum	*Amanita verna*
Bacillus spp.	200 mm	-
Escherichia coli	-	-
Kleibsella spp.	-	-
Pseudomonas solanacerum	16mm	-
Shigella dysenteriae	13mm	-

Seleroderma citrinum was shown higher zone of inhibition against with *Bacillus* spp. Lowest in *Shigella* spp. But did not show any zone of inhibition against with *Escherichia coli* and *Klebsiella* spp. But *Amanita verna* did not show any inhibition against with *Bacillus* spp., *Escherichia coli* and *Klebsiella* spp., Pseudomonas solanacerum *and Shigella* spp.

Antifugal activity of metabolites

Test fungi	Zone of inhibition shown by	
	Scleroderma citrinum	*Amanita verna*
Phythium spp.	16mm	-
Rhizoctonia spp.	16mm	-
Fusarium moniliforme	20mm	10mm
Fusarium spp.	19mm	8mm
Geotrichum spp.	12mm	-
Phytopthora infestans		
Aspergillus niger	14mm	-

In this case *Scleroderma citrinum* was shown higher zone of inhibition against with *Fusarium moniliforme* lowest in *Geotrichum* spp. But *Amanita verna* was shown zone of inhibition only against with *Fusarium moniliforme* and *Fusarium* spp.

Discussion

The metabolites of ectomycorrhizal fungi, *Scleroderma citrinum* found highly antifungal activity against *Fusarium monoliforme, Fusrium* spp., *Pythium* spp., *Phytopthora infestans, Geotrichum* spp. And antibacterial activity against *Bacillus* spp., *Pseudomonas solanacerum* and *Shigella dysenteriae*. Such antifunal and antibacterial activity of ectomycorrhizal fungi also reported by DH Marx in 1969, Perrin and Garbaye 1983. Similarly Duchesne *et al.* 1988, Buscot *et al.* 1992 also reported on antifungal and antibacterial activity of ectomycorrhizal fungi. The inoculation of *Pissolithus arrijus* and *Scleroderma verrucossum* in pine nursery as well as in out plant was prove better in field trial (Shrestha 1999). *Amanita verna* has shown inhibitory effect only in *Fusarium moniliforme* and *Fusarium* spp. These type of inhibitory effect has studied by Kasuya *et al.* (1996). Due to antimicrobial activity of mycorrhizal plants seems to be less affected by plant pathogens.

Conclusion

From this study, it has shown that Mycorrhizae is not only beneficial to tree growth by way of increased nutrient uptake and exposing a greater absorbing surface but also protect roots ends from attack by parasitic fungi.

The presence of antagonistic soil microorganisms can influence survival of the symbionts as well as root growth of the host plant. Fungicides used in plant disease control can inhibit mycorrhizal fungi under some conditions or may stimulate mycorrhizal development in other by reducing microbial competition. Fungi toxicants young seedlings in nurseries may have an inhibitory effect on the development of mycorrhizae.

Utilization of mycorrhizae not only promote the growth of the plant it also protect against such plant pathogens. Thus ectomyorrhizal fungi play as a biocontrol agent. This supported the conclusion that selected strains have a potential to be use for biocontrol of coniferous seedling pathogens and crop pathogens.

By exploring such fungi we can substitute chemical fertilizer. Thus application of science, technology and management naturally enhances the productive and regulative benefits of forests with the corresponding economic gains in terms of contribution to gross national products and employment opportunities to many. More of these gains will occur when the products and services whether these are wood, herbs, animals, biodiversity, or nature tourism are further proceeded at small, medium & industrial scales. Furthermore, the gains in the forestry and forest industries sectors through linkages to other section of the economy will have multiplier effects. Thus, the Nepalese context the environmental and economic gains of applying mycorrhizal fungi in forestry and crops could help not only in alleviating property in Nepal and by also in revitalizing the economy as a whole.

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Assessement of Productivity of Pipla *(Piper longum* L.) in Dang

Chiranjivi Regmi and Ishwor P. Khanal

Abstract

Pipla (Piper longum) is an important medicinal plant. The fruits, roots and the thicker parts of stem are used in Ayurvedic and allopathic medicine for cough, bronchitis asthma and as sedative in insomnia and epilepsy. There is a high demand of this crop in the internal as well as international market. Pipla grows in wild in mid hills and plains of Terai and inner Terai of Nepal. Although some farmers grow it on the hedges of their Bari land, it is mainly collected from the wild. It can very well fit in the agroforestry systems of Terai and inner Terai because it grows very well under shade. The field experiment was laid out under the sisoo plantation in Randomized Complete Block Design (RCBD) in Beljhundi, Dang in September, 1999. There were two treatments using support and without support with six replications. The planting material was brought from Dhakeri, Banke. The crop was harvested in October 2002, because Pipla is the perennial plant and starts giving fruits only on the third year after plantation. The average yield of dry fruits obtained from the treatment without support was *175* kg/ha and with support was 21 kg/ha. The results show that Pipla grows well under sisoo and gives good yield in Dang.

Introduction

Piper longum L., commonly known as Pipla, is one of the indigenous plant of Nepal. It grows in wild throughout the Terai, inner Terai and in the Tars and riverbelts of mid hills of Nepal. Pipla is used as traditional medicine for the treatment of coughing and fever caused by cold (Bajracharya 1979). In Ayurvedic and Allopathic medicine it is used to prepare the drugs for the disease of respiratory tract i.e. cough, bronchitis asthma, etc. (Chadha 1976). It is also used in the kitchen as an important spice specially to prepare the meat items. Local people collect fruits of Pipla from the wild for domestic use. However, a large quantity of Pipla is imported from India and Indonesia. It is an economically important medicinal plant that has great demand in internal as well as national markets.

Different parts of Pipla is used in medicine. The fruits, the root and the thicker parts of the stem are used in the Ayurvedic medicine and Alleopathic medicine (Anonymous 2002). Fruits of *P. longum* has shown the presence of the alkaloids piperine (4.5%). Dried fruits of *P. longum* on steam distillation gave 0.7% of an essential oil with spicy odour resembling that of pepper and ginger oils (Chadha 1976).

Piper longum is a perennial climber belonging to Piperaceae family. It can be grown in the shade under the forest trees. It grows in tropical and subtropical climate. It is found in wild in the Terai, the inner Terai and the mid hills of Nepal. The fruits of *Piper longum* are collected from wild plant so far in Nepal. Therefore, there is not enough information on productivity and cost of cultivation of this medicinal plant. Similarly, there is no information on technical know how about its cultivation.

Dang valley is located in the inner Terai. This valley is surrounded by the ranges of Siwalik from all sides. The average temperature of the valley ranges from 15°C to 20°C with maximum temperature approximately about 30°C in summer and minimum about 3°C during winter. It's altitudes varies from 500 to 750 m above vary sea level. Most of the rainfall is during monsoon (June-August) and small quantity of rainfall is during winter. Dang is the one of the appropriate area for the cultivation of *Piper longum.*

Farmers will grow different medicinal and aromatic plants (MAPs) including Pipla in their marginal land and agroforestry. Several community forestry users groups (CFUGs) also have shown their interest to grow MAPs in their community forests but they do not have any access to the information about cultivation techniques and the performance of medicinal plants including that of Pipla. Farmers would like to see the performance of MAPs before they go for cultivation. Therefore, there is an urgent need for conducting field trials first in small area to see the performance of selected MAPs.

Mahendra Shanskrit University (MSU) has shown great interest for the promotion of medicinal plants and has provided enough land for the conservation and promotion of MAPs. Royal Nepal Academy of Science and Technology (RONAST) has initiated a joint research and development project "Conservation and Management of Selected Medicinal and Aromatic Plants in Dang" since 2056 B.S. This paper is the outcome of this project.

Objective

The objective of this study is to asses the productivity of Pipla in the agroforestry condition prevalent in Dang.

Methods and Materials

The field experiment was conducted at the premises of Mahendra Sanskrit University, Beljhundi, Dang. As the Pipla is a shade loving climber, the experiment was carried out under the sisoo plantation with a view to develop technology suitable for the agroforestry of Dang.

The experiment was conducted in Randomized Complete Block Design (RCBD). Although Pipla is a climber it grows well with or without support. There were two treatment (i) with support and (ii) without support. There were 3 replications for each treatment. The plot size per treatment was maintained 24 square meters.

The planting material was collected from Dhakeri, Banke. The stems of Pipla were cut into small pieces of 10 - 15 cm in size with three nodes. They were planted by giving space 1.5 m row to row and 1 m plant to plant. The plantation was carried out on September 14, 1999 (2056 Bhadra 32). There was no fruiting in first year and negligible fruiting was observed in second year. The crop was harvested on October 20-24, 2002 (2059 Kartik 7).

Results and Discussion

The results presented here are from the yield of third year after plantation. The fresh fruits were air-dried in the oven. The average water content in the fresh fruits was found to be 71% and that of dry matter only 29%. The productivity was calculated for one hectare based on these data. The results obtained are presented in table 1 below.

Table 1. Productivity *of Piper longum* with and without support (kg/ha).

Treatment	Plot No.	Yield (gm/plot)		Productivity kg/ha (air dry)	Average productivity
		Fresh fruits	Air dry fruits		kg/ha (air dry)
With support	1	1075	323	179.4	
,,	2	1750	525	291.7	
,,	3	1710	513	285.0	232
,,	4	1070	321	178.3	
,,	5	1070	321	178.3	
,,	6	1670	501	278.3	
Without support	7	1070	321	178.3	
,,	8	1020	306	170.0	
,,	9	1070	321	178.3	
,,	10	1050	315	175.0	175
,,	11	1020	306	170.0	
,,	12	1060	318	176.3	

The results show that average yield (productivity) of dry fruits of Pipla ranges from 306-528 gm/plot (24 sq m). When calculated per hectare it comes 170-292 kg/ha.

Treatment wise the average yield of dry fruits of Pipla was found to be 232 kg/ha in the treatment with support and only 175 kg/ha in the treatment without support. The yield is higher in the treatment with support by 30% as compared to that of without support. It is obvious the Pipla is a climber and with that support it becomes more productive. However, it can give satisfactory yield even if there is no support.

Chandha (1976) has reported that Pipla is cultivated below the Cherrapunji region of India where the Pipla start bearing fruits three to four years after planting and give yield 560 kg/ha in the first year to 1680 kg/ha of dry fruits. In this case he also has reported that the plantation were heavily manured with cow dung cake. However, in our experiment the yield was very low 232 kg/ha even in the treatment with support in the first year of bearing. It might be explained by the fact that our experiments were carried without using any manure and irrigation but in the natural conditions. Its shows that there is an enormous potentiality of increasing the yield of Pipla in the conditions of Dang if high inputs are provided for the cultivation of Pipla in Dang. The results of our experiment show that Pipla gives satisfactory yield under sisoo plantation. Therefore, cultivation of Pipla can be recommended in the agroforestry system of Dang and similar areas.

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Performance of Ashwagandha (*Withania somnifera)* in Dang

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Introduction

Ashwagandha (Withania somnifera) is an erect, evergreen, tomentose shrub, 30-150 cm. high, belonging to the Solanaceae family. It is cultivated for the medicinal roots. Ashwagandha is mentioned as an important drug in the ancient Ayurvedic literature. It is used for the treatment of sexual impotency. It is also useful in the treatment of inflammatory conditions, ulcers and scabies when applied locally. The leaves are bitter and given in fever. A paste made from the leaves is prescribed for syphilitic sores .The powdered drug is grayish with pungent odour and acid taste. It is characterized by the presence of a large number of starch-grains, cork-cells, cortical and xylem- parenchyma, tracheids, vessels and wood fibres. The pharmacological activity of the roots is attributed to the presence of several alkaloids. In addition to the alkaloids, the roots are reported to contain starch, reducing sugars, hentriacontane, glycosides, dulcitol withaniol(0.08%), acid and a neutral compound. (Chadha 1976).

Although the agroclimatic conditions are suitable to grow ashwagandha in the Terai and the Inner terai of Nepal, there is no information about its cultivation so far in the country. It grows well in sandy loam or light soil with good drainage. Temperature should be between 20 °C to 35 °C for growing this medicinal plant. Considering the economic importance and appropriate agroclimatic conditions of Dang, the first attempt was made to introduce ashwagandha and evaluate its performance.

Objective

To see the performance of ashwagandha in the condition of Dang.

Methods and Materials

The field experiment was conducted at the premises of Mahendra Shanskrit university, Beljhundi, Dang during 2056-2059 B.S. The experiment was laid out in RCBD.There was only one treatment with two replications every year. The seeds of ashwagandha were provided by Dabur Nepal. The seeding was done by broadcasting method. The thining was carried out when the plants had 4-5 leaves. The plant density was maintained 4-5 plants per sq. m .The weeding was done every month. The irrigation was applied only once during November. The seeding was carried out in June by in small experimental plots of 10-20 sq. m 2 and the harvesting was done in January-February each year. No fertilizers were applied in experimental plots.

Results and Discussion

The roots were harvested and the weight of fresh roots were recorded. The roots were air dried in the oven at 70 °C over night to know the water content in the fresh roots. The average water content in the roots was found to be 70%. The yield was calculated based on the dry matter. The results of this experiment are presented in table 1.

Table I. The Yield of Aswagandha (Withania somnifera) dry roots.

Year of experiment	Plot N.	Area(sq.m^2)	Yield gm/plot	Yield kg/ha
2056B.S.	La	20	650	325
2056	lb	20	500	250
Average			575	288
2057	2a	15	200	133
2058	2b	15	150	100
Average			175	117
2058	3a	10	200	200
2058	3b	10	275	275
Average			237	237

The results of the experiment show that the highest yield 325 kg/ha was obtained in first year of experiment and the lowest yield 100 kg/ha was obtained in the second year. In the third year the average yield was in between of first and second years. The lowest in the second year might be due to the climatic conditons of the year.

The average yield was obtained about 117- 288 kg/ha. The average yield of dry roots of Aswagandha is low as compared to the average yield (300-500 kg/ha), obtained by the commercial growers of South India. The high yield in India is due to the application of well developed technologies for the commercial cultivation of Aswgandha. It indicates that the application of good cultivation practices can promote the cultivation of ashwagandha in Dang successfully.

The results have confirmed that Aswagandha can be grown in Dang successfully but more research has to be carried out before recommending for its cultivation in large scale.

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Antibacterial Property of Medicinal Plants Against Gram Negative Bacteria

Tista Prasai, Binod Lekhak and Madhav Prasad Baral

Abstract

With an estimation of World Health Organization that as many as 80% of the world's population living in rural areas rely on herbal traditional medicines as their primary health care, the study on properties and uses of medicinal plants are getting growing interests. Based on local use for common diseases and ethnobotanical knowledge, an attempt has been made to assess the antibacterial properties of medicinal plants was made in the present study. Crude ethanolic extraction of eight different medicinal plants viz. *Achryanthes bidentata, Alnus nepalensis, Aloe vera, Ficus religiosa, Musa paradisica, Myrica esculenta, Oxalis corniculata* and *Rhododendron arboreum* were screened and tested against seven different gram negative enteric bacteria viz. *E.coli, Klebsiella* spp, *Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Shigella* spp and *Vibrio cholerae. Rhododendron arboreum* gave the highest yield of 40% while *Aloe vera* had lowest yield 10% of crude extracts. Among eight plants tested, four plants viz. *Alnus nepalensis, Ficus religiosa, Myrica esculenta* and *Rhododendron arboreum* were found to have effective against *E.coli, Klebsiella* spp, *Proteus vulgaris, Salmonella typhi, Shigella* spp and *Vibrio cholerae*. All the plants were ineffective against *Pseudomonas aeruginosa*. The largest zone of inhibition was obtained with *Rhododendron arboreum* against *Vibrio cholerae* (19mm) and smallest minimum bactericidal concentration (MBC) value of 6.25 mg/l was obtained with *Alnus nepalensis* and *Myrica esculenta* against *Salmonella typhi*.

Introduction

Men have used herbal medicines from time immemorial. Ancient Egyptian, Indian, Chinese and others used as a variety of plant forms and product for curing all kinds of ailments. Following the advent of modern medicine, herbal medicines suffered a set back, but during last two or three decades advances in phytochemistry and in identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines (FAO 1990).

WHO has estimated that 80% of the world's population relies chiefly on traditional medicine. A major part of traditional therapies involve the use of plant extracts or their active constituents (Akerele1993). The major part of the traditional therapy involves the use of plant extract or their active constituents and they are actually the plants containing the chemical constituents like alkaloids, flavonoids, steroids, tannins, essential oils, resins, fatty acids, gums etc. which are capable of producing definite physiological actions on the human body. These plants, their parts or their compounds are used to cure diarrhoea, dysentery, cholera, wounds infection, colds, coughs, bronchitis, headaches, indigestion, sprains, muscular pains. Based on local use for common diseases and ethnobotanical knowledge, an attempt to assess the antibacterial property of medicinal plants was made in this study. The main objective of this research is to screen and evaluate antibacterial activity of crude ethanol extracts and to find out the minimum bactericidal concentration (MBC) value of crude ethanol extracts against gram-negative bacteria.

Materials and Methods

The different parts of eight different plants were collected from different part of Kathmandu valley. Medicinal plants were identified according to various literatures (Flora of Kathmandu Valley by HMG/N (1986) and Medicinal plants of Nepal by HMG/N (1970 and 1997), and including other pertinent taxonomic literature.

Test organisms were received from Central Department of Microbiology, Tribhuvan University Kirtipur. The organisms were tested for their purity and confirmed by testing their morphological, cultural and biochemical characteristics. The plating media viz. Nutrient agar, Nutrient broth, Muller Hinton agar were provided by Hi media laboratories limited, India. Crude ethanolic extraction of eight different medicinal plants viz. *Achryanthes bidentata, Alnus nepalensis, Aloe vera, Ficus religiosa, Musa paradisica, Myrica esculenta, Oxalis corniculata* and *Rhododendron arboreum* were screened and tested against seven different gram negative enteric bacteria viz. *E.coli, Klebsiella* spp, *Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Shigella* spp and *Vibrio cholerae.*

Collection of samples:

The medicinal plants used for the experiment were *Achryanthes bidentata, Alnus nepalensis, Aloe vera, Ficus religiosa, Musa paradisica, Myrica esculenta, Oxalis corniculata* and *Rhododendron arboreum.* Collected plants were washed thoroughly and then chopped into small pieces, shade dried and grinded into powder form. Dried powder was extracted continuously for 8-10 hours using 95% ethanol. The extracting solvent was removed from the extract under reduce pressure with rotary vacuum evaporator. Extract, thus obtained was dissolved in sterile distilled water to prepare solution of 100 mg/ml that was used for evaluation of antibacterial activity.

Preparation of standard culture inoculum of test organism:

Three or four isolated colonies on fresh nutrient agar culture plate were transferred to the sterile nutrient broth and incubated until the turbidity was adjusted to that recommended by WHO in 1991 for antibacterial susceptibility testing.

Determination of Zone of Inhibition (ZOI):

The freshly prepared inoculum was swabbed on the Muller Hinton Agar surface with the help of sterile cotton swabs. Wells of 6mm diameter were bored on the medium and 50 μl of the solution of medicinal plant extract was loaded carefully using micropipette. It was left to stand allowing the solution diffuse fully in the agar medium. The plates were incubated at 37^0C for 24 hours and the zone of inhibition were measured using a scale and mean were recorded.

Determination of Minimum Bactericidal Concentration (MBC):

Freshly prepared nutrient broth was used as a diluent and the crude extract was diluted by two-fold serial dilution. Exactly 50 μl of standard culture inoculum was added to each test tube except the negative control tube. All test tubes were then incubated at 37^0C for 24 hours. The tube content were sub cultured in fresh nutrient agar medium separately and Minimum bactericidal concentration were determined as that showing no growth.

Results

Percentage yield:

Dried powder of different parts of medicinal plants was extracted with 95% ethanol by using soxhlet apparatus for continuous extraction for 8-10 hours. After extraction the solvent was completely removed by using Rotary Vacuum Evaporator. The percentage yields of ethanol crude extract from medicinal plants were shown in the Table 1. *Rhododendron arboreum* gave the highest yield of 40% while *Aloe vera* had lowest yield 10% of crude extracts.

Table 1. Percentage Yields of Ethanol Crude Extracts from Medicinal Plants

S.N	Name of medicinal plants	Percentage yields
1.	*Achryanthes bidentata*	12%
3.	*Alnus nepalensis*	25%
2.	*Aloe vera*	10%
4.	*Ficus religiosa*	20%
5.	*Musa paradisica*	15%
6.	*Myrica esculenta*	22%
7.	*Oxalis corniculata*	20%
8.	*Rhododendron arboreum*	40%

Evaluation of Antibacterial Activity:

Extract thus obtained was dissolved in sterile distilled water to prepare solution of 100 mg/ml that was used for evaluation of antibacterial activity. Three average reading of the diameter of zone of inhibition were taken and their mean values were calculated. The positive antibacterial property was recorded for those medicinal plants showing zone of inhibition of diameter 8 mm or more against particular test organism (Table 2).

Table 2. Antibacterial Activities of Ethanolic Extracts of Different Medicinal Plants against Tested Bacteria

S. N	Name of Medicinal Plants	Test Organism						
		E.coli	*Klebsiella* spp	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Shigella* spp	*Vibrio cholerae*
1.	*Achryanthes bidentata*	-	-	-	-	-	-	-
2.	*Aloe vera*	-	-	-	-	-	-	-
3.	*Alnus nepalensis*	+	+	+	-	+	+	+
4.	*Ficus religiosa*	+	+	+	-	+	+	+
5.	*Musa paradisica*	-	-	-	-	-	-	-
6.	*Myrica esculenta*	+	+	+	-	+	+	+
7.	*Oxalis corniculata*	-	-	-	-	-	-	-
8.	*Rhododendron arboreum*	+	+	+	-	+	-	+

Notes:
Positive sign indicates inhibition of bacteria
Negative sign indicates no inhibition (Bacterial growth)

Among eight plants tested, four plants viz. *Alnus nepalensis, Ficus religiosa, Myrica esculenta* and *Rhododendron arboreum* were found to have effective against *E.coli, Klebsiella* spp, *Proteus vulgaris, Salmonella typhi, Shigella* spp and *Vibrio cholerae*. All the plants were ineffective against *Pseudomonas aeruginosa*. The largest zone of inhibition was obtained with *Rhododendron arboreum* against *Vibrio cholerae* (19mm) and smallest minimum bactericidal concentration (MBC) value of 6.25 mg/l was obtained with *Alnus nepalensis* and *Myrica esculenta* against *Salmonella typhi*. The mean diameter of zone of inhibition and minimum bactericidal concentration are shown in table 3:

Table 3. Mean diameter of Zone of Inhibition (ZOI) and Minimum Bactericidal Concentration (MBC) against tested bacteria

S.N	Name of bacteria	**Antibacterial Activity**							
		Alnus nepalensis		*Ficus religiosa*		*Myrica esculenta*		*Rhododendron arboreum*	
		ZOI (mm)	MBC (mg/l)	ZOI (mm)	MBC (mg/l)	ZOI (mm)	MBC (mg/l)	ZOI (mm)	MBC (mg/l)
1.	*Escherichia coli ATCC-25922*	14	>50	12	50	13	50	18	50
2.	*Klebsiella spp*	13	50	12	50	10	50	12	50
3.	*Proteus vulgaris* ATCC-49132	14	12.5	12	50	14	25	12	25
4.	*Pseudomonas aeruginosa* ATCC-27853	-	-	-	-	-	-	-	-
5.	*Salmonella typhi*	17	6.25	15	25	15	6.25	18	50
6.	*Shigella spp*	10	>50	12	>50	10	50	10	50
7.	*Vibrio cholerae*	16	25	10	50	10	25	19	12.5

Discussions

Medicinal plants and herbal products are popularly employed worldwide in a variety of health care settings and as home remedies. Several civil communities, in particular of developing countries basically rely on traditional healers and medicinal plant practitioners to meet their primary health needs; and in industrialized countries plant products are gaining popularity as alternative complementary therapies (WHO 1999).

The medicinal plants like Achryanthes *bidentata, Alnus nepalensis, Aloe vera, Ficus religiosa, Musa paradisica, Myrica esculenta, Oxalis corniculata* and *Rhododendron arboreum* are being used traditionally for the treatment of diarrhoeal diseases, typhoid fever, urinary tract infections and wound infections, which are often caused by Gram negative bacteria. These Gram-negative bacteria commonly transmit through water and food materials. Several literatures suggest that extracts of particular medicinal plants are quite effective against various kinds of such pathogenic bacteria.

In order to have significant use of plants or their parts, they should be extracted using appropriate organic solvents. The total yield may be influenced due to various factors like age type and parts of the plant, time of collection and extraction, consistency and dryness of extract etc. The yield of ethanolic extract of studied plants ranged from 40 percent (*Rhododendron arboreum*) to 10 percent (*Aloe vera*). Plants materials with flowers give higher yield.

Antibacterial activity of medicinal plants can be evaluated basically by two methods first by measurement of size of zone of inhibition (ZOI) and second by quantitative determination minimum bactericidal concentration (MBC) on solid agar medium. Diameter of zone of inhibition estimates potency of the particular plant extract while minimum bactericidal concentration determines the bactericidal activity of the particular plant extract.

In this study *Alnus nepalensis, Ficus religiosa, Myrica esculenta, Rhododendron arboreum* revealed their activity against most of Gram-negative bacteria except *Pseudomonas auroginosa*. Some plants *Achryanthes bidentata, Aloe vera, Musa paradisica, Oxalis corniculata* failed to show antibacterial effect, which may be due to presence of oil in their extract that makes them insoluble in water.

However, the size of inhibition produced by plant extracts against particular organism depends upon various factors extrinsic and intrinsic parameters. If extrinsic parameters are standardized and fixed, errors can be minimized. But intrinsic factors such as nature of medicinal plants including its component, solubility etc. Due to variable diffusibility in agar medium, the high antibacterial potency may not be demonstrated as ZOI commensurate to its efficacy. Therefore, MBC values have also been computed in this study. MBC is the lowest concentration of antimicrobial substance required to produce a sterile culture (Cheesbrough 1993). Actually the concentration of antimicrobial substance at the periphery of ZOI is the minimum concentration required to inhibit or kill the particular bacteria. *Alnus nepalensis* and *Myrica esculenta* revealed lowest MBC (6.25 mg/l) expressing its highest effectiveness against *Salmonella typhi*. Similarly *Rhododendron arboreum* is also effective against *Vibrio cholerae* (MBC 12.5 mg/l). The medicinal plants can be carefully applied against diarrhoeal diseases and some other gram-negative bacterial pathogens. However more careful assessment and research is needed for household level applications.

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Antagonistic Study of Ectomycorrhizal Fungi Isolated from Baluwa Forest (Central Nepal) Against with Pathogenic Fungi and Bacteria

Geeta Shrestha Vaidya, Keshav Shrestha and Hakan Wallander

Abstract

Two ectomycorrhizal fungi *Pisolithus* sp. and *Scleroderma* sp. were isolated from collected sporocarps of Baluwa Forest of Kavre district of Nepal. These were evaluated against the following fungi and bacteria *Aspergillus niger, Aspergillus ochraceous, Asperglus usustus, Fusarium udum, Fusarium oxysporum, Geotrichum* sp. *Pythium* sp. and *Rhizoctonia* sp. In case of *bateria Agrobacterium tunifaciens, Escherichia coli, Pseudomonas solanacerum, Pseudomonas aeruginosa and Shigella dysentriae* and *Salmonela typhi.* Most of them were collected from the hospital and some of them were isolated from the soil. Some plant pathogenic fungi were provided by Nepal Agricultural Research Council, Nepal. The antifungal and antibacterial action of ectomycorrhizae in vitro test was carried out by direct cross inoculation and extraction of metabolites from liquid culture and activity by agar well diffusion method. *Pisolithus* sp. and *Scleroderma* sp. had higher activity against *Pythium* sp., *Rhizoctonia* sp.,*Fusarium* sp., *Agrobacterium tunifaciens, Pseudomonas solanaserum, Kleibsella* sp. *Staphylococcus aureus, Shigella dysenteriae* and, *Escherichia coli* but no activity was found against *Aspergillus granulosis, Aspergillus niger and Aspergillus ustus.* In this study *Pisolithus* sp. has shown higher inhibition in *Pythium* sp. and *Bacillus* sp. but *Scleroderma* sp. has shown higher inhibition in *Pythium* sp. and *Salmonella typhi.* So, from this present study mycorrhizal plants seems to be less affected by plant pathogens due to its antagonistic characters and it also shows the inhibition against human pathogenic bacteria. So, it can control some human disease also.

Introduction

Ectomycorrhizal roots may be less susceptible than non mycorrhizal roots to infection by root pathogens by secreting antibiothiathogens. Mycorrhizal fungi may also afford protection by stimulating host root cells to elaborate inhibitions that may maintain the symbiotic state, and that also serve to inhibit infection by pathogens. Many ectotrophic mycorrhizal fungi produce antifungal and antibiotics in pure culture, effective against many root pathogenic fungi, various bacteria and also many human pathogenic bacteria.

Baluwa forests like in Kavre district was totally croded before and covering with red soil. Its altitude ranges from 980-1020m. But Fodder department of Nepal Agriculture Research Council, Khumaltar had initiated for plantation since six years and the project was for three years. They planted mostly *Bauhinia* sp (Fig. 1). *Leuconia* sp and *Pinus* sp. but only 30% *Pinus* sp could suceeded and another few species could suceeded. Since ectomycorrhizal fungi have not yet been studied from this area. Nepal is very rich in biodiversity. Numerous vegetation including mycorrhiza is virgin and still to be analyzed. The present study can help in tracing mycorrhiza having potential and broad spectrum antagonistic activity against pathogenic microorganisms. Therefore, the main objective of this study was to collect, identify and isolate from the ectomycorrhizal mushrooms such as *Pisolithus* sp. and *Scleroderma* sp. in other to examine their antifungal/antibacterial against mostly common plant pathogens such as *Pythium* sp., *Rhizoctonia* sp., *Fusrium udum, Fusarium oxysporum, Geotrichum, Aspergillus granulosis, Aspergillus niger* and *Aspergillus ustus.* Same way plant pathogenic bacteria and human pathogenic bacteria such as *Agrobacterium tunifaciens, Bacillus* sp., *Escherichia coil, Klebsella* sp., *Pseudomonas aeruginosa, Shigella dysenteriae, Staphylococcus aureu and Salmonela typhi.* This ectomycorrhizal fungi could help to survive in adverse environmental conditions. It promotes the growth of the tree plant by way of increased nutrient uptake and exposing a greater absorbing surface. It makes plant drought and frost resistant and protects root ends from attack by *parasite* fungi.

Fig. 1. Baluwa forest (Kavre district) showing the sampling site.

Methodology

Collection and Identification

Sexual spores were collected from Baluwa forest of Kavre district (Fig. 2). They were identified according to Adhikari (1999) and Brundrett *et al.* (1996).

Some plant pathogens were contributed by Plant Pathology Division, Nepal Agriculture Research Institute (NARI), Khumaltar, Nepal and some were isolated from soil at the research laboratory of Royal Nepal Academy of Science and Technology (RONAST) and most of the human pathogenic bacteria were contributed from microbiology Lab., Tribhuvan University Teaching Hospital (TUTH), Maharajgunj. Isolation of ectomycorrhizal fungi from collected sexual spores and selection criteria was done according to Shrestha (1999).

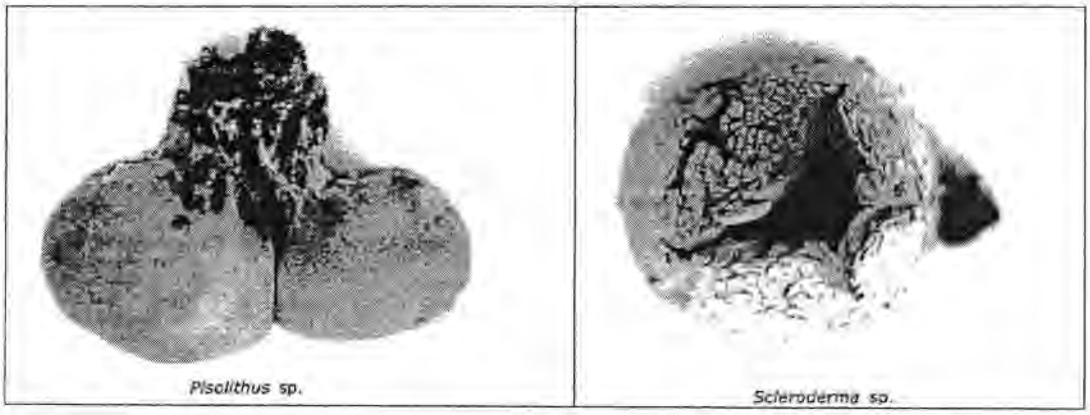

Fig. 2

Antagonistic Action Study

A. Direct cross inoculation

Ectomycorrhizal fungi were isolated from *Pisolithus* sp. and *Scleroderma* sp. and were incubated at 27°C. Tested organisms should be allowed to growth about 1 to 2 cm. Then plant pathogens were inoculated at four different sites on the plate and again incubated for 1 - 2 weeks at same temperature but the inoculation periods depends upon the type of the organisms. Then observed for inhibition of the growth.

B. Extraction of metabolites

Mycorrhizal fungi was inoculated potato dextrose liquid media and incubated for 30 - 60 days at 27° C. Every day with shaking or whenever possible. After fully growth fungal mat were separated by filtering through muslin chees cloth. The filtrates were subjected to solvent extraction with chloroform. The solvent was recovered and the resulting residue contained metabolites having different concentration.

C. Agar well diffusion

For bacteria: Bacterial broth was prepared to match the turbidity standard and it was seeded on MHA plate and left for few minutes at room temperature. Then wells were made on inoculated plate with sterile cork borer and solution was loaded in these wells. It was left for 30 minutes for diffusion. Then the plates were incubated at 3727°C for 18-24 hrs. After incubation plates were observed for zone of inhibition.

For Fungi: Dry plates of PDA were taken and different pathogenic fungi were seeded and then wells were made same like in bacteria. Tested fungal spores were loaded in center of wells and incubated at 27°C for 1-2 weeks. After incubation observed for growth of inhibition.

Results and Discussion

The cross inoculation method has shown that *Pisolithus* sp., was highly active against *Pythium* sp., *Rhizoctonia* sp., *Fusarium udum* and moderately active against *Fusarium oxysporum* and *Geotrichum* sp. (Table 1). *Scleroderma* was highly active against *Pythium* sp., *Rhizoctonia* sp., *Fusarium oxysporum* moderately active against *Fusarium udum* and low active against Geotrichum sp. (Table 1).

Table 1. Inhibitory test of two ectomycorrhizal fungi against pathogenic fungi by direct cross inoculation method.

Ectomycorrhizal fungi	*Pythium* sp.	*Rhizoctonia* sp.	*Fusarium* udum	*Fusarium oxysporum*	*Geotrichum* sp.
Pisolithus sp.	+++	+++	+++	++	++
Sclerodrms sp.	+++	+++	++	+++	+

+++ = highly inhibited, ++ = moderately inhibited and + = low inhibited

The metabolites of *Pisolithus* sp. in concentration 10 mg/mL show higher zone of inhibition *Rhizoctonia* sp., *Fusarium udum, pythium* sp and slightly decrease in *Fusarium oxysporum* and *Geotrichum* sp against *Scleroderma* sp. In same concentration show higher zone of inhibition in *Rhizoctonia* sp., and it shows equally inhibition in *Pythium* sp. And *Fusarium udum* but decrease in *Fusarium oxysporum* and *Geotrichum* sp. But *A. nigher, A. ustus*, and *A. granulosis* did not show zone of inhibition (Table 2).

Table 2. Antifungal activity of metabolites

Test Fungi	Zone of inhibition (mm) shown by	
	Pisolithus sp.	*Scleroderma* sp.
Aspergillus granulosis	-	-
Aspergillus niger	-	-
Aspergillus ustus	-	-
Fusarium udum	20	18
Fusarium oxysporum	16	15
Geotrichum sp.	14	13
Pythium sp.	18	18
Rhizoctonia sp.	22	20

The metabolites of *Scleroderma* sp. shows higher zone of inhibition in same concentration against with *Salmonella typhi, Kleibsiella* sp., *Bacillus* sp., *E. coli, Pseudomonas aeruginosa, Agrobacterium tunifaciens* and lowest in *Staphylococcus aureus* and *Shigella dysenteriae* in agar well difusion method (Fig. 3 & 4).

Fig. 3.

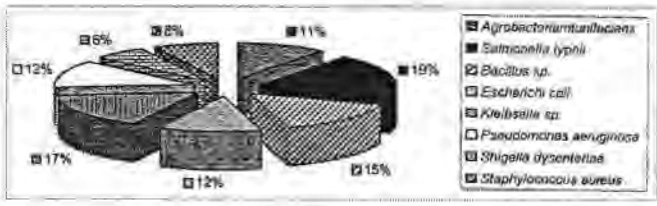

Fig. 4. Pie chart shows the percentage of inhibition shown by *Pisolithus* sp. Against with pathogenic bacteria

In same way in case of *Pisolithus* sp. it shows higher inhibitory properties in same concentration against pathogenic bacteria such as *Bacillus* sp., *Kleibsiella* sp., *Salmonella typhi E. coil, Agrobacterium tunifaciens, Pseudomonas aeruginosa* lowest in *Staphylococcus aureus* and *Shigella dysenteriae in agar well difusion method.* (Fig. 5).

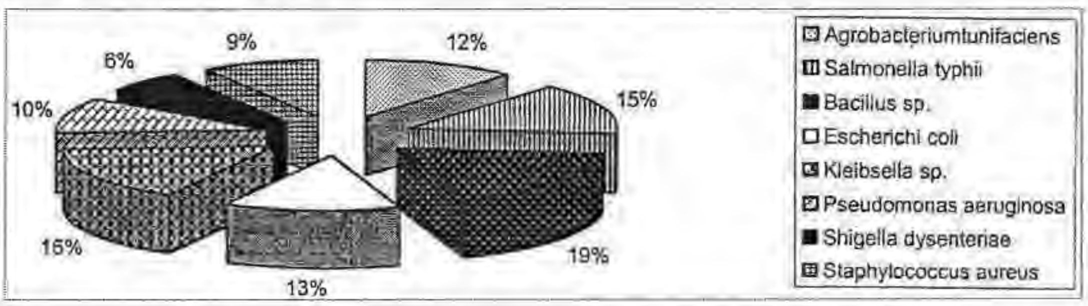

Fig. 5. Pie chart shows the percentage of inhibition shown by *Pisolthus* sp. Against with pathogenic bacteria.

In this table *Pisolithus* sp. shows highly antagonistic characters against fungi than *Scleroderma* sp.

Due to inhibitory effect of these ectomycorrhizal fungi against with pathogenic fungi and bacteria, the inoculation of such ectomycorrhizal fungi having antagonistic characters could be useful for biocontrol measures for such pathogens. Such antifungal and antibacterial properties having ectomycorrhizal fungi have been reported by (D.H. Marx 1969 and Perrin & Garbaye 1983). Same way (Duchesne *et al.* 1988 & Buscot *et al.* 1992) also reported on antifungal and antibacterial activity of ectomycorrhizal fungi. The inoculation of *Pisolithus arrijus* and *Scleroderma verrucossum* in pine *nursery* as well as in out plant was proved better in field trial (Shrestha 1999). (Bakshi & Kumar 1968) also reported that ectomycorrhizae having an antimicrobial property and makes plants drought and frost resistant and protects root ends from attack by pathogenic organisms. These ectomycorrhizal fungi could be use for antibiotic and antifungal drug.

Due to antimicrobial activity, mycorrhizal plants seems to be less affected by plant pathogens. So, utilization of mycorrhiza not only promotes the growth of the plant but also it protects against such plant pathogens.

Conclusion

Ectomycorrhial fungi could be widely used for the management and soil fertility and also could be used for erosion control. So the use of ectomycorrhizal fungi (mushrooms) could be use for best organic fertilizers for different forests and could be used for substitute for chemical fertilizers and can make greenery in bare land as well as their nature habitats by managing and using these resources in a sustainable way. This ectomycorrhizal fungi not only use for biofertilizers but also we could used for human drugs such as antibiotic and antifungal for many human diseases.

Performance *of Pinus Roxburghii* Inoculated with Pure Culture of Four Indigenous Ectomycorrhizal Fungi

Geeta Shrestha Vaidya and K. Shrestha

Abstract

Many ectomycorrhizal fungi were isolated from *the* spores and sporocarps collected from different pine forests of Nepal. Among them, *Astraeus hygrometricus, Boletus* sp., *Lycoperdon pyriforme* and *Pisolithus arrijus* were compared for their effectiveness under natural field conditions.Many of them were subjected for pure culture synthesis and selected from the pot culture expenment. Four fungi were inoculated in pine nursery *(Pinus roxburghii)* for their performance at two sites, Godawari and Chalnakhel. Their performance were observed after six, nine and twelve months of transplantation. The results revealed that inoculated seedlings attained better shoot height and had better root length than their non mycorrhizal counterparts. More lateral and mycorrhizal roots, more shoot weight and more root weight than non inoculated ones. The inoculated transplants had higher survival rate in the field as compared to those which were not inoculated. Among the above fungi, *Pisolithus arrijus* performed better as regards to growth and survival of seedlings.

Introduction

Chir pine *(Pinus roxburghii)* is an important multipurpose species which establishes itself well on new clearings, landslides and in eroded areas (Mohan & Puri 1956) and has been successful in checking erosion in areas where grazing is no controlled (Shrestha 1975).

The ectomycorrhizal (EM) fungi help the plants in their nutrition by facilitating the absorption of mineral nutrients. The important role of mycorrhiza plays as regards to the growth and development of plant species has already been reviewed widely (Melin & Nilsson 1958, Mosse *et al.* 1981). Pines cannot grow without mycorrhizae (Mikola & Gaillard 1978). In many countries the use of natural myorrhizal fungi in the raising of pine seedlings has still been a common practice (Semakhanfov 1962, Mikoli 1970, Bakshi 1980). These measures have been and are still adopted in Nepal too. This method may carry diseases and pests and is erratic. Mycorrhiza formation is required for the survival and growth of many forest tree species (Marx & Keny 1991, Smith & Read 1997). The fungi increase the uptake of nutrients especially in infertile soils by extending the absorbing surface of the roots (Hatch 1937). The hyphae of mycorrhizal fungi are widely distributed through the soil and contribute significantly towards nutrient uptake and cycling in the soil and in many forest ecosystems (Brundrett *et al.* 1996). They also provide resistance to plants against adverse soil conditions and some root pathogens (Zak 1964, Bakshi & Kumar 1968, Marx l989 Marx & Cavey 1969). The survival and success of plantation has been low probably due to the lack of proper EM fungal inoculation. So, mycorrhizal associations are considered as key factors in forest ecosystem. Therefore, the studies were initiated using pure cultures of ectomycorrhizal fungi for inoculation. This has been successfully used on field scale by a number at investigators (Moser 1961, Hacskaylo & Vazzo 1967, Marx & Bryan 1975) and seedling quality field performance is largely governed by processes occurring under the soil surface in the root zone of seedlings (Charles *et al.* 1987).

There is a growing concern in Nepal about the serious degradation in the quality of the forests and significant reduction in the area of the forest land. So, Nepal immediately needs intensive reforestation activities.

Methodology

Sporocarps were collected from pine forests of three districts (Dadeldhura, Makwanpur and Palpa) of pine forest of Nepal in spring and were identified according to Adhikari (2000) and Brundrett *et al.* (1996). Among different types of ectomycorrhizal fungi. *Astraeus hvgromerricus Boletu* sp., *Lycoperdon pyriforme* and *Pisolithus arrijus* were selected from pot experiment for field trials according to Shrestha (1999a). The selection of mycorrhizal fungi is extremely important for pure culture inoculation on large scale, Shrestha (1999b).

Description of the Experimental Sites:

Two sites namely Chalnakhel and Godawari. Were selected for field trials. Chalnakhel lies in Kathmandu district while Godawari in Lalitpur district.

The altitude of Godawari is 1515 m. The mean maximum temperature ranges between 28 °C to 18 °C and minimum temperature is between 16 °C to -2 °C. Total rainfall recorded at Godawari is 1863.5 mm. Godawari valley, situated at the foot hills of Phulchoki, is more humid and cooler than Kathmandu valley during summer and winter months. The deviation in rainfall could be attributed to the favourable arrangement of the folds of Phulchoki mountain for bringing more precipitation down to Godawari valley. Monsoon period starting from early June and ending by late September has over 80% of the total rainfall. Few spells of rains occur during winter from January to February Godawari valley commonly experiences frosts in early December to February. In this place *Pinus roxburghii* and *Pinus wallichiwia* occur.

The altitude of Chalnakhel is 1288 m. It is characterized by typical monsoon climate with rainy summer and dry winter. The mean maximum temperature ranges between 32 °C to 20 °C and the mean minimum temperature is between 18 °C to 0 °C. The total rainfall recorded at Chalnakhel is 1301.9 mm. While in Kathmandu valley, frosting is less common. In Chalnakhel, *Pinus roxburghii* and *Pinus patula* occur due to their altitude difference.

Chemical analysis: Chemical analysis of the soil samples from Chalnakhel and Godawari were performed to check their pH, nitrogen, phosphorus, potassium and organic matter. Division of Soil Science, Nepal Agriculture Research Council (NARC) performed these analysis according to the following methods:

- Total Nitrogen (N) by Kjeldhal method
- Available Phosphorus (P) by modified Olsen's bicarbonate method
- Available Potassium (K) by Flame photometer method

Experiment 1

Hundred seedlings of *P. roxburghii* inoculated with 2mg of each of *Astraeus hygrometricus, Boletus* sp. *Lycoperdon pyriforme, Pisolithus arrijus* and natural mycorrhiza were mixed with soil seperately and then were kept in polypropylene bags. The soil inoculum having natural mycorrhiza was inoculated in 1:10 ratio. The seeds of *P. roxburghii* were planted in polypropylene bags. Seedlings that had been aseptically raised in the glass house and were allowed to develop until they mostly attained a height of 10-30 cm and then planted out in the field. All the seedlings were numbered so that their field performance could be followed on each site.

Results

The seedlings were harvested after seven months and examined for shoot height , root length, mycorrhizal root and oven dry weight of shoots and roots. Results have been shown in Table 1.

Table 1(a). Effect of different ectomycorrhizal fungi within seven months

Treatments	Shoot height (cm)	Root length (cm)	Lateral root per plants	Mycorhizal root (%)	Oven dry wt. per plant	
					Shoot (g)	Root (g)
T1	35.00	23.5	15	38	0.712	0.389
T2	32.00	23.1	13	35	0.659	0.208
T3	33.50	24.5	14	36	0.768	0.301
T4	36.00	24.8	16	41	0.894	0.435
T5	22.50	18.2	10	31.5	0.592	0.135

T1 = *Astraeus hygrometricus*, T2 = *Boletus* sp., T3 = *Lycoperdon pyriforme*, T4 = *Pisolithus arrijus*, T5 = Natural mycorrhiza

In case of *P. roxburghii seedlings, Pisolithus arrijus* fungi as inoculums had a significantly more height growth, mycorrhizal root and dry weight of shoot and root compared to those *Astraeus hygrometricus*, *Boletus* sp., *Lycoperdon pyrforme, Pisolithus arrijus* and natural mycorrhiza.

Table 1(b). Significance value (f) of various treatments between column and row

Source of Variation	Sum of square	Degree of freedom	Means square	Ratio of variance	F test
Between column	16004.9	5	3200.98	3200.98	
Between	2.7	11	0.24545	0.35099	9121.96
Residual	19.3	55	0.35099	0.35099	
				0.24545	1.42998
Between column	13807.128	5	2761.4256	2761.4256	
Between	4.07	11	0.37	63.025	
Residual	3466.42	55	63.025	63.025	
				0.37	170.337
Between column	14820.78	5	2964.156	2964.156	
Between	3.58	11	0.3254	0.4078	7268.65
Residual	22.43	55	0.4078	0.4078	
				0.3254	1.2532
Between column	17785.364	5	3557.072	3557.072	
Between	9.087	11	0.826	0.4968	7159.96
Residual	27.326	55	0.4968	0.826	
				0.4968	1.6626
Between column	9441.787	5	1888.3574	1888.3574	
Between	11.268	11	1.02436	0.4391	4300.51
Residual	24.153	55	0.4391	1.02436	
				0.4391	2.3328

Significance level of 0.995, the critical value of F is 1.9174, 2.3683 (by taking the value of F corresponding to 5, 11 and 60 degree of freedom) as there is no value of 5.5 in denominator the closest value for 60 considered. In the above calculation, it has shown two different type column wise and row wise. In the above calculation all the colunm wise, the calculated value of X^2 (all treatments) are more than the tabulated value. All the treatments are considered to be poor in other the calculated as row wise. Among these calculated value of X^2 (all treatment) 4 is best due to lesser tabulated value.

$V_1 = (6 - 1)\ 5$
$V_1 = (12- 1)= 11$
$V_2 = 72 - (5+ 11) = 55$
$V_1 = 5$ and $V_2 = 55$ nearest 60 the tabulated value = 2.37
$V_1 = 11$ (nearest 12) and $V_2 = 55$ nearest to 60 the tabulated value = 1.92

Experiment 2

In this experiment only 50 seedlings of *P. roxburghii* were used and the same mycorrhizae as in experiment 1 were used and rest of the method was also same.

The height of each plant was recorded on the same day after planting in May 1999. All plots were regularly weeded by hand in accordance with the usual practice in pine plantation (Momoh & Gbadegesin 1980). The soil samples of the two sites were analysed for pH, Nitrogen, Phosphorus, Potassium and organic matter before planting (Table 2).

Table 2. Physico-chemical properties of the soil samples collected from the study sites.

Sampling Area	pH	N %	P_2O_5 kg/ha	K_2O kg/ha	OM %
Chalnakhel	4.4	0.201	33.48	824	6.16
Godawari	4.9	0.138	18.26	824	4.15

In above table it has shown that the chemical properties of soil (N, P and organic matter) of Chalnakhel has shown higher than that of Godawari soil but K is same in both sites and pH is acidic in Chalnakhel than that of Godawari.

The experiment was done for twelve months from May 1999 to Mar. 2000. The first assessment of the experiments were done on same day of planting. The second assessment was done in November 1999. The third and fourth assessments were done in February 2000 and May 2000. Within this one year period, the plants had survived one rainy season and one dry season in the field and had a total of twelve months in the field.

The results clearly showed that the survival rate of the plants was higher on inoculated with *P. arrijus* than *A. hygrometricus. L. pyriforme, Boletus* sp. and natural mycorrhizal soil inoculum in Chalnakhel than in Godawari (Fig. 1 and 2). Similarly, *P. arrijus* showed better performance than the other species of mycorrhizae at both sites. All the three tested mycorrhizae performed better in Chalnakhel than in Godawari (Fig. 3 and 4).

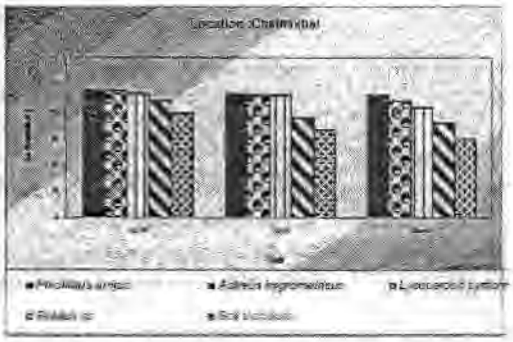

Fig. 1. *P. roxburghii* inoculated with *P. arrijus*

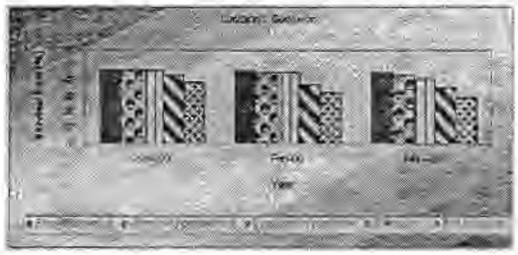

Fig. 2. *P. roxburghii* inoculated with *P. arrijus*

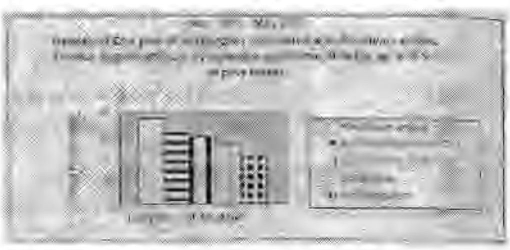

Fig. 3. *Pinus roxburghii* inoculated with *P. arrijus*

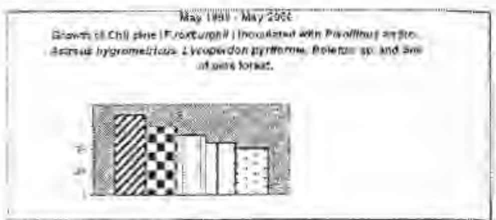

Fig. 4. *P. roxburghii* inoculated with *P. arrijus*

Discussion

In this present study *P. arrijus* was found to lead to better survival and better growth of *P. roxburghii* in both sites than *A. hygromericus, L. pyriforrne, Boletus* sp. And natural mycorrhiza which was also reported h Trappe (1962b). *Boletus* sp. has shown a mycorrhizal fungus (Bakshi 1974).

Bery and Marx (1976) also discovered that the fungus gave better growth of pinus taeda than other naturally occuring fungi in the southern United States. Marx (1990) also found that ectomycorrhizal development on seedlings was encountered among nurseries and development of ectomycorrzae depends on the types of the soil also. In the present study also it is clearly seen that all ectomycorrhizae grew better in Chalnakhel than in the soil of Godawari nursery due to higher phosphorus, nitrogen and organic matter contained in the soil of Chalnakhel than in the soil of Godawari nursery. The quality of the soil for plants as well as for the mycorrhizae has also been reported by (Shrestha 2000). Similar results were reported by Gray & Gerdman 1973, Jackson *et al.* 1972 in which mycorrhizal plants have been found to thrive well and grow much better than the non-mycorrhizal plants in soils with low quantities of available phosphorus. Marx and Kenny (1991) have reported that the trees of many species including pines will not grow and develop normally without fungi, Marx & Cordell (1988) have found that pine seedling with abundant *Pisolithus* ectomycorrhizae formed in the nursery survived and grew better after out planting than seedlings with natural occurring fungi. The present study has also found similar results. *P. arrijus* show better performance in the field. By exploring such fungi we can substitute chemical fertilizers and can use them for rehabilitation of the forest as well as far biological control.

Acknowledgement

This work was initiated with the research grant provided by International Foundation for Science (IFS), Sweden. Prof. D.H. Marx of the Forest Science Laboratory, Athens, George and Dr. Bernie, Dell, Perth, Australia provided relevant literatures. We were also grateful to the forest nurseries Chalnakhel and Godavari for providing field and manpower and to Mr. Purna Bahadur Tamang for his assistantship during field works.

Antibacterial Activity of the Wild Mushrooms Against Human Pathogens

G. Shrestha Vaidya, S. Thapa, A. Shrestha and K.Shrestha

Abstract

Antimicrobial activity of two wild mushrooms groups namely *Ganoderma* spp. *and Agaricus* spp. against pathogenic bacteria of human was tested. The activity of the fungal mass was studied by direct cross inoculation technique and that of chloroform extract of the fungi grown in liquid culture media was tested by agar well diffusion technique. The study revealed that fungal body of *Ganoderma* was ineffective against all the tested bacterial pathogens, while the extract of the same mushroom was effective *against Salmonella typhi, S. paratyphi, S. dysenteriae, Pseudomonas aeruginosa, Vibrio cholerae, Klebsiella oxytoca, P. vulgaris, P. mirabilis* and *Staphylococcus aureus,* and ineffective against *Escherichia coli* and *Klebsiella pneumoniae.* In direct cross inoculation, *Agaricus* spp. totally inhibited *Salmonella typhi, S. Paratyphi, Escherichia coli, P. aeruginosa, V. Cholerae, K pneumoniae, P. vulgaris* and *P. mirabilis,* and the rest were inhibited partially. The chloroform extract of *Agaricus* spp. was effective against all of the tested bacteria.

Introduction

Mushrooms help to increase immunity power of a cancer patient. Some fungi also help to increase immunity. Mushrooms are also rich sources of natural antibiotics (Stamets 2001). It also secretes extra cellular metabolites that inhibit certain bacteria, fungi, viruses and parasites.

Plants are more susceptible than animals to fungal diseases. The use of diseased plants does not affect human health whereas diseases of fungi do (Martin 2001). The microbial antagonists produced by *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* are common to man and plants that produce antibiotics to fight infection from microorganisms.

During the last decades several pathogenic mncroorganisms developed resistance to the available antibiotics. Infections by multidrug resistant isolates of *Candida* spp., *Staphylococcus epidermidis, S. aureus, Streptococcus* spp., *Enterococcus* sp. and *Escherichia coli,* among others, became more and more frequent stimulating the search for new antibiotics with novel mechanisms of action (Kotra & Mobashery 1998).

Nepal is very rich in biodiversity. The present study can help in tracing mycorrhiza having potential and broad-spectrum antagonistic activity against pathogenic microorganisms. The study aims to reveal the antibacterial property of wild mushrooms, *Agaricus* spp and *Ganoderma* spp against various human pathogens isolated from patients. Some of the species of *Agaricus* and *Ganoderma* are widely used for therapeutic properties and they have not been thoroughly studied yet as the source of antimicrobial compounds. *Ganoderma* and *Agaricus* species are abundant in characteristic tropical rain forests of Nepal.

Methodology

Collection of Sporocarps (Mushrooms)

Several species of mushrooms were collected from forests of Nepal including various locality of Lalitpur and Kavre districts. The forests of Nepal are very rich in several species of mushrooms. *Agaricus* spp. were collected from Kavre and *Ganoderma* spp. from Lalitpur district. The most suitable time for the collection of sporocarps is from July to October. Therefore, sporocarps of various developmental stages were collected during that season. Young sporocarps were used for direct isolations. Fully matured specimens were also collected for identification. Identification of these mushrooms were done according to Brundett *et al.* (1996) and Svrcek (1983). Isolation *of* fungi from collected sporocarps were done. In this process a shallow (1-2 mm) slit across the middle of the cap surface were cut and small pieces of interior tissues were cut with a fine sterile tipped scalpel or needle and then transfer to the potato dextrose media in plates and were incubated at room temperature and selection criteria was based on Shrestha (1999 a, 1999 b).

Study on the antibacterial property of the mushrooms by direct cross inoculation method

The fungi were inoculated on Potato Dextrose Agar (PDA) plates and incubated at 27°C for about 2-4 days. After incubation and growth of fungi, human pathogenic bacteria were inoculated at four different sites on the same plate and incubated again for 24 hours at 35°C and observed for the inhibition of bacteria (Shrestha and Piya 2002).

Extraction of metabolites of the mushrooms

Pure cultures of mushrooms were inoculated into Modiifed Melin Norkan's (MMN) broth and incubated at 27°C for about a month with shaking whenever possible. After a month of incubation and heavy growth of the pure culture of the fungi, fungal mat was separated by filtering through muslin or cheesecloth. The solvent was subjected to solvent extraction with chloroform. The solvent was recovered and the resulting residue was used for the study (Shrestha & Piya 2002).

Study on the antibacterial property of extracted metabolites of the mushrooms by agar well diffusion technique

Bacterial broth was prepared to match the turbidity standard 0.5 of Nephelometer and swabbed on Muellar Hinton Agar (MHA) plate and, left for few minutes at room temperature. Two wells were made on inoculated plate with alcohol-flamed sterile cork borer and extracted fungal metabolite in suspended in chloroform in one of the wells. For the control, another well was loaded with chloroform. The plates were left for about 30 minutes for diffusion and incubated at 37°C for about 24 hours. After incubation, the zone of inhibition around the wells was noted (Shrestha & Piya 2002).

Result

Antibacterial activities of the mushrooms *Ganoderma* spp. and *Agaricus* spp.were detected by direct cross inoculation method and their extracts by agar well diffusion method. The pathogenic bacteria used in the study were *S. typhi, S. paratyphi, Shigella dysenteriae, Escherichia coli, P. aeruginosa, Vibrio cholerae, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus vulgaris, Proteus mirabilis* and *Staphylococcus aureus*.

Antibacterial activity of *Ganoderma* spp.

The result of the antibacterial activity of *Ganoderma* by direct cross inoculation revealed that the fungal body was ineffective against all of the tested bacterial pathogens but the metabolic extract was found to be effective against most of the tested bacterial pathogens (Fig. 2). The most inhibited bacteria was *S. typhi*, while it was ineffective against *Escherichia coli* and *Klebsiella pneumoniae*.

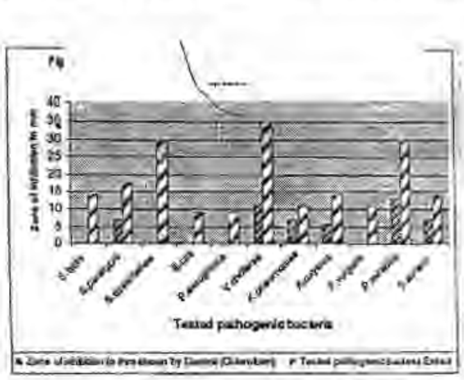

Fig. 1. Antibacterial activity of the chloroform extract of *Agaricus* species

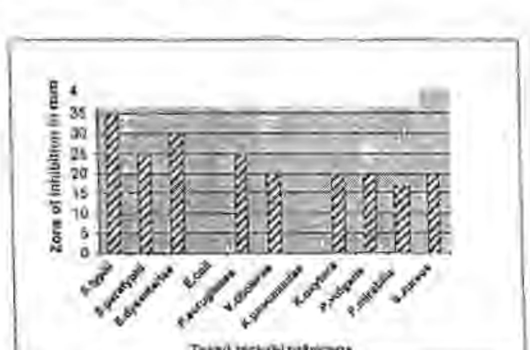

Fig. 2. Antibacterial activity of the chloroform extract of *Ganoderma* species

Antibacterial activity of *Agaricus* spp.

Fungal body of *Agaricus* spp. totally inhibited the growth of *Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Klebsiella pneumoniae, Proteus vulgaris,* and *Proteus mirabilis*. But the pathogens *Shigella dysenteriae, Klebsiella oxytoca* and *Staphylococcus aureus* were inhibited only partially (Table 1).

Table 1. Antibacterial activity of *Agaricus* spp.

Pathogenic bacteria	Inhibition by *Agaricus* spp.
Salmonella typhi	++
Salmonella paratyphi	++
Salmonella dysenteriae	+
Escherichia coli	++
Pseudomonas aeruginosa	++
Vibrio cholera	++
Klebsiella pneumonia	++
Klebsiella oxytoca	+
Proteus vulgaris	++
Proteus mirabilis	++
Shigella aureus	+

The metabolic extract *of Agaricus* spp. was found to be effective against all of the tested pathogens (Fig. 1). The results showed that the pathogen *S. dysentriae* was the most inhibited one, while *K. pneumoniae* was least inhibited.

Discussion

In the present study, direct inoculation of *Ganoderma* spp. as a whole was found to be ineffective against all the bacteria tested, while the extract of the same was effective against most of the bacterial species tested in this study. The chloroform extract of *Ganoderma* spp. was found to be effective against *Salmonella typhi, Salmonella paratyphi, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio cholerae, Klebsiella oxytoca, Proteus vulgaris, Proteus mirabilis* and *Staphylococcus aureus* and ineffective against *Escherichia coli* and *Klebsiella pneumoniae*. Among all the tested bacteria, *Salmonella typhi* was highly inhibited. The similar test was also done by Sudirman (1997) using methanolic extract of *Ganoderma* spp. against *Bacillus subtilis* and was found to be effective. Similar test had been done by Coletto *et al.* (1991) with the mycelia and cultural filtrates of *G. recinaceum* and *G. lucidum*, which showed the activity against *B. subtilis*. *G. recinaceum* also inhibited *Staphylococcus aureus*. Another study showed that *G. applanatum* exhibited antimicrobial activity against Gram-positive *B. cereus, S. aureus*, and less activity against the Gram- negative *E. coli*, and *P. aeruginosa* (Smania *et al.* 2001). The test against *E. coli* in our study is supported by the findings of Smania *et al.* (2001), but the finding for *P. aeruginosa* of the same study was found to be contrasting with our study.

The direct cross inoculation of *Ganoderma* was found to be ineffective against all the tested bacterial pathogens. From this study, it is revealed that the metabolic extract of *Ganoderma* is effective against pathogenic bacteria and the fungal body as a whole is ineffective.

The fungal body and the metabolic extract of *Agaricus* inhibited, either partially or totally. All tested pathogenic bacteria, *Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Klebsiella pneumoniae, Proteus vulgaris*, and *Proteus mirabilis* were totally inhibited by the direct cross inoculation, whereas the metabolic extract was found to be most effective against *Shigella dysenteriae*. It is to be considered that the effectiveness of the fungal extract and the fungal body vary even against the same organism.

Hexane and chloroform-methanol extracts of *Agaricus* inhibited *Salmonella* in the study of Osaki *et al.* (1994). A bactericidal substance was also isolated in the same study. An antimicrobial activity *of Agaricus* cf. *nigrecentulus* against *Staphylococcus saprophyticus* was also shown by Rosa *et al.* (2003). Sidorova and Velikanov (2000) confirmed the presence of bioactive metabolites in several species of *Agaricus*, and also found that the water extract of *Agaricus* inhibited the growth of Gram-positive bacteria and yeasts.

Acknowledgement

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Study of Rhizospheric Soil Microflora of Baluwa Forest of Kavre District (Central Nepal)

Geeta Shrestha Vaidya, Keshav Shrestha,
Hâkan Wallander and Buddhi R. Khadgi

Abstract

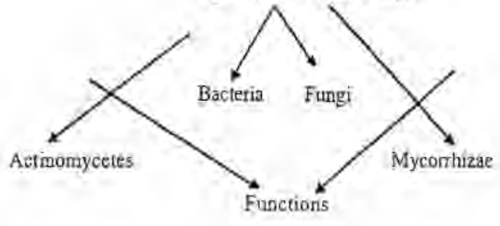
Soils contain enormous numbers of diverse living organisms assembled in complex and varied communities Microorganisms are present in great numbers on rhizospheric soil, and they play vital roles in numerous physiological processes. Associations of microorganisms participating in saprotrophic, pathogenic and symbiotic root activities, mediate these dynamic processes. The soil microflora and the vegetation of an ecosystem are Closely interrelated. Plants influence soil biotic process by delivering organic compound into the soil, where as soil microbes have an impact on plant growth through the decomposition and mineralisation of plant materials. Baluwa forest is an eroded newly planted site. In the present study, soil samples were taken from two sides of *Bauhinia purpurea* and *Leuceania leucocephala.* These soils were analyzed for nitrogen, phosphorus, potassium, pH and organic matter. The total numbers of microorganisms were enumerated by plate count method. Then six species of bacteria, ten species of fungi, five species of actinomycetes were isolated on suitable media and three species of endomycorrhizae were also extracted, from same soil and identified. Besides, the antagonistic action of some of these microbial strains was studied against pathogenic bacteria and fungi. These strains could be sources of antibiotics useful in the control of plant and human diseases.

Introduction

Nepal is very prone to soil erosion and is susceptible to sediment disasters mainly caused by slope failure, landslides, debris flows and bank erosion. Productivity of the soils is deteriorating due to accelerated surface soil erosion (Jagannath *et al.* 1998). Diverse microorganisms are essential for a sustainable biosphere. It is important to preserve and conserve biodiversity of rhizospheric microorganisms as well as their natural habitats. The most extensive microbial growth takes place in the organic-rich surface layers, especially in and around the rhizosphere. Rhizospheric microorganisms produce a large number of bioactive secondary metabolites. So biological diversity is one of the major challenge that human have deal in the next millennium. The rate and time of species turnover have changed abruptly due to human interference and habitat destruction. This is probably one of the biggest threats to biodiversity. The production of food for human beings will be impossible without the biological diversity. The challenge resides in the capacity to use the biological diversity creating the conditions of its continuity for future generation.

Soil is one of our most valuable resources. With ideal temperature and moisture conditions soils are excellent culture media for many kinds of microorganisms. This is especially true of cultivated and improved soils. It regulates global biogeochemical cycles. Soil organisms (Biota) carry out a wide range of processes that are important for soil health and fertility in both natural and managed agricultural and forest soils. The total number of organisms, the diversity of species and the activity of the soil biota will fluctuate as the soil environment changes.

So, diverse microorganisms are essential to a sustainable biosphere. They can be used for biological control of plant and animal pests and drugs.



The functions of the microorganisms are as follows:

1. Organic matter turnover
2. Nutrient transfer
3. Soil structure improvement
4. Disease transmission & prevention
5. Pollutant degradation

Methodology

Sampling site

Samples were collected from two sites such as *Bauhinia* purpurea and *Leuceania leucocephala sites* of Baluwa forest of Kavre district of central Nepal. This forest is situated 40 km. East of Kathmandu city on the side of Arniko highway to the Tibetan border. Samples were collected in sterile plastic bags with the help of a sterile spoon. Then, it was immediately brought to the laboratory for detail analysis. The nitrogen, phosphorus and potassium percent of soil were measured. Then, soil pH was measured. Microorganisms exist in mixed populations from the rhizospheric region of soil. The plate count method was used to determine the number of cells that will multiply under certain defined conditions.

Biological analysis

I) Identification of bacteria:

The plate count method: In this method, 1 gm of rhizospheric soil sample was transferred to 10 mL of D/W to get dilution of 10^{-1}. Then 1 mL of solution from the tube containing 10^{-1} dilution was transferred into another tube containing 9 mL of D/W so that it was 10^{-1} diluted. It goes dilution up to 10^{-2} were prepared.

After incubating the bacterial colonies for 24 hours in nutrient agar, their colonial characteristics were studied. Then, Gram staining and spore staining were performed according to Benson (1979). Different biochemical tests were performed according to Mackie and McCartney (1989). After performing the Biochemical, physiological and morphological tests, the isolated bacteria were identified on the basis of the characters as given in Bergey's Manual of Systematic Bacteriology (1984).

II) Identification of fungi:

Fungal colonies were grown in potato dextrose agar, Czapek's Dox agar and Modified Melin-Norleman's agar for one week. Then, their colony characters were studied. Morphological characters were studied with the help of cellophane method and by using malachite green as a dye (Benson 1979). After that, fungi were identified by referring a manual of Soil Fungi, Gilman (1998).

III) Identification of actinomycetes

Actinomycetes colonies were grown in glycerol asparagines agar for one week. Then, their colony characters were studied. Morphological characteristics were studied by buried cover slip method, using crystal violet as a dye. Actinomycetes were identified with the help of Bergey's Manual of Systematic Bacteriology (1984). Then, the growth of actinomycetes was tested at high temperature (55°C) and at high salt concentration (7% NaCl).

IV) Antagonistic action study

Direct cross inoculation: Antagonistic action of some of these microorganisms done with direct inoculation method (Shrestha Vaidya *et al.* 2002, Shrestha Vaidya *et al.* 2005).

Tested organisms should be allowed to growth about 1 to 2 cm. Then plant pathogens were inoculated at four different sites on the plate or inoculate in one side if the growth pattern of the organism is faster and again incubated for 1-2 weeks at same temperature but the inoculation periods depends upon the type of the organisms. Then observed for inhibition of the growth.

V) Extraction and identification of AM spores

Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting according to Gerdemann (1963).

Chemical analysis

Chemical analysis of the soil samples from *Bauhinia purpurea* and *Leuceania lecucocephal* were performed to check their nitrogen, phosphorus, potassium, organic matter and pH according to (Vaidya & Shrestha 2005).

Biochemical tests for the identification of bacteria

Organism	Gm stain	Catalase	Oxidase	O/F	VP	Urease	Citrate	Glucose	Sucrose
Staphylococcus sp.	+Cocci	+	-	F	-	+	+	+	+
Pseudomonas aeruginosa	(-) rod	+	+	O	-	+	+	+	-
Escherichia coli	(-) rod	+	-	F	-	-	-	+	-

Biochemical tersts for the identification of *Bacillus* sp.

Organisms	Gm stain	Catalase	Oxidase	O/F	Spore	Indole	VP	Urease	Citrate	Glucose	Lactose
B. subtilis	+Rod	+	+	O	ST/O	-	-	+	+	+	+
B. cereus	+Rod	+	-	F	C\O	-	+	-	+	+	-

Results

S.N.	Description of the soil	pH	OM %	N %	P_2O_5 Kg/ha	K_2O kg/ha
1	*Bauhinia* site	4.0	0.54	0.031	441	473
2	*Leuceania site*	4.0	0.94	0.023	605	466

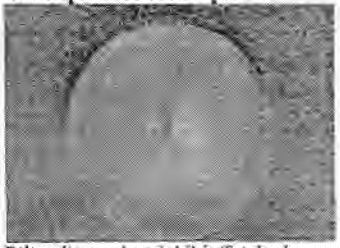
In above table it has shown that the chemical analysis soil samples (N, P, K and Organic matter) of *Leuceania* site has shown better than of soil samples of *Bauhinia* site but pH was same at both sites.

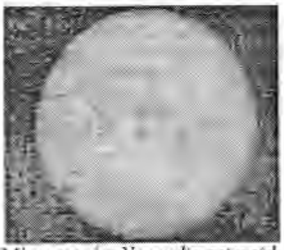
Soils were collected from both sites of *Bauhinia* and *Leuceania* rhizospheric soils. From these soils, isolated Fungi, Actinomycetes, Endomycorrhizae and Bacteria have been shown in Table 1, Table 2, Table 3 and Table 4. The total numbers of these organisms have been shown in Fig. 1 and Fig. 2.The percentage of these organisms isolated have been shown in pie chart Fig. 3 and Fig. 4. Some micro-organisms have been studied their antagonistic with pathogenic organisms.

Total actinomycetes isolated from soil samples at different temperatures

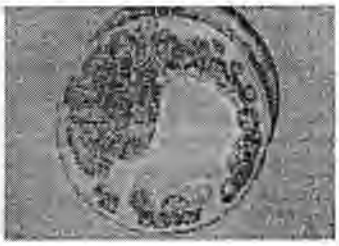
S.N.	Actinomycetes	Growth at 45 °C	Growth at 7% NaCl
1	*Nocardia asteroid*	-ve	+ve
2	*Norcardia* sp	+ve	+ve
3	*Streptomyces* sp	+ve	+ve
4	*Microtetraspora ferruginea*	-ve	-ve
5	*Pilimelia anulata*	-ve	-ve

Examples of AM spores


Pilimelia anulata inhibit *Trichoderma* sp.


Microscopic. *Nocardia asteroid*


Aaulospora scorbiculata


Pilimelia anulata inhibit *Aspergillus niger*


Glomus constrictum

Table 1. Total Fungi isolated from *Bauhinia* and *Leuceania* sites

S.No.	Fungi isolatged	*Bauhinia* site	*Leuceania* site
1	*Aspergillus niger*	√	
2	*Aspergillus fumigatus*		√
3	*Cladosporium harbarum*	√	
4	*Fusarium oxysporum*	√	
5	*Fusarium moniliforme*		√
6	Curvularia sp.	√	
7	*Paecilomyces varioti*		√
8	*Penicillium granulatum*	√	
9	*Rhizopus cohnii*		√
10	*Trichoderma* sp.		√

In above table *Aspergillus niger, Cladosporium harbarum, Fusarium oxysporum, Curvularia* sp. and *Penicillium granulatum* were isolated from *Bauhinia purpurea* site and *Aspergillus fumigatus, Fusarium moniliforme, Paecilomyces varioti, Rhizopus cohnii* and *Trichoderma* sp. from *Leuceania leucocephala* site.

Table 2. Total Actinimycetes isolated from *Bauhinia* and *Leuceania* sites

S.No.	Fungi isolated	*Bauhinia* site	*Leuceania* site
1	*Nocardia asteroid*	√	
2	*Streptomyces* sp.		√
3	*Pilimelia anulata*		√
4	*Nocardia* sp.	√	
5	*Microtetraspora ferruginea*		√

In this table Actinomycetes were found more in *Leuceania* site than that of *Bauhinia site*

Table 3. Total Endomycorrhizae (AM spore) isolated from *Bauhinia* and *Leuceania* sites.

S.No.	Fungi isolated	*Bauhinia* site	*Leuceania* site
1	*Glomus constrictum*	√	
2	*Acaulospora scobitulata*		√
3	*Glomus macrcarpon*		√

In this table more AM spore were found in *Leuceania* site than that of *Bauhinia* site

Table 4. Total Bacteria isolated from *Bauhinia* and *Leuceania* site

S.No.	Fungi isolated	*Bauhinia* site	*Leuceania* site
1	*Staphylococcus* sp.	√	
2	*Bacillus cereus*	√	
3	*Bacillus subtilis*		√
4	*Salmonella typhi*		√
5	*Escherichia coli*	√	
6	*Pseudomonas aeruginosa*		√

Among these above isolated organisms *Pilimelia anulata Nocardia* sp., *Bacillus subtilis, Penicilium varioti* were studied their antagonistic action against with pathogenic fungi and bacteria such as *Aspergillus flavus, Aspergillus niger, Trichoderma viridi, Escheria coli, Salmonella* and *Shigella.*

Table 5. Results of cross inoculation methods

S.No.	Cross inoculated fungi & bacteria	*Pilimelia anulata*	*Nocardia* sp.	*Bacillus subtilis*	*Penicillium varioti*
1	*Aspergillus flavus*		++	+++	+
2	*Aspergillus nigere*	+++	+	+	
3	*Trichoderma viridi*	+++	+	+++	+
4	*Escherichia coli*	++	+++	+	+++
5	*Salmonella*	+	+++	++	++
6	*Shigella*	++	++	+	+++

Note: +++ = Highly inhibited, ++ = Moderately inhibited and + = slightly inhibited

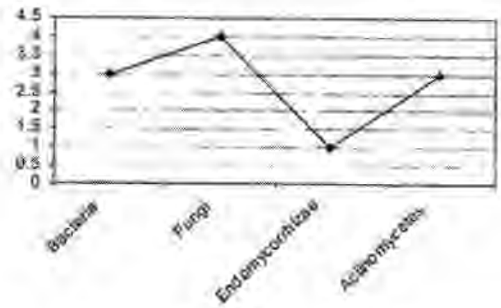


Fig. 1. Microbial diversity shows number of organisms isolated from *Bauhinia* site

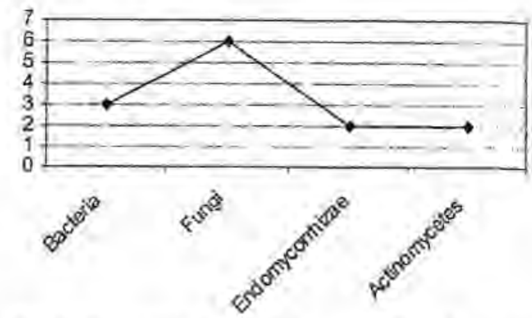


Fig. 2. Microbial diversity shows number of organisms isolated from *Leuceania* site

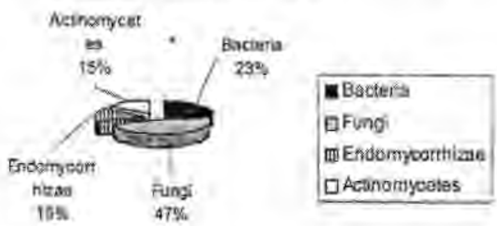


Fig. 3. Pie chart shows the percentage of different organisms isolated from *Leuceania* site

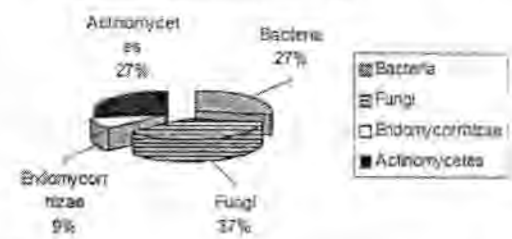


Fig. 4. Pie chart shows the percentage of different organisms isolated from *Bauhinia* site

Discussion

Microbial biodiversity is a vast frontier and a potential goldmine for the biotechnology industry because it offers countless new genes and biochemical pathways to probe for enzymes, antibiotics and other useful molecules. In this research, *Pseudomonas aeruginosa* inhibited the growth of *Aspergillus fumigatus, Escherichia coli, Salmonella typhi.* Similarly, Kandela *et al.* (1997) studied a number of physical and chemical properties of the purified pink pigment, the pyorubrin, that is produced by local collection of *Pseudomonas aeruginosa* strains. Such studies are important due to the biological significance of pyorubrin activity against other bacteria. Vasudeva *et al.* (1963) detected antibiotic, bulbiform in soil produced by *Bacillus subtilis.* Similarly, Singh *et al.* (1965) detected antibiotic, bulbiform in seeds in soils, which is formed by *Bacillus subtilis.* In this study also *Bacillus subtilis* inhibited the growth of *Aspergillus flavus* and *Trichoderma viridae. Pillimelia anulata* inhibits *Aspergillus niger* which causes diseases in human and plants. So, from this we could use *Pilimelia anulata* for controlling these diseases.

Mirchink Greshnykh (1961) detected antibacterial antibiotic in soil produced by *Penicillium purpurogenum.* In this research also *Penicillium varioti* inhibited *Escherichia coli, Salmonella* and *Shigella,* which are pathogens. In this research, *Pilimelia anulata* inhibited *Trichoderma* sp., *Aspergillus niger, Salmonella* sp., *Escherichia coli, Shigella* sp. that are pathogens. *Nocardia* sp. inhibited the growth of *Salmonella* sp., *Escherichia coli, and Shigella* sp., *Aspergillus flavus* that are pathogens. Similarly, Rangaswami and Vidyasekaren (1963), detected antifungal antibiotic in corn rhizosphere. In this study we have found that more beneficial organisms in *Leuceania* site which has more organic matter and N, P, K than that of *Bauhinia* site. So nutrients present in soil is also affect the presence of microorganisms in the soil. Same way the chemical analysis of the soil included the determination of soil acidity and the quantification of exchangeable in accordance with Brunner *et al.* (2002). The soil-acidity is determined by chemical activity of protons in the soil solution. It is expressed as pH-value and has an influence on the bioavailability of nutrients (Na, Ca, Mg, K) as well as the diversity and activity of soil organism (Walthert *et al. 2004)*.

Conclusion and Recommendation

Nepal keeps wide biodiversity and is almost virgin as far as these kinds of studies are concerned. Therefore Possibility of finding new antibiotics, new species of actinomycetes, bacteria and fungi are useful for the growth of the plant by different way such as soil structure improvement, organic matter turnover, and nutrient transfer and disease prevention. So, from all these function we can conclude that the functions of all these microorganisms act as a pollutant degradation.

So the diverse microorganisms are essential to a sustainable biosphere and they can be used for biological control of plant and animal pests. Not only that it can use for antibiotic drugs for human diseases. Because most antibiotics of chemotherapeutic value are derived from organisms isolated from soil, this environment has been of considerable interest in regard to its content of antimicrobial principle. Interest in biological control has increased recently fuelled by public concerns over the use of chemicals in the environment in general, and the need to find alternatives to the use of chemicals for disease control. The key to achieving successful, reproducible biological control is the gradual appreciation that knowledge of the ecological interactions taking place in soil and root environments is required to predict the conditions under which biocontrol can be achieved. This type of work requires a study not only of any potential biocontrol agent but also its interactions with the crop, the natural resident micro biota and the environment as well. Therefore, isolation and maintenance of cultures of bacteria, fungi, mycorrhizae and actinomycetes is an efficient way to conserve their biodiversity. So, it is vitally important to preserve and conserve microbial diversity.

Acknowledgements

We would like to express our sincere gratitude to Prof. Dr. Dayananda Bajracharya, Vice Chancellor and Prof. Dr. Krishna Manandhar, Secretary of RONAST for providing the facilities. Authors are grateful to Mr. Purna Bahadur Tamang for his assistantship.

Banko Jankari, 16, No. 2, 17-24, 2006

Mushroom Diversity in the Sagarmatha National Park and its Buffer Zone Area

Prabina Rana and Anjana Giri

Abstract

Nepal is rich in mycodiversity. However, only a few scientific researchers have been conducted. It has been observed that mycological studies are concentrated to lower altitude as compared to higher altitude. This paper aims to document the wild mushrooms and its diversity in the Khumbu region. A total of 150 mushroom species belonging to 37 families and 65 genera were collected from Lukla [2840 meters above mean sea level (m above msl)] to Pangboche (4000 m above msl). The largest family recorded were Boletaceae and Russulaceae having 18 species followed by Tricholomataceae (16 sps.), Polyporaceae (9 sps.) etc. Most of the collected mushroom species were found on soil. The appearance, occurence and dominance were found to be controlled by different factors such as altitude, vegetation, temperature humidity, etc. The diversity of mushroom species were found the highest (84 sps.) at an altitudinal range of 3500 – 4000 m above msl followed by 2500 – 3000 m above msl (52 sps.) and 3000 – 3500 m above msl (14 sps.).

Introduction

Nepal, although bound within a narrow belt from north to south, possesses distinct phytogeographical zones related to altitude and other factors. The differences in humidity and precipitation have led to the changes in the floristic composition from the west to east and north to south faces. The contrasting topographies, varieties of climate and vegetational zone offer heterogeneous and dense habitats for mushroom flora. (Bhandary 1985). Furthermore, the dominant or mixed or pure forest types *(Shorea – Pinus - Quercus - Abies - Rhododendron - Betula – Junipers)* from tropical to alpine zones with their ecological environment provide different micro-ecological conditions suitable for the origin, development and growth of diverse and specific mycotaxa. Due to its phytogeography, the western part of the country is much drier than the eastern and central part. Hence, the concentration of different groups of fungi varies from region to region. Moreover, the fungal diversity is favored in central and eastern as compared to western region. Besides, temperature has a universal influence and acts as a limiting factor for the growth and distribution of mushrooms. Mushrooms grow mainly from May to October, but they flourish well during July and August (Adhikari 2000). The life of a fleshy mushroom is normally reckoned in days, while that of a bracket polypore extends to months. However, a great majority of mushrooms have fruiting bodies which last for only a single season. In *Coprinus ephemerus* Bull.ex Fr.) Fr. the fruiting body lasts only a few hours developing during the night and vanishing by early morning. Woody fungi, such as *Fomes fometarius* (Fr.) Fr. and *Ganoderma applanatum* (Fr.ex Wallr.) produce perennial basidiomes every year. The annual layers may reach upto fifty layers. Variations in the production of fruiting bodies is quite marked (Kaul 1999). In some cases they may be produced every year, differing only in abundance. *Hypholoma storea* was found on a decaying part of a living beech trunk in 1805 by Fries and it appeared in the same crack again in 1833 (Swanton and Spinal 1922). On the other hand, the basidiomes of *Fistulina hepatica* Huds.: Fr. appeared year after year from crack in an oak tree.

Although, Nepal is rich in mycodiversity a very few scientific researchers have been conducted. It has been observed that mycological study is mainly concentrated to lower altitude as compared to high altitude. The present study aims to document the wild mushrooms and it's diversity in the Khumbu region.

Materials and Methods

Study area

The Khumbu Valley is a territory along the Nepal and Tibet (China) border. The Valley is a part of Solu-Khumbu District (Northeastern region of Nepal). It encompasses the Sagarmatha National Park (1,148 sq.km) and its buffer zone area (Fig. 1). The park includes the upper catchment areas of the Dudh Kosi and Bhote Kosi Rivers. Due to its altitudinal diversity, various type of vegetation is found in different climatic zones (temperate, subalpine, alpine, nival and permanent snow zones). The dominant vegetation at the lower elevation of the park below 3000 m above msl is composed mostly of Blue pine and Hemlock forest. The lower subalpine region above 3000 m above msl. comprises *Pinus wallichiana, Abies spectabilis* and *Juniper recurva* forests. The upper subalpine, above 3600 m asl consists of Birch - Rhododendron forest *(Betula utilis, Rhododendron campanulatum* and R. *campylocarpum)* and the lower alpine region above the timber-line at 3800–4000 m above msl houses scrubs *(Juniper* spp, *R. anthopogan & R. lepidotum).*

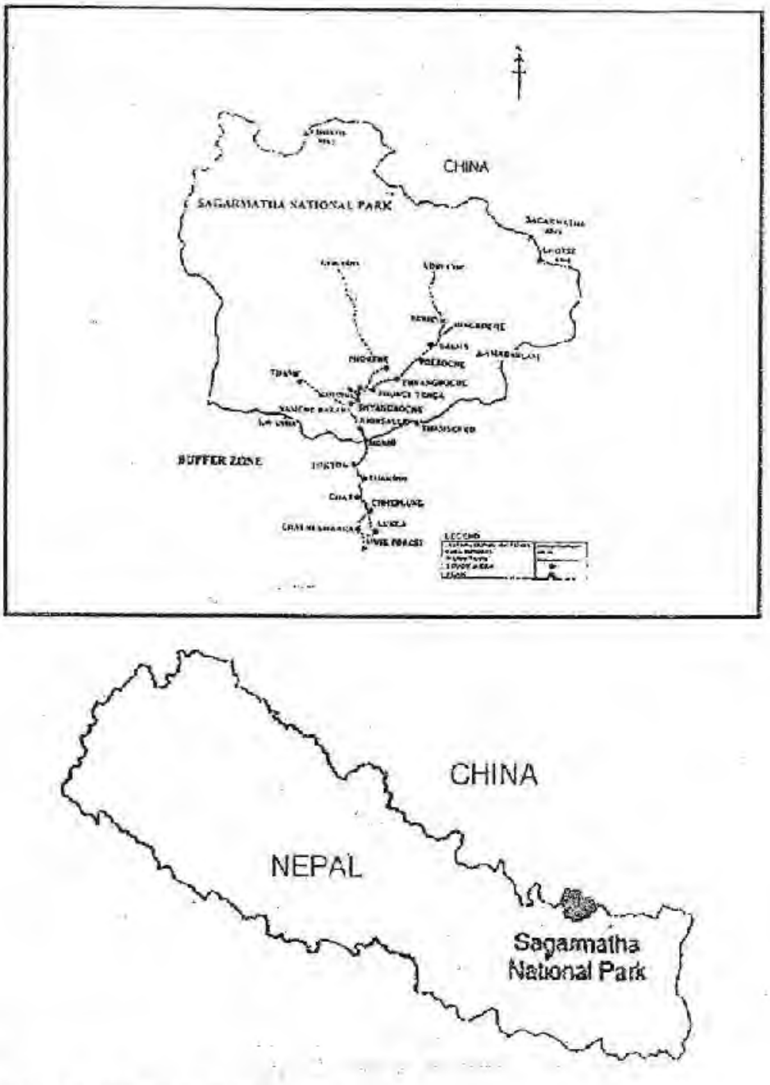


Fig. 1. Map showing the study area

Data collection

The mushroom specimens were collected from Lukla (2,800 m above msl) to Pangboche (4,000 m above msl during the month of August to September 2004. The mushroom specimens were collected carefully by digging them with the help of a sharp knife. Data on habit and habitat, ecological parameter such as altitude, forest type, etc. were recorded in the field (Table 1). The mushroom specimens were photographed in their natural habitat before they were picked up. Each collection was placed in butter paper bags and tag numbers were assigned to them. The morphological characters such as spore colour, pileus and tube colour, colour change induced after bruising or cutting and exposing to chemicals such as Potassium hydroxide (KOH), Ferrous sulphate ($FeSO_4$) were noted. The specimens were either sun dried or dried by placing them on tin foil over a local oven. Dried specimens were placed in butter paper bags. Naphthalene balls were used as insect repellents. The dried specimens were identified in the laboratory with the help of literatures and studying their macro and microscopic characters. The collected specimens have been now deposited in the Nepal Academy of Science and Technology (NAST) Laboratory.

Results and Discussion

A total of 150 mushroom specimens belonging to 37 families and 65 genera were collected from Lukla (2600 m above msl) to Pangboche (4000 m above msl). Out of the collected mushroom specimens except 5 species, all were identified upto the generic level and 68 were identified upto species level (Table 2). The largest families were Boletaceae and Russulaceae having 18 species, followed by Tricholomataceae (16 sps.), Polyporaceae (9 sps.) and Cortinariaceae (8 ups.) and so on. During the survey, different micro-habitat of mushrooms were also recorded.

Table 1. Different localities with their GPS readings and dominant vegetation

S. N	Locality	Longitude	Latitude	Altitude	Vegetation
1	Muse Forest (Lukla)	86°43.40'	27°41.87'	2783	Mixed forest of *Rhododendron* sps., *Quercus semicarpifolia*, *Tsuga dmosa* and *Pinus wallichiana*
2	Ghat	86°42.98'	27°42.44'	2604	Dominated by coniferous forest *(Pinus wallichiana)*
3	Phakdin	86°42.76'	27°44.92'	2480	Pine forest (*Pinus wallichiana*)
4	Tok Tok	86°42.89'	27°46.78	2725	Mixed forest of *Pinus wallichiana* and *Rhodendron* sps.
5	Jorsalle	86°43.07'	27°47.26'	2690	Pure Pine forest (*Pinus wallichiana*)
6	Namche	86°42.60'	27°48.16'	3150	Mixed forest of *Rhododendron* sps. and *Pinus wallichiana*
7	Phurte	86°42.36	27°48.27'	3615	Mixed forest of *Abis spectabilis, Tsuga (dumosa, Betula utilis, Rhododendron* sps. *and Juniper* sp.
8	Sangboche	86°42.69'	27°48.79'	3700	Mixed forest of *Juniper* sp. and *Rhododendron* sps.
9	Khunde	86°42.59'	27°49.02'	3900	Mixed djbicst of *Pinus wallichiana, Abis spectabilis* and *Rhododendron* sps.
10	Khumjung	86°43.70'	27°48.92'	3800	Mixed forest of *Abies spectabilis Rhododendron* sps. and *Betula utilis*
11	Jamikiau Forest	86°44.01	27°50.00'	3825	Mixed Forest of *Abies spectabilis, Rhododendron* sps., *Betula utilis and Juniper* sp.
12	Lausasa	86°44.11'	27°49.63'	3770	Mixed forest of *Rhododendron* sps. and *Abies spectabils*
13	Phungi Tenga	86°45.12'	27°50.12'	3440	Mixed forest of *Abies spectabilis Rbododendron* sps and *Betula utilis*
14	Thyangboche	86°46.02	27°50.26'	3776	Mixed forest of *Rhododendron* sps. and *Abies spectabils.*
15	Deboche	86°46.37'	27°50.47'	3665	Mixed forest of *Betula utilis, Rhododendron* sps., *Abies spectabilis* and *Juniper* sps.
16	Pangboche (Omakha forest)	86°46.43'	27°50.57'	3890	Mixed forest of *Abies spectabilis, Betula utilis* and *Rhododendron* sps.

Most of the mushrooms were found on soil (80%) followed by trees/plants (14%), forest litter (2%), dead wood (2%), dung (1.3%) and mushrooms (0.7%).

Table 2. Collected wild mushrooms with their scientific names, family, locality and altitude

S No	Col. No.	Col Date	Locality	Altitude (m)	Local name	Scientific Name	Family	Remarks
1	1	13.8.2004	Muse forest	2783	Chiple Chyau	*Armillaria mellea*	Tricholomataceae	Locally edible
2	3	13.8.2004	Muse forest	2783		*Crepidotus* sp.	Crepidotaceae	
3	4	13.8.2004	Muse forest	2783		*Mycena capillaries*	Marasmiaceae	
4	10c	13.8.2004	Muse forest	2783		*Laccaria amethystine*	Tricholomataceae	
5	10d	13.8.2004	Muse forest	2783		*Tylopilus* sp.	Boletaceae	

6	10e	13.8.2004	Muse forest	2783		*Ganoderma* sp.	Ganodermataceae	
7	10f	13.8.2004	Muse forest	2783		*Conocybe* sp.	Bolbitiaceae	
8	10gh	13.8.2004	Muse forest	2783		*Lactarius piperatus*	Russulaceae	
9	10i	13.8.2004	Muse forest	2783		*Boletus edulis*	Boletaceae	
10	10j	13.8.2004	Muse forest	2783		*Boletus* sp.	Boletaceae	
11	10k	13.8.2004	Muse forest	2783		*Microporus* sp.	Polyporaceae	
12	10L	13.8.2004	Muse forest	2783		*Lactarius subdulcis*	Russulaceae	
13	20	13.8.2004	Muse forest	2783	Rato Anda Chyau	*Amanita hemibapha*	Amanitaceae	Locally edible
14	21	13.8.2004	Muse forest	2783	Seto Anda Chyau	*Amanita vaginata*	Amanitaceae	Locally edible
15	22	13.8.2004	Muse forest	2783	Kalo Martip	*Boletus pulverulentus*	Boletaceae	Locally edible
16	23	13.8.2004	Muse forest	2783	Sano Anda Chyau	*Amantia* sp.	Amanitaceae	Locally edible
17	24	13.8.2004	Muse forest	2783		*Russula olivacea*	Russulaceae	
18	25	13.8.2004	Muse forest	2783		*Russula* sp.	Russulaceae	
19	26	13.8.2004	Muse forest	2783		*Russula* sp.	Russulaceae	
20	27	13.8.2004	Muse forest	2783		*Cortinarius* sp.	Cortinaraceae	
21	30	13.8.2004	Muse forest	2783		*Tricholoma* sp.	Tricholomataceae	
22	32	13.8.2004	Muse forest	2783		*Gyrodon merulioides*	Boletaceae	
23	33	13.8.2004	Muse forest	2783	Seto Martip	*Boletus auripes*	Boletaceae	Locally edible
24	40	13.8.2004	Muse forest	2783		*Marasmuis* sp.	Marasmiaceae	
25	41	14.8.2004	Lukla/Ghat	2604		*Strobilomyces stro*	Strobilomycetaceae	
26	42	14.8.2004	Lukla/Ghat	2604		*Phylloporus bellus*	Boletaceae	
27	43	14.8.2004	Lukla/Ghat	2604		*Pulveroboletus ravenelli*	Boletaceae	
28	50	14.8.2004	Lukla/Ghat	2604		*Tylopilus alboater*	Boletaceae	
29	51	14.8.2004	Lukla/Ghat	2604		*Strobilomyces* sp.	Strobilomycetaceae	
30	54	14.8.2004	Lukla/Ghat	2604		*Tricholoma* sp.	Tricholomataceae	
31	56	14.8.2004	Lukla/Ghat	2604		*Collybia* sp.	Marasmiaceae	
32	61	14.8.2004	Pkhakdin	2500		*Laccaria bicolor*	Tricholomataceae	
33	70	14.8.2004	Pkhakdin	2500		*Boletinus cavipes*	Boletaceae	
34	81	15.8.2004	Tok Tok	2725		*Coltricia perennis*	Hymenochaetaceae	
35	82	15.8.2004	Jorsalle	2685		*Pholiota* sp.	Strophariaceae	
36	84	15.8.2004	Tok Tok	2725		*Boletinus asiaticus*	Boletaceae	
37	85	15.8.2004	Tok Tok	2725		*Boletinus* sp.	Boletaceae	
38	86	15.8.2004	Tok Tok	2725		*Paxillus* sp.	Paxillaceae	
39	87	15.8.2004	Jorsalle	2685		*Heterobasidion* sp.	Polyporaceae	
40	89	15.8.2004	Jorsalle	2685		*Nematoloma* sp.	Strophariaceae	
41	90	15.8.2004	Jorsalle	2690		*Marasmius* sp.	Marasmiacedae	
42	91	15.8.2004	Jorsalle	2690		*Heterobasidion annosum*	Polyporaceae	
43	100	15.8.2004	Jorsalle	2690		*Gloeophyllum sepiarium*	Formitopsidaceae	
44	101	15.8.2004	Jorsalle	2690		*Amanita* sp.	Amanitaceae	
45	102	15.8.2004	Jorsalle	2690		*Laccaria proxima*	Tricholomataceae	
46	105	15.8.2004	Jorsalle	2690	Chiple Chyau	*Armillaria* sp.	Tricholomataceae	Locally edible
47	110	16.8.2004	Jorsalle/ Namche	2800		*Namatoloma* sp.	Strophariaceae	
48	120	16.8.2004	Jorsalle/ Namche	2800		*Amanita* sp.	Amanitaceae	
49	121	17.8.2004	Phurte	2840	Takatale	*Nematoloma capnnides*	Strophariaceae	Locally edible
50	125	17.8.2004	Phurte	2840		*Russula* sp.	Russulaceae	
51	126	16.8.2004	Jorsalle/ Namche	2800		*Clitocybe* sp.	Tricholomataceae	
52	128	16.8.2004	Namche	3150		*Suillus placidus*	Boletaceae	
53	129	16.8.2004	Namche	3065		*Amanita gemmata*	Amanitaceae	

54	130	16.8.2004	Namche	3150		*Suillus sibiricus*	Boletaceae	
55	140	16.8.2004	Namche	2900		*Lyophyllum* sp.	Tricholomataceae	
56	141	16.8.2004	Namche	3150	Pothi Karshya	*Collybia butyraceae*	Marasmiaceae	Locally edible
57	201	17.8.2004	Phurte	3585	Rato Martip	*Boletus* sp.	Boletaceae	Locally edible
58	202	17.8.2004	Phurte	3585	Bhale Chyau	*Amanita* sp.	Amanitaceae	
59	203	17.8.2004	Phurte	3585	Pakar Shyamo	*Russula metachroa*	Russulaceae	
60	205	17.8.2004	Phurte	3547		*Cortinarius* sp.	Cortinariaceae	
61	206	17.8.2004	Phurte	3546	E. Shyamo	*Gomphus clavatus*	Gomphaceae	Locally edible
62	207	17.8.2004	Phurte	3615	Che Shyamo	*Ramaria flava*	Ramariaceae	Locally edible
63	208	17.8.2004	Phurte	3615	Pothi Karshya	*Tricholoma saponaceum*	Tricholomataceae	
64	209	17.8.2004	Phurte	3615	Sindi Shyamo	*Russula* sp.	Russulaceae	Poisonous
65	210	17.8.2004	Phurte	3585		*Clararia acuta*	Clavariaceae	
66	211 A	17.8.2004	Phurte	3585		*Ramaria* sp.	Ramariaceae	
67	211 B	17.8.2004	Phurte	3585		*Cyathus striatus*	Nidulariaceae	
68	212	17.8.2004	Phurte	3885		*Hygrophorus* sp.	Hygrophoraeveae	
69	213	17.8.2004	Phurte	3885	Sindi Shyamo	*Russula* sp.	Russulaceae	Poisonous
70	221	20.8.2004	Khunde	3770		*Lycoperdon* sp.	Lycoperdaceae	
71	222	20.8.2004	Khunde	3830		*Coprinus* sp.	Coprinaceae	
72	227	20.8.2004	Khunde	3900		*Hygrocybe strangulate*	Hygrophoraceae	
73	229	20.8.2004	Khunde	3900		*Hygrocybe coccinea*	Hygrophoraceae	
74	230	20.8.2004	Khunde	3900		*Hygrocybe* sp.	Hygrophoraceae	
75	233	20.8.2004	Syanboche	3940		*Laccaria laccata*	Tricholomataceae	
76	234	20.8.2004	Khunde	3900	Sindi Shyamo	*Entoloma* sp.	Entolomataceae	Poisonous
77	237	20.8.2004	Khunde	3900	Kujir (Keti)	*Cantharellus cibarius*	Cantharellaceae	
78	238	20.8.2004	Khunde	3900		*Scleroderma* sp.	Sclerodermataceae	
79	240	20.8.2004	Khunde	3800	Fhe Shyamo	*Boletus* sp.	Boletaceae	Locally edible
80	241	20.8.2004	Khunde	3900	Kujir (Keta)	*Chroogomphus tomentosus*	Gomphidiaceae	
81	243	20.8.2004	Khunde	3900	Aallu Chyau	*Scleroderma citrinum*	Sclerodermataceae	Locally edible
82	250	21.8.2004	Khumjung	3800		*Boletus* sp.	Boletaceae	
83	251	21.8.2004	Khumjung	3800		*Pholiata nameko*	Strophariaceae	
84	258	21.8.2004	Khumjung	3800	Dhurkho Chyau	*Auricularia polytricha*	Auriculariaceae	Locally edible
85	259	21.8.2004	Khumjung	3800		*Auricularia* sp.	Auriculariaceae	
86	260	21.8.2004	Khumjung	3800	Tuk Shya	*Lactarius violaceus*	Russulaceae	
87	261	21.8.2004	Khumjung	3800		*Dermocybe phoenicea*	Cortinariaceae	
88	262	21.8.2004	Jamikhiau	3840		*Nyctalis* sp.	Tricholomataceae	
89	265	21.8.2004	Jamikhiau	3825		*Russula* sp.	Russulaceae	
90	267	21.8.2004	Jamikhiau	3825		*Lycoperdon* sp.	Lycoperdaceae	
91	268	21.8.2004	Jamikhiau	3825	Che Shyamo	*Clavulina cinerea*	Clavulinaceae	Edible
92	269	21.8.2004	Jamikhiau	3825		*Amanita* sp.	Amanitaceae	
93	270	21.8.2004	Jamikhiau	3825	Jip Chyambu	*Tylopilus eximus*	Boletaceae	
94	271	21.8.2004	Jamikhiau	3825	Dangba	*Paxillus involutus*	Paxillaceae	
95	273	21.8.2004	Jamikhiau	3825		*Cordyceps* sp.	Clavicipitaceae	
96	274	21.8.2004	Jamikhiau	3825		*Trichoglossum* sp.	Geoglossaceae	
97	275	21.8.2004	Jamikhiau	3825	Petok	*Unidentified*		Edible
98	279	21.8.2004	Jamikhiau	3860		*Coprinus radians*	Coprinaceae	
99	280	21.8.2004	Jamikhiau	3860	La Shyamo	*Hydnum repandum*	Hydnaceae	Edible
100	281	21.8.2004	Jamikhiau	3825		*Tyromyces* sp.	Polyporaceae	
101	300	22.8.2004	Khunde	3890		*Lactarius* sp.	Russulaceae	
102	302 A	23.8.2004	Lausasa	3770		*Stropharia semiglobata*	Strophariaceae	

103	302 B	23.8.2004	Lausasa	3570	*Cortinarius* sp.	Cortinariaceae
104	303	23.8.2004	Lausasa	3570	*Lactarius deliciosus*	Russulaceae
105	304	23.8.2004	Lausasa	3570		Polyperaceae
106	306	23.8.2004	Lausasa	3570	*Pleurotus dryinus*	Pleurotaceae
107	308	23.8.2004	Lausasa	3570	*Clitocybe* sp.	Tricholomataceae
108	311	23.8.2004	Lausasa	3570	*Amanita* sp.	Amanitaceae
109	312	23.8.2004	Lausasa	3570	*Ganoderma applanatum*	Ganodermataceae
110	320	23.8.2004	Lausasa	3570	*Gomphus floccosus*	Gomphidiaceae
111	321	23.8.2004	Lausasa	3570	*Polyporus* sp.	Polyporaceae
112	400	24.8.2004	Phungi Tenga	3340	*Clitocybe* sp.	Tricholomataceae
113	401	24.8.2004	Phungi Tenga	3340	*Tyromyces* sp.	Polyperaceae
114	403	24.8.2004	Phungi Tenga	3340	*Otidea onotica*	Pyronemataceae
115	404	24.8.2004	Phungi Tenga	3370	*Cortinarius* sp.	Cortinariaceae
116	405	24.8.2004	Phungi Tenga	3440	*Cortinarius* sp.	Cortinariaceae
117	407	24.8.2004	Phungi Tenga	3590	*Lycoperdon pyriforme*	Lycoperdaceae
118	408	24.8.2004	Thyangboche	3702	*Pholiota aurivella*	Strophariaceae
119	409	24.8.2004	Thyangboche	3702	*Clitocybe* sp.	Tricholomataceae
120	410	24.8.2004	Thyangboche	3702	*Coprinus micaceus*	Coprinaceae
121	411	24.8.2004	Thyangboche	3702	*Psathyrella* sp.	Coprinaceae
122	413	24.8.2004	Thyangboche	3645	*Russula paludosa*	Russulaceae
123	415	24.8.2004	Deboche	3700	*Marasmium* sp.	Marasmiaceae
124	420	24.8.2004	Deboche	3700	*Fomitopsis* sp.	Formitopsidaceae
125	421	24.8.2004	Deboche	3700	*Inocybe* sp.	Cortinariaceae
126	425	24.8.2004	Deboche	3700	*Lactarius* sp.	Russulaceae
127	427	24.8.2004	Thyangboche	3702	*Russula densifolia*	Russulaceae
128	430	24.8.2004	Deboche	3738	*Lycoperdon* sp.	Lycoperdaceae
129	450	24.8.2004	Deboche	3700	*Amanita pachycolea*	Amanitaceae
130	500	25.8.2004	Deboche	3665	*Russula* sp.	Russulaceae
131	501	25.8.2004	Deboche	3665	*Entoloma* sp.	Entolomataceae
132	502	25.8.2004	Deboche	3665	*Lactarius rolemus*	Russulaceae
133	504	25.8.2004	Deboche	3665	*Polypore*	Polyporaceae
134	505	25.8.2004	Deboche	3665	*Lycoperdon perlatum*	Lycoperdaceae
135	507	25.8.2004	Deboche	3665	*Pseudohydnum gelatinosum*	Tremellaceae
136	508	25.8.2004	Deboche	3665	*Polypore*	Polyporaceae
137	509	25.8.2004	Deboche	3665	*Clavaria* sp.	Clavariaceae
138	550	25.8.2004	Omakha forest	3890	*Pseudohydnum gelatinosum*	Tremellaceae
139	551	25.8.2004	Omakha forest	3890	*Hohenbuebelia* sp.	Tricholomataceae
140	552	25.8.2004	Omakha forest	3890	*Cortinarius traganus*	Cortinariaceae
141	553	25.8.2004	Omakha forest	3890	Unidentified	
142	554	25.8.2004	Deboche	3665	*Aleuria aurantia*	Pyronemataceae
143	570	26.8.2004	Deboche	3665	*Vascellum* sp.	Lycoperdaceae
144	571	26.8.2004	Deboche	3665	*Lycoperdon* sp.	Lycoperdaceae
145	572	26.8.2004	Deboche	3665	*Scutellinia scutellata*	Pyronemataceae
146	579	26.8.2004	Thyangboche	3445	*Coprinus neveus*	Coprinaceae
147	581	26.8.2004	Thyangboche	3445	*Coprinus* sp.	Coprinaceae
148	583	26.8.2004	Phungi tenga	3255	*Agaricus xanthodermus*	Agaricaeae
149	590	26.8.2004	Thyangboche	3445	*Peziza badia*	Pezizaceae
150	591	26.8.2004	Thyangboche	3445	*Cystoderma* sp.	Dermolomataceae

In comparison to forest types, it was found that Muse forest (Lukla) bears a maximum number of mushroom species (24 sps.) followed by Deboche forest (18 sps.), Phurte (15 sps.), Jami Khiau (13 sps.), Khunde (12 sps.), Jorsalle (12 sps.), Lausasa (10 sps.), Thyangboche (10 sps.), Fungi Tenga (7 sps.), Ghat (7 sps), Khumjung (6 sps.), Namche (5 sps.), Tok Tok (4 sps.), Pangboche (4 sps), Phakdin (2 sps.) and Syangboche (1 sp.) (Table 2).

In Nepal, the distribution of mushrooms in a particular locality depends on topographl soil type, rainfall and temperature, and vegetation pattern. The appearance and occurrence of mushrooms and their dominance are controlled by different factors such as altitude, phytogeography, etc. The mushroom specimens were found high on south and south west facing slope. The diversity of mushroom specimens was found high at an altitudinal range between 3500- 4000 m above msl followed by 2500-3000 m above msl and 3000-3500m above msl.

Between 3500-4000 m above msl, the dominant vegetation is mixed forest of *Abies, Rhododendron, Betula, Junipers.* Altogether, 84 species, belonging to 30 families were recorded within this range. The diversity of Russulaceae, Coprinaceae, Lycoperdaceae, Polyporaceae was found high. It was observed that the highest diversity of mushroom was recorded within this region. This may be due to cloud formation, which is a decisive factor for the vegetation of Nepal (Beug and Miehe 1999). The cloud zones are a source of moisture which creates microhabitats and favour high species richness (Rahbek, 1995). The cloud forest is rich in diversity (Falkenberg and Voltonilini 1994).

Similarly, between 3000-3500 m above msl, the main vegetation is the mixed forest of *Abies, Rhododendron, Pinus* and *Betula.* A total of 14 species of 11 families were recorded within this altitudinal range. The diversity of the family Boletaceae, Coprinaceae, Cortinariaceae, etc. was found high. The dominant or mixed forest types of *Rhododendron, Abies* and *Betula,* provide different micro-ecological condition suitable for the origin, development and growth of diverse mycotaxa such as saprohytes, parasites (Adhikari 2000). Similar types of result (eg. *Coprinus* sp., *Mycena* sp., *Nyctalis* sp., etc.) were recorded in the present study. Lower number of species (14 sps.) collected within this altitudinal range might be associated with steep cliff formation, having lesser number of trees, dry slopes and it's proximity to the main trail. Similar type of results was also obtained by Devkota (2005) at Lumle area.

Between 2500-3000 m above msl, the main dominant species is Pine. A total of 52 species belonging to 15 families were recorded. Within this ranges the diversity of Boletaceae was found high followed by Tricholomataceae, Amanitaceae and Strophariaceae, etc. The litter debris of the pine tree controls and regulates the temperature of soil moisture, texture and nutrition for the growth of Amanitaceae, Boletaceae, Russulaceae, Tricholomataceae (Adhikari 2000). Similar type of results was obtained in the present study (Fig. 2). The collection of high number of mushroom species within this range may be due to the presence of rich humicolous soil and different vegetation types *(Pinus wallichiana* and *Quercus* sp.). Such type of finding has been supported by Brooks (1969) and Arnoids (2001).

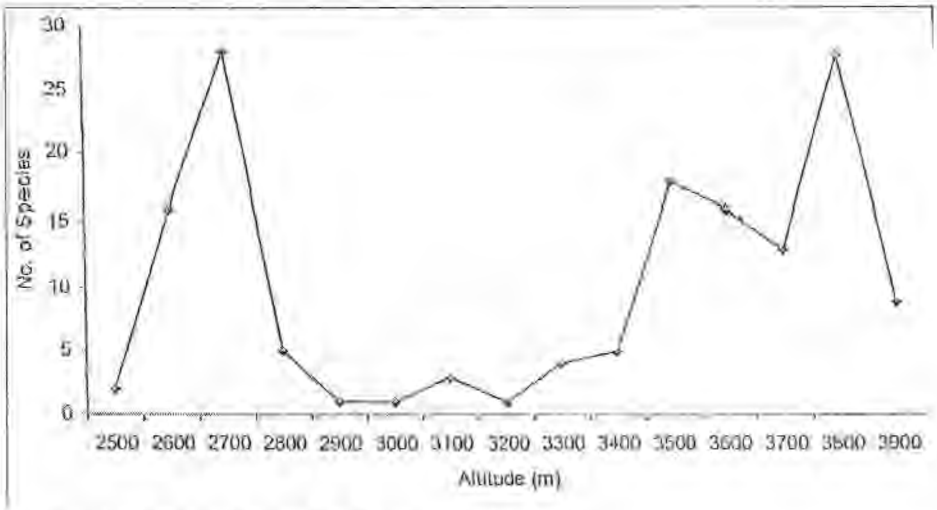


Fig. 2. Altitudinal distribution of collected mushroom species

Out of the 37 families and 65 genera, 26 families had only one genus while the remaining 11 families had more than one genus. (Fig. 3). The highest number of genera was found in the families Tricholomataceae and Boletaceae. Mushrooms differ in their temperature and humidity requirements and hence appear in the early or late rainy season. The total growing period of different species also depends on temperature and humidity requirements. It was observed that some species of mushrooms appear early in the lower altitude and later in the higher altitude. Temperature seems to be a deciding factor in this case. Similar type of observation was also made by Kumar *et al.* (1990) and Devkota (2005).

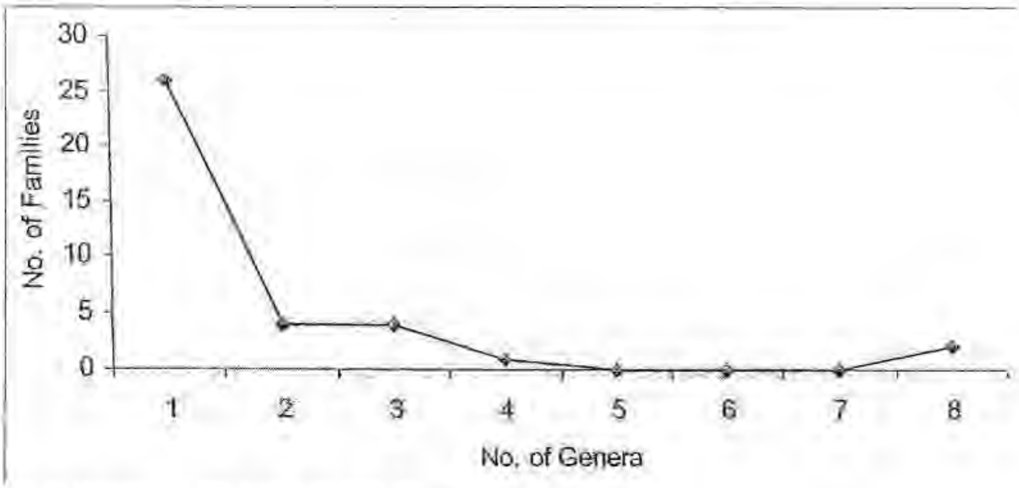


Fig. 3. Number of families with their respective genera

Conclusion

The fungi survive in adverse condition dlue to their mycelium, and drought resisting *structure* in nature. These structures allow them to appear in their season. The local people residing near the Ghat area (bullet zone) have been experiencing the loss of several species of mushroom that were found few years back. According to their views change in habit, urbanization and climate change could be the contributing factor for the loss of mushroom species. Loss of habitat poses an eminent threat to a large number of biological species including mushrooms. It was observed that vegetation inside the Sagarmatha National Park (SNP) has been well conserved as compared to the buffer zones. Different families as well as species of mushrooms were observed to grow in different ecological niches and altitudinal ranges. Conservation of natural habitat and regeneration of forest would be a major positive contribution to enrich the growth of mushrooms. Besides, community forests within the buffer zone should be managed in a sustainable manner. The study further helps to contribute for the documentation of the mushroom resources found in that region.

Domestication of the edible, medicinal and other species is necessary for sustainable socio-economic development. The edible mushroom is consumed by most of the local inhabitants. In a country like ours, where agriculture is the most dominant profession of the people, the agricultural waste is quite abundant. Most of the agricultural and forest waste are burnt or allowed to rot. The conversion of this waste, to some extent, can be utilized by growing edible mushrooms in the form of protein. In the world, only few mushrooms are cultivated commercially. To commercialize these wild edible mushrooms, it is vital to assess the nutritive value, conduct toxicity assay and develop cultivation techniques of the most prized wild edible mushroom of that region. Nepal Academy of Science and Technology (NAST) have already initiated research work in assessing the nutrient value of wild edible mushroom of this region.

Acknowledgements

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New Record of Fleshy Fungi from Khumbu Region, Nepal

Prabina Rana, Anjana Giri and Sanjib K. Shrestha

Abstract

Pulverboletus ravenelii (Berkeley and Curtis) Murrill, a wild mushroom of class Basidiomycetes recently collected from Ghat under coniferous forest (dominanted by *Pinus wallichiana)* on 14 Aug. 2004, at an altitude of 2604 metre above sea level (m asl) has been recorded as new to Nepal.

Introduction

The first exploration and collection of Nepalese fungi was initiated by J.D Hooker in 1848. Thereafter, Nepalese and foreign mycologists have contributed in this field. A previously undescribed Boletus encountered during a taxonomical and ecological investigation of the mycoflora of Khumbu region, is assigned to the genus *Pulverboletus* as *P. ravenelii.* The genus was kept under family Boletaceae. The specimen was collected from Ghat under the coniferous forest at an altitude of 2604 m asl. The area lies between Lukla and Phakdin (buffer zone of Sagarmatha National Park) at a longitude of 86°42.98' and latitude of 27°42.44'.

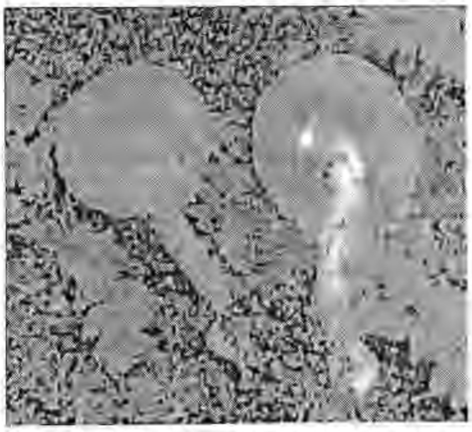
Materials and Methods

The specimen was collected from Ghat under the coniferous forest (dominated by *Pinus wallichiana)* on 14 Aug, 2004. The photographs of specimen were taken in its natural habitat. The morphological characters such as spore print and colour, habitat, pileus and tube colour, etc. were examined in the field. The specimen was first sun dried and taken to the laboratory of RONAST for microscopic study and identification. The measurement of size of spores, basidia, cystidia were taken by ocular micrometer. Identification was done on basis of literatures of Adhikari, M.K. 2000; Imazeki *et al.* 1988; Dilcinson, C. and Lucas, J. 1979; Miller, O.K. 1984; Svreck, M. 1975, Murrill, W.A. 1909, and Kreiger, H. 1967. The specimen is preserved at the RONAST Laboratory, Khumaltar.

***Pulverboletus ravenelii* (Berkeley and Curtis) Murrill**

Pileus 3.6-9.0 cm broad, convex to broadly convex; margin tucked in, entire, surface viscid to subviscid but typically overlain with a dry veil, tomentose to pulverulent due to velar layer, glabrous to appressed fibrillose when veil fragments disappear, yellowish color with fibrils of reddish brown, veil elements yellow in young basidiocarps, unchanging or becoming a paler yellow when old. *Context* 1-2 cm thick, whitish to pale yellow, slowly changing to blue when exposed sometimes becoming brown.

Tubes 0.5-1.5 cm long, adnate, bright yellow when young, becoming darker yellow with age, turning blue to greenish blue when bruised, sometimes changing to cinnamon-brown to blackish; *pores* 0.5-1.0 mm broad, round to angular, concolorous with tubes, bluing when bruised.



Stipe 6.4-12.0 cm long, 0.7-1.5 cm thick at the apex, equal or occasionally tapering toward the apex or irregular in shape, solid, with white to pale yellow rhizoids at the base; surface dry to moist. *Context* pale ochraceous to whitish except intensely yellow in base, unchanging or developing a pinkish tinge when exposed.

Nutritional Value of Some Wild Edible Mushrooms Collected from Sagarmatha National Park and its Adjoining Area

Prabina Rana and Anjana Giri

Abstract

Eleven wild edible mushrooms, *Ramariaflava* (Schaeff.: Fr.) Quel., *Paxillus involutus* (Batsch : Fr.), *Gomphus clavatus* (Pers. Fr.) S.F. Gray, *Gomphus floccosus* (Schw.) Singer, *Leccinum* sp., *Ramaria botrytis* (Pers.: Fr.) Ricken, *Hygrophorous* sp., *Tylopilus eximus* (Peck) Sing., *Chroogomphus tomentosus* (Murr.) O.K. Miller, *Amanita hemibapha* (Berk. & Br.) Sacc., *Boletus* sp. were studied for their chemical composition and nutritional value. These mushrooms have been consumed by the local people residing in the Sagarmatha National Park and its adjoining area for a long time, In this study, proximate composition; moisture, ash carbohydrate, crude fat, crude protein, crude fibre and minerals such as calcium, phosphorous and, iron found in these mushrooms were investigated. The results showed that some of the wild edible mushrooms that were investigated in the present study have nutritional values comparable to the common cultivated mushroom species like *Agaricus bisporus* (Lange) Imbach, *Pleurotus ostreatus* (Jacq. :Fr.) Kummer, *Lentinula edodes* (Berk.) Pegler, *Volvariella volvacea* (Bull.: Fr.) Singer, etc.

Introduction

Wild edible mushrooms are natural resource with a high nutritional and economic value. Attitudes towards these mushrooms are very different around the world. According to Boa (2004) about 1200 species of fungi are used in 85 different countries for their gastronomic value and medicinal properties. The use of mushrooms as food is probably as old as human civilization. They were preferred only for culinary characteristics, while the nutritive value of mushrooms was recognized much later (Crisan & Sands 1978; Garcha *et al.* 1993).

An edible mushroom contains high level of dietary fiber, substantial amount of protein, vitamins and minerals but is low in fat. They also have various health benefits such as antioxidative, antitumour and hypercholesterolemic effects (Wong & Cheung 2001). Therefore, edible mushrooms are regarded as an ideal health food. The chemical compositions of these mushrooms determine their nutritional value and sensory properties. It differs according to species but also depends on the substratum, atmospheric condition, age and part of the fructification (Manzi *et al.* 2001). In general edible mushrooms contain 160-350 (gm/kg dry weight) protein, 20-60 (gm/kg dry weight) fat and 280-339 (gm/kg dry weight) carbohydrate. They are the useful sources of iron, phosphorous, potassium, etc. The nutritional value of protein is usually very high in the majority of fungi (Ilievska & Petrovska 2000). Ogundana and Fagade (1981) cited by Adejumo & Awosanya (2003) indicated that mushroom consists of about 16.5% dry matter out of which 7.4% is crude fiber, 14.6% is crude protein and 4.48% is fat and oil.

In Nepal research has been concentrated in precommercial experimentation of commercially important edible mushrooms such as *Pleurotus ostreatus, Volvariella volvacea, Agaricus bisporus* and *Lentinula edodes*, etc. However, focus has not yet been centered on the potential wild edible mushrooms. The present study was undertaken to assess the nutritive value of prized wild edible mushrooms identified with the help of Sherpa community residing within the study area. The results of this study could further help to draw the attention of government and private institutions to develop cultivation practices of the most prized wild edible mushroom which have good nutritive value.

Study Area

The Khumbu valley is a territory along the Nepal and Tibet (China) border. The valley is a part of Solukhumbu district (northeastern region of Nepal). It encompasses the Sagarmatha national park (1,148 sq. km) and its buffer zone area (Fig. 1). The park includes the upper catchment areas of the Dudh Kosi and the Bhote Kosi rivers. Due to its altitudinal diversity, various types of vegetation are found in different climatic zones (temperate, subalpine, alpine, nival and permanent snow zones). The dominant vegetation at the lower elevation of the park below 3000 meter above sea level (m asl) is composed

Spore print yellowish brown. Spores 9-11 X 5.0-5.5 μm, ellipsoid, moderately thick walled, ochraceous in KOH.

Basidia 22.0-31.5 X 8.5-13.5 μm, four-spored, hyaline, claväte. Hymenium typically yellow in KOH. *Hymenial cystidia* 30-56 X 5-12 μm, scattered to numerous, clavate to subcylindric, thin-walled, hyaline to lemon yellow in KOH.

Specimen examined - Solitary in moist soil under pine forest of Ghat, especially in open areas among grasses. Generally found from July to September at an altitude of 2604 m asl. Not previously recorded, hence new to Nepal.

Distribution: Taiwan, China, Japan, Malaysia, North America

Acknowledgement

We are grateful to Ev-K2-CNR/RONAST for their full support. We are indebted to Mr. Morten Christensen, PhD scholar of the Royal Veterinary & Agricultural University, Denmark for his assistance in making the microscopic study and identification.

mostly of blue pine and hemlock forest. The lower subalpine region above 3000 m asl comprises of forests of *Pinus wallichiana, Abies spectabilis* and *Juniper recurva.* The upper subalpine, above 3600 m asl, consists of birch-rhododendron forest *(Betula utilis, Rhododendron campanulatum* and *R. campylocarpum)* and the lower alpine region above the timber-line at 3800-4000 m asl, houses scrubs *(Juniper* spp., *R. anthopogan & R. lepidotum).*

Materials and Methods

Collection of mushrooms

The prized wild edible mushrooms identified with the help of Sherpa community were collected from Lukla (2,800 m asl) to Pangboche (4,000 m asl) during the month of August to September 2006 (Fig 1). The fully matured mushroom species were collected from different parts of the study area by uprooting their substrata with the aid of a scalpel or sharp knife. The collected mushroom specimens were photographed in their natural habitat before they were picked up. Data on their habit and habitat, ecological parameters such as altitude, forest type, etc. were recorded in the field. Each collection was placed in butter paper bags and tag numbers were assigned to them. Morphological characters were recorded and chemical tests were performed in the field. The specimens were either sun dried or dried by placing them on tin foil over a local oven. Each dried mushroom specimen was placed in a separate butter paper bag. The specimens were identified in the laboratory at NAST, Khumaltar with the help of standard literature (Adhikari 2000, Imazeki & Hongo 1979, Purukayastha & Chandra 1985, Svreck 1983, McKenny 1971, Mcknight & Mcknight 1987) and studying the macroscopic and microscopic characters. The specimens have been housed at NAST.

Fig. 1. Map of the study area

Chemical analysis of mushrooms

Mushrooms, taxonomically and locally identified as prized wild edible items were collected, shed dried and further subjected to nutritional analysis. Proximate analysis (moisture, carbohydrate, crude protein, crude fat, crude fiber and ash) was performed at the Department of Food Technology and Quality Control in accordance with AOAC (1995). All the calculations were carried out on dry weight basis of mushrooms. The minerals such as phosphorous, calcium and iron were read on atomic absorption spectrophotometer.

Results and Discussion

Mushroom fructifications are composed of two basic parts: pileus and stipe which can take various shapes, size and colour (Szweykowska & Szweykowski 2003). The flesh-filled fungal fructifications differ in colour and consistency depending on the species (Deremek & Pilat 1988 cited by Bernas *et al.* 2006). The chemical composition of edible mushrooms determines their nutritional value. It differs according to species but also depends on the substratum, atmospheric conditions, age and part of the fructification (Pryzybylowicz & Donoghue 1988, Vetter 1994, Shah *et al.* 1997, Marizi *et al.* 2001).

Out of 26 wild edible mushrooms (Gin & Rana 2007), proximate and chemical analyses were concentrated only in 11 species. Mushrooms were selected on the basis of region-wise availability and popularity among local residents of the study area. The selected edible mushrooms are: *Ramaria flava* (Schaeff: Fr.) Quel., *Paxiltus involutus* (Batsch : Fr.), *Gomphus clavatus* (Pets.: Fr.), *Leccinum* sp., *Ramaria botrytis* (Pers.: Fr.) Ricken, *Hygrophorous* sp., *Gomphus floccossus* (Schw.) Singer, *Tylopilus eximus* (Perk) Sing., *chroogomphus tomentosus* (Murr) O.K. Miller, *Amanita hemibapha* (Berk. & Br.) Sacc. and *Boletus* sp. During analysis of mushroom samples nine parameters such as crude protein (%), crude fat (%), crude fibre (%), moisture (%), ash (%), carbohydrate (%), calcium (mg/100g), phosphorous (mg/100gm), iron (mg/100gm) were accomplished.

Chemical composition of wild edible mushrooms proximate analysis

Carbohydrates

Forest mushrooms differ greatly in carbohydrate content. A considerable proportion of the carbohydrate compounds occur in the form of polysaccharides with particles of different size fungal polysachharides are represented by glycogen and such indigestible forms as dietary fibres, cellulose, chitin, mannans and glucans (Manzi *et* at. 2000) which are important in the proper functioning of the human alimentary track. In the present study the values of carbohydrates range from a low of 31.02% in *Amanita hemibapha* to a high of 62.63% for *Chroogomphus tomentosus* (Table 1) on a dry weight basis.

Carbohydrates are the main components of mushrooms apart from water. Similarly, carbohydrate content in mushrooms is found to vary from 27.6- 71.1% on dry weight basis (Rautuvaara 1947, cited by Purkayastha 1985). The chemical analyses of these mushrooms demonstrate that they have respectable amount of food value. Similarly, in our results the amount of carbohydrate is higher as compared to other components.

Crude protein

Mushrooms are rich sources of proteins and amino acids. Most of the amino acids are found in mushrooms (Kurtzman 1978). Protein is an important component of dry matter of mushrooms and they constitute more than half of total nitrogen, and their contents depend, among other things, on the composition of the substratum, size of pileus, harvest time and species of mushroom.

In the present study, *Chroogomphus tomentosus* had the lowest level of crude protein (11.84%) while *Ramaria flava* had the highest level *(28.32%)* (Table I). Dried mushrooms in general contain 19-40% crude protein (Kurtzman 1978). In this study all mushrooms fall within this range except *Paxillus involutus* (16.46%), *Chroogomphus tomentosus* (11.48%) and *Ramaria botrytis* (16.96%). The results of crude protein show values comparable to some cultivated mushrooms like *Agaricus bisporus* (26.3%), *Pleurotus ostreatus* (10.5%), *Lentinula edodes (17.5%)* and *Volvariella volvacea* (29.5%).

Crude fat

The content of fat in mushrooms is low. They mainly contain unsaturated fatty acids (over 70%). According to Crisan and Sands (1978) the crude fat of mushrooms has representatives of all classes of lipid compounds including free fatty acids, monoglycerides, diglycerides, triglycerides, sterols, sterol esters and phospholipids.

In the present study, *Ramaria botrytis* had the lowest (0.39%), while *Amanita hemibapha* had the highest (6.48%) crude fat constituents (Table 1). The average fat content of mushrooms ranges from 2-8% of the dry weight, but it can vary from less than 1% to as high as 15-20% (Crisan & Sands 1978). The fat contents of three mushrooms, *Gomphus clavatus* (0.97%), *Leccinum* sp. (0.89%) and *Ramaria botrytis* (0.39%) are less than 1% while the remaining mushrooms fall within the average range. The results of fat content of mushrooms show values comparable with some cultivated mushrooms such as *Agaricus bisporus* (1.8%), *Pleurotus ostreatus* (1.6%) and *Volvariella volvacea* (5.7%).

Moisture

Moisture is the most variable component in the proximate analysis of mushrooms and is significantly affected by environmental factors such as temperature and relative humidity during growth and storage as well as by the relative amount of metabolic water which may be produced during storage (Crisan & Sands 1978).

In the present study, *Gomphus floccosus* had the lowest moisture content (8.30%) while *Hygrophorous* sp. had the highest (11.95). Whereas, *Ramaria flava* and *Gomphus clavatus* have more or less similar moisture content (Table 1). The moisture content of the fresh mushrooms varies between 85-95% and 90% may be considered as an average, while air dried mushrooms contain 10-12% moisture. In the present analysis the moisture content of dried mushrooms mostly fall under this range. The higher moisture contents in mushrooms promote favorable condition for microbial growth and enzyme activity.

Crude fibre

Edible mushrooms contain high level of dietary fibre. The importance of an adequate amount of fibre in human diet is emphasized by nutritionists. In the present study, *Hygrophorous* sp. had the lowest fibre content (7.73%) while *Tylopilus eximus* had the highest (19.84%) (Table 1).

According to Crisan and Sands (1978) the fibre content in almost all mushrooms is very high and varies from 3-32%. The range of crude fibre content in some of the mushrooms of our study are similar to those of cultivated mushrooms like *Agaricus bisporus* (8.0- 10.4%), *Pleurotus ostreatus* (7.5-8.7%), *Lentinula edodes* (7.3-8.0%) and *Volvariella volvacea* (4.4-1 3.4%).

Ash

The quantity of ash in different mushrooms varied from 13.24 to 28.88%. *Chroogomphus tomentosus* had the lowest ash content while *Paxillus involutus* had the highest ash content (Table 1).

Table 1. Proximate analyses of mushrooms collected from the study area

SN.	Mushroom species	Proximate constituents (%)					
		Crude protein	Crude fat	Moisture	Ash	Carbohydrate	Crude fibre
1	*Amanita hemibapha*	25.87	**6.48****	10.23	26.4	**31.02***	**13.37**
2	*Boletus* sp.	27.75	1.99	9.40	24.42	36.44	19,05
3	*Chroogomphus tomentosus*	**11.84***	2.42	9.69	**13.42***	**62.63****	8.78
4	*Gomphus clavatus*	22.68	0.97	10.86	18.44	47.05	8.69
5	*Gomphus Floccosus*	20.97	1.89	8.30*	16.36	40.35	14.02
6	*Hygrophorous* sp.	22.97	1.53	**11.95****	**19.18**	**40.05**	7•73*
7	*Leccinim* sp.	21.33	0.89	10.73	18.06	48.99	10.17
8	*Paxillus involutus*	16.46	3.37	10.27	28.88**	41.02	12.55
9	*Ramamia botrytis*	16.96	**0.39***	10.15.	23.5	53.32	7.97
10	*Ramaria flava*	28.32**	1.35	10.88	16.53	42.96	8.85
11	*Tylopilus eximus*	25.89	1.78	11.46	24.36	36.51	**19.84****

* Lowest value ** Highest value

Mineral contents

The fructifications of mushrooms are characterized by a high level of well assimilable mineral constituents (Mattila *et al.* 2001). According to Vetter (1994) the level of macro constituents such as sodium, potassium and phosphorous are constant, while the contents of calcium, magnesium and sulphur depend on the composition of the substratum. Mushrooms probably contain every mineral present in their growth substrate (Crisan & Sands 1978). Manning (1985) reported that a significant proportion of the recommended daily dietary need of phosphorus and iron can be supplied by the mushrooms. Mineral content of mushroom is generally higher than many fruits and vegetables (Manning 1985).

Calcium

In the present study, *Boletus* sp. had the lowest calcium content (1.82 mg/100 gm) while the *Gomphus floccosus* had the highest (33.09 mg/100 gm) (Table 2). The range of calcium content in these mushrooms is similar to cultivated species like *agaricus bisporus* (23 mg/l00 gm) and *Pleurotus ostreatus* (33 mg/100 gm).

Phosphorous

The present study showed that *Ramaria flava* had the lowest phosphorous content (62.51 mg/100 gm) while *Paxillus involutus* had the highest phosphorous content (944 mg/100 gm) (Table 2). The results show that some of the species are rich in phosphorous. This is in agreement with the value of some cultivated mushrooms like *Agaricus bisporus* (790-1425 mg/l00 gm) and *Lentinula edodes* (476 mg/100 gm).

Iron

The present investigation showed that *Leccinum* sp. had the lowest iron content (0.576 mg/100 gm) while *Amanita hemibapha* had the highest (307.26 mg/100 gm) (Table 2). The range of iron content in some mushrooms exhibit a comparable range with cultivated mushrooms such as *Agaricus bisporus* (0.2-19.0 mg/100 gm), *Pleurotus ostreatus* (15.2 mg/100 gm) and *Lentinula edodes* (8.5 mg/l00 gm).

Table 2. Mineral analyses of mushrooms collected from the study area.

SN	Mushroom Species	Minerals (mg/l00gm)		
		Calcium	Phosphorous	Iron
1	Amanita hemibapha	20.09	721.75	307.26**
2	Boletus sp.	1.82*	163.77	10.66
3	Chroogomphus tomentosus	4.01	264	2.81
4	Gomphus clavatus	26.22	389.49	25.37
5	Gomphus floccosus	33.09	518.9	54.56
6	Hygrophorous sp.	6.17	649.37	13.23
7	Leccinum sp.	6.10	480	0.576*
8	Paxillus involutus	5.40	944*	6.16
9	Ramaria botrytis	6.30	441	6.21
10	Ramaria flava	6.66	62.5l*	4.07
11	Tylopilus eximus	2.92	359	0.93

Conclusion

Wild edible mushrooms are a natural resource with high nutritional and economic value. The proximate and chemical analysis showed a difference in the chemical composition of different mushrooms. The chemical composition (crude protein, carbohydrate, crude fat, crude fibre, ash, iron, calcium and phosphorous) of these mushroom samples were comparable to some of the cultivated common edible mushrooms such as *Pleurotus ostreatus, Volvariella volvacea, Agaricus bisporus* and *Lentinula edodes* and the overall nutritional values of these mushrooms were good. The present study indicates that here seems to be a good potentiality for developing cultivation practices of these prized edible mushrooms. Government and private institutions should promote and develop cultivation practices of the wild edible mushrooms which have good nutrient value and are prized by the locals. Further, in depth study of these mushrooms on type of vitamins, amino acid profile, minerals, heavy metals and radioactive substance as well as toxicity assay are recommended.

Wild edible mushrooms play important roles in the local ecosystems in terms of decomposition of organic materials and formation of ectomycorrhizae with forest trees. Mushrooms are also important natural products supporting local economies of the region. Local people in the study area collect these prized mushrooms from different forests localities. Improper harvest of mushrooms can damage forest habitat through the effects of excessive foot traffic, which leads to forest compaction, damage to fungal mycelium and can affect the mushroom production.

Moreover, the local people have been experiencing decline of some edible mushrooms within the study area. Unmanaged harvesting and climate change could be a contributing factor for this decline. In order to ensure continued production of these wild edible mushrooms from their natural habitat effective conservation methods and proper harvesting techniques are needed.

Acknowledgements

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Effect of Various Organic Matters on Growth of an Arbuscular Mycorrhizal Fungus

Geeta Shrestha Vaidya, Keshav Shrestha, Buddhi R. Khadge, Hâkan Wallander and Nancy C. Johnson

Abstract

The success of plantations on the eroded slopes is highly dependent on the extent of mycorrhizal colonization of the plants. In this study we have investigated the role organic matter on growth of an arbuscular mycorrhizal (AM) fungus in eroded slopes in Nepal such as Baluwa forest of Kavre District. Different types of organic matters (five grams each of leaf powder of *Thitonia diversifolia, Eupatorium adenophorum, Lantana camara* and five grams of farm compost) and triple-superphosphate were mixed with 45 g of eroded soil collected from various sites with no vegetation. The eroded soil without amendments was used as control. The mixture was placed in mesh bags (50 µm mesh) that allowed fungal colonization. The mesh bags were buried around trees of *Bauhinia purpurea* and *Leuceania diversifolia* that had been planted at the eroded site. The mesh bags were harvested after 6 months and the amount of external mycelium of AM fungi in the mesh bags was quantified by analyzing the AM spores and measuring the density of AM spores. Lantana leaf powder showed better effect than other organic matters and control has showed poor effect.

Introduction

Mycorrhizal fungi are essential for plant establishment in degraded soils. Most plants live in symbiosis with mycorrhizal fungi that improves water and nutrient uptake in exchange for carbohydrates supplied by the plant. Arbuscular mycorrhizal fungi form symbiosis with grasses, herbs and nitrogen fixing bushes and trees. These fungi are especially important for phosphorus uptake and they produce a glycoprotein (glomalin), which acts as a glue that binds soil particles together to form water stable aggregates. Successful colonization of plants by mycorrhizal fungi is especially important in degraded soils where nutrient availability is low and where it is important with rapid plant establishment and plant growth to stabilize the soil (Shrestha 1999). In such soils the inoculum potential (e.g. spores) is low and it is essential to add the inoculum propagules and provide beneficial conditions (e.g. improve organic matter content of the soil) to the mycorrhizal fungi for successful plant establishment (Shrestha 1999, Shrestha Vaidya & Shrestha 2005).

The supply of carbon from the plant is the primary driving force for growth of arbuscular mycorrhizal (AM) fungi in soil but AM hyphae for specific components, in the soil ecosystem are limited. So organic matter is one which has been found to stimulte hyphal growth (St. John *et al.* 1983; Joner & Jakobsen 1995). The productivity of these soils is deteriorating due to accelerated surface erosion (Jagannath *et al.* 1998).

Many biotic and abiotic factors influence growth and biomass portioning of AM fungi. Farming systems (Boddington & Dodd 2000), soil moisture (Anderson *et al.* 1983), organic matter (Ryan *et al.* 1994), pH (Porter *et al.* 1987, Van Aarle *et al.* 2002) and temperature (Koske 1987) can influence the distribution of AM hyphae and spores. Managing soil could thus be a potential way to optimise proliferation of indigenous AM fungi (Boddington & Dodd 2000). Particularly, addition of organic matter can have a beneficial effect on the growth of indigenous AM fungi in nutrient-limited soils (Caravaca *et al.* 2002, Gaur & Adholeya 2002). Organic amendments enhance spore production (Johnson & McGraw 1988, Douds *et al.* 1997), extra-radical proliferation of hyphae (Joner & Jakobsen 1995) and improve colonization of roots (MuthnkumarandtJdain 2000). Ryan *et al.* (1994) attributed increased AM fungal biomass to the beneficial effects of organic matter on soil structure, water status and on synergistic microbial activities in the soil. Organic matter addition to the soil at eroded sites could thus be an approach to enhance the beneficial effect of AM fungi on soil stabilization and plant establishment. The eroded soils in Nepal are generally impoverished regarding soil organic matter and soils have low phosphorus availability due to high phosphorus fixing capacity (Brown *et al.* 2000).

The aim of this study was to investigate whether growth, spore formation and glomalin production by indigenous AM fungi at eroded sites in Nepal can be stimulated by the addition of organic matter or phosphorus amendments.

Materials and Methods

Eperimental site

A field experiment was conducted in Baluwa Forest of Kavre district in Central Nepal. This forest is situated 40 km east of Kathmandu city on the side of Arniko highway to the Tibetan border. This place was completely eroded as a result of land slide before but about five years ago it was planted with *Bauhinia purpurea* and *Leuceania diversifolia* which are important fodder plants in Nepal. Plantation was performed by the Fodder Department of Nepal Agricultural Research Council, Khumaltar. At the time of our experiment approximately 30% of the plants had survived.

Soil samples

Soil samples were collected from Bisankhunarayan. The site was degraded due to hatching and poaching and finally it become completely eroded due to heavy rainfall and landslides. Forty five grams of the eroded soil was put in nylon mesh bags and mixed with different types of organic matter. We used 5 g. of dried leaves and dried compost (10% by weight) as organic matter additions. Fully expanded leaves of three easily available agroforestry plant species namely *Tithonia diversfolia, Lantana camara* and *Eupatorium adenophorum* were collected from farmers' fields and compost was collected from a local farmer. We also included one treatment with rock phosphate. In this case 45 mg of triple superphosphate were mixed with 50 g of eroded soil. This represents approximately 40-50 kg phosphorus per hectare . The mesh bags that were used as controls were filled with *50* g of eroded soil without any amendments. In total, six treatments were included (control, *Tithonia diversifolia, Lantana camara, Eupatorium adenophorum*, compost and rock phosphate) The mesh bags were buried to a depth of about 10 cm where plant roots had the highest density.

Spore analysis

The AM fungal spores within 25 g of the soil and amendment mixtures inside the mesh bags were extracted, identified and quantified. Spores were extracted using wet sieving and sucrose density gradient centrifugation (Kinney & Lindsey 1987). Spores were mounted in polyvinyl alcohol on slides and examined using a compound microscope. Species were identified to species using taxonomic characteristics described in INVAM (2005) and Schenck and Perez (1990).

A sample of soil weighing 25 g was mixed with a substantial volume of water and then decanted through a series of sieves (750, 250, 100 and 50 micron) after allowing heavy soil particles to settle down for a few seconds. This washing and decanting process was repeated until the water was clear. Roots and coarse debris were collected on petridishes. Then the fine kaolin clay remaining in the last sieve (50 μ) was transferred to centrifuge tube then it was added with water in equal weight of each four tubes and then centrifuged it for 3 minutes at 2000 RPM. After this the supernatant and floating debris was discarded.

The next step involved resuspending the pellet in 50% sucrose by vigorously shaking the tightly stoppered tubes. The samples were then centrifuged for 1 minute at 2000 RPM to separate spores from denser soil components. Immediately after centrifugation, spores in the sucrose supernatant were poured onto the finest sieve (50 μ) and carefully washed with water to remove the sucrose.

After rinsing, the spores were washed onto a petridish thoroughly before vacuum filtration. In this process we used Millipore filter paper for spore counting.

Spores on microscope slides were squashed to reveal inner wall layers and then were put under dissecting microscope for identification. Identification were made following *Brundrett et al.* (1996) and Schenck & Perez (1990).

Results and Discussion

We studied the soil of this site for endomycorrhizae as well as chemical analysis including pH. From all these results we got the soil types very poor having only few AM spores (mean 40 spores per 250 g soil) and very low N, P, K, organic matter and pH.

Eroded soil had extremely low levels of N, P, K, pH and organic matter (Table 1). Addition of all types of organic matter had a strong positive influence on AM biomass especially during the wet season. The addition of organic matter had a positive influence on formation of AM spores. The mesh bags with compost, rock phosphate, or eroded soil contained significantly lower spore numbers and spore volumes compared to the three treatments with dried leaves. So in this study *Lantana* has shown better effect than *Tithonia* and *Eupatorium*. *Tithonia* has also shown good effect than *Eupatorium* but compost was better than triple superphosphate (Fig.1) and volume of the spores has been shown in Fig. 2.

Table 1. Nutrient analysis of eroded soil

Soil type	Total N (%)	P (kg/ha)	K (kg/ha)	Organic matter (%)	pH
Eroded Soil	0.41	0.67	1.712	73	4.0

N = Nitrogen, P = Phosphorus and K = Potassium

The mesh bags had the following amendments: control (no amendment), rock phosphate, dried leaves of *Tithonia diversifolia, Lantana camara and Eupatorium adenophorum* and farmers compost. Values are means (n 16) and bars represent SE.

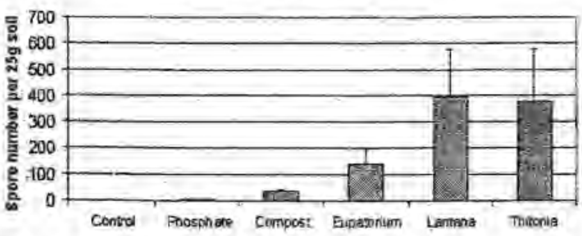

Fig. 1. Analysis of endomycorrhizal spores in each mesh bag having different organic matter

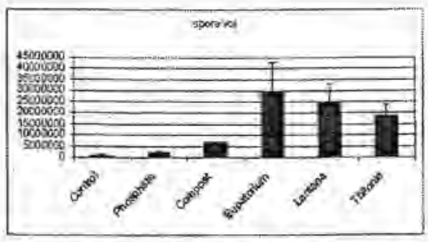

Fig. 2. Volume of AM spores in each mesh bag having different organic matters

In this study eight types of spores have been found: *Glomus hyaline, G. Amber, G. fasiculatum, G. aggrigate, G. canadense, Aculospora hyaline A. spinosa and A. scrobiculata.* Some spores have been shown in Fig. 3 and 4.

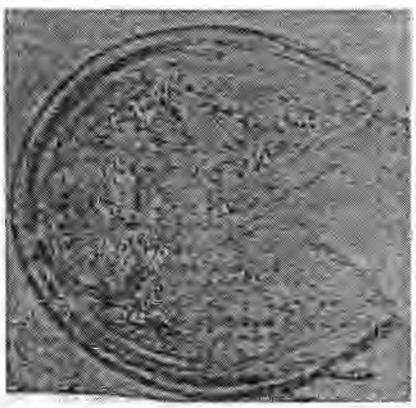

Fig. 3. *Acaulospora scrobiculata*

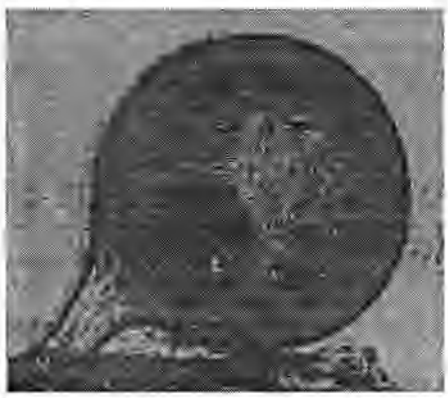

Fig. 4. *Glomus macrocarpum*

Use of nylon mesh bags remain popular in both natural and cultivated systems for decomposition studies involving comparisons of placement and management effects on mass loss and nitrogen and phosphorus release from organic materials (Bocock & Gilbert *1957*, Douglas *et al.* 1980, House *et al.* 1984, Holland & Coleman 1987 , Wagger 1989).

Nziguheba *et al.* (1998) reported that a high quality organic material such as *Tithonia* applied either alone or in combination with tri-superphosphate increased labile and moderately labile soil phosphorus over a 16 week period. Similar results have been found in this study. *Tithonia diversifolia* has shown better results than triple-supephospate similar results were reported by Jama *et al.* (1999). He has reported that the higher maize yield with *Tithonia* than mineral fertilizer.

Gachengo *et al.* (1999) had shown the comparison of crop yields, nutrient recovery, and soil extractable phosphorus and nitrogen obtained in the field trial reflected the differences in quality of the organic materials added as green manures. The higher yields for the first crop with applications of higher quality *Tithonia* are due to a combination of more phosphorus and nitrogen added and faster release patterns of phosphorus and nitrogen from *Tithonia* as compared to *Senna*.

We have seen that growth of AM in degraded soil is highly stimulated by the presence of organic matter. Different types of organic matters had similar positive influence on AM growth. Addition of rock phosphate on the other hand had no effect on AM growth. Number of AM spores was also positively influenced by organic matter addition. Added leaves of *L. camara* and *T. diversifolia* had a stronger positive influence on AM spore formation than other organic materials.

Joner and Jacobsen (1995) have shown beneficial role of organic matter which may also be related to an improvement of physical properties like increased soil porosity and reduced mechanical resistance to hyphal growth through the soil. Giovanetti and Avio (1985) found that additions of different materials, which increased the pore volume in soil, had a beneficial effect on mycorrhizal growth response, colonization and spore numbers which is related to our study.

Our results have shown that *Lantana* and *Tithonia* are better than other treatments. Similar findings were reported by Nziguheba *et al.* (2000). He reported that the effect of organic materials on phosphorus was dependent on the quality of the residue.

Acknowledgements

We would like to acknowledge to Dr. D.P. Serchan, Chief of Soil Division, Nepal Agricultural Research Council for providing *Tithonia* samples. Our thanks go to Mr. Purna Bahadur Tamang of Nepal Academy of Science and Technology, Khumaltar, Lalitpur for assistance in our study.

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Study of Biodiversity of Arbuscular Mycorrhizal Fungi in Addition with Different Organic Matter in Different Seasons of Kavre District (Central Nepal)

Geeta Shrestha Vaidya, Keshab Shrestha, Buddhi R. Khadge

Abstract

Different types of organic matter (dried and powdered 5 gm leaves of *Tithonia diversifolia, Eupatorium adenophorum, Lantana camara*, farmers compost) and triple-superphosphate were mixed with eroded soil collected from sites with no vegetation. Eroded soil without amendments served as control. The mixture was placed in mesh bags (50 µm mesh) that allowed fungal colonization but excluded roots. Two sets of mesh bags were buried around *Bauhinia purpurea* and *Leucaena diversifolia* that had been planted in an eroded site. The first set was buried in June 2003 and harvest in December 2003 (the monsoon period) and the second set was buried in December 2003 and harvested in June 2004 (The drier period). We found that more Arbuscular mycorrhizal (AM) fungal biomass and spores were produced during the wet season than during the dry season. The different types of organic matter had similar influence on the amount of AM biomass but the species composition was varied with the types of organic matter. In wet season nine species of AM spores and in dry season ten species of AM spores were found. In dry season *Scutellospora nigra* was found which was different from wet season.

Introduction

Nepal is very prone to soil erosion and is susceptible to sediment disasters mainly caused by slope failure, landslides, debris and bank erosion. There is an urgent need to control erosion and prevent potential sediment disaster in Nepal (Shrestha Vaidya *et al.* 2002). Therefore, mycorrhizae are essential for the establishment of tree seedlings and for their food growth and development in soils in low nutrients. Because mycorrhizae is beneficial to tree growth as they increase nutrient uptake and expose a greater absorbing surface (Shrestha Vaidya *et al.* 2002 and 2005).

The arbuscular mycorrhizal (AM) symbiosis is an association between most terrestrial plants and a class of fungi (Glomeromycota) which occurs in the roots of host plants (Schussier *et al.* 2001). AM fungi are normally considered to improve plant mineral nutrition in particular, phosphorus (P), water uptake, and resistance to root pathogens (Smith & Read 1997).

Addition of organic matter such as green manure is a common practice to improve soil nutrient content and soil structure. Organic residues from plants such as *Tithonia diversfolia* and *Lantana camara* have been found to be especially beneficial since they are reported to have a high content of N and P, which is mineralized rapidly from the organic material. Nziguheba *et al.* (2000) found that P is released more rapidly from such organic residues than from triple superphosphate.

Successful colonization by mycorrhizal fungi is especially important in degraded soils where nutrient availability is low; furthermore, AM fungi improve soil structure because they produce extraradical hyphal networks and their hyphae contain and release glomalin, which is a putative glycoprotein, assayed from soil as glomalin related soil protein (GRSP) that is correlated with aggregate water stability (Wright & Upadhyaya 1998, Rillig 2004; Rillig & Munimey 2006). Improved soil structure increases water infiltration and can reduce soil erosion (Tisdall & Oades 1982).

Many biotic and abiotic factors influence growth and biomass partitioning of AM fungi. Farming systems (Boddington & Dodd, 2000), soil moisture (Anderson *et al.* 1983), organic matter (Ryan *et al.* 1994), pH (Porter *et al.* 1987; vanAarle *et al.* 2002) and temperature (Koske 1987) are all examples of factors that can influence the distribution of AM fungal hyphae and spores. Managing soil could thus be a potential way to optimise proliferation of indigenous AM fungi (Boddington & Dodd 2000). Particularly, addition of organic matter can have a beneficial effect on the growth of indigenous AM fungi in nutrient limited soils (Caravaca *et al.* 2002; Gaur & Adholeya 2002). Organic amendments enhance spore production (Johnson & McGraw 1988; Douds *et al.* 1997), extra radical proliferation of hyphae (St. John *et al.* 1983; Joner & Jakobsen, 1995), and improve colonization of roots (Muthukuniar & Udaiyan 2000). Giovanetti & Avio (1985) suggested that this beneficial effect might be related to increased pore volume in soil which has a beneficial effect on AM colonization the mycorrhizal growth response and AM spore numbers. Furthermore, Ryan *et al.* (1994) attribute increased AM fungal biomass to the beneficial effects of organic matter on soil structure, water status, and on synergistic microbial activities in the soil. Organic matter addition to the soil in eroded sites could thus be an approach to enhance the beneficial effect of AM fungi on soil stabilization and plant establishment.

Material and Methods

This field experiment was conducted in Baluwa Forest Kavre district in Central Nepal.

This forest is situated 40 km East of Kathmandu city on the side of Arniko highway to the Tibetan border. The study site was completely eroded as a result of a land slide in 1998 it was planted with *Bauhinia purpurea* and *Leucaenia diversifolia* which both are important fodder plants in Nepal The Fodder department of the National Agriculture Research Council, Khumaltar, Nepal, performed Plantation. At the time of our experiment approximately 30% of the plants had survived. The soil at the experimental site is in this area are dominated by Rhodustults and Hapolustults (both members of the Ultisols soil order) (Brown et al. 2000). The chemical characteristics of the soil have been examined earlier by the Division of Soil Science, Nepal Agricultutal Research Council (NARC). It was found that the soil was acidic (pH 4.0) and poor in nutrients and in organic matter. The total content of N, measured by the Kjelldahl method, was 4.1 mg g^{-1} and the organic matter content was 0.73%. The low pH and the high content of Al and Fe in these soils suggest a very high P fixing capacity G. Shrestha Vaidya *et al.* 2007).

Experimental design and methods

Growth of AM fungi under field conditions was estimated with ingrowth mesh bags, similar to a design used earlier to estimate growth of mycorrhizal fungi in forests (Wallander *et al.* 2001), sand dunes (Olsson & Wilhelmsson 2000), (G. Shrestha Vaidya *et al.* 2007) and pre-Saharan desert shrubland ecosystems (Labidi *et al.* 200x). The mesh bags were constructed of nylon mesh (50 □m mesh size) to allow fungal colonization but excluded roots, because the latter cannot penetrate the mesh. These bags were used to clearly separate hyphal from root effects in the field. The mesh bags were filled with eroded soil mixed with different forms of organic matter or rock phosphate (see below).

The eroded soil was collected from a degraded site at Bisankhu Narayan (Godavari) in Nepal. This site was completely eroded due to a landslide after heavy rainfall and no vegetation was present on the site. Forty five gm of eroded soil were placed in nylon mesh bags and mixed with different types of organic matter or left unmixed as control: 5 gm of dried leaves or dried compost (10% by weight) was used as organic matter additions. Fully expanded leaves of three common agroforestry plant species *(Tithonia diversifolia, Lantana camara* and *Eupatorium adenophorum)* were collected from border rows in a farmer's fields and from roadsides and the compost were collected from a local farmer. The compost was made from disposed vegetable waste, cow dung, straw and husk. One of the treatment Triple-superphosphates. In this case 45 mg Triple-superphosphate was mixed with 50 gm of eroded soil. This represents approximately 40-50 kg P per hectare which is an amount usually used by local farmers. The mesh bags that were used as controls were filled with 50 gm of eroded soil without any amendments. In total, 6 treatments were included (control, *Tithonia diversifolia, Lantana camara, Eupatorium adenophorum*, compost and Triple superphosphate).

The experiment lasted for one year: one set of mesh bags was buried from June 2003 through December 2003 (the monsoon period) and a second set was buried in the same locations from December 2003 through June 2004 (the dry period). During the dry period some rain was recorded in January (2- 3 days). Plenty of rain was recorded during the wet season although no estimates of the amounts were made. Mesh bags containing each of the six treatments were buried 10 cm from the base of eight *Bauhinia purpurea* trees and eight *Leucaena diversifolia* trees (approximately 1.5-2m high) for a total of eight replicates in each tree species. A total of 192 bags were used (2 harvests x 6 treatments x 2 tree species x 8 replicates). Spore production differed between tree species and the tree species are therefore separated in the spore analysis. The mesh bags were buried to a depth of about 10 cm where the density of roots was high (Shrestha Vaidya *et al.* 2007).

Elemental analysis of Plant and Soil material

The fresh leaves of the plant species were air dried and ground to pass a 0.5 mm sieve. The concentrations of Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn of dried plant leaves, dried compost and eroded soil were analysed with ICP-AES. The C and N were analysed with an elemental analyser (Elementar Analysensysteme GnmH. Model vario MAX CN).

The AM fungal spores within 25 gm of the soil and amendment mixtures inside the mesh bags were extracted, identified and quantified. Spores were extracted using wet sieving and sucrose centrifugation (McKenney & Lindsey 1987). Spores were mounted in polyvinyl alcohol on slides and examined using a compound microscope. Species were identified to species using taxonomic characteristics described on the INVAM website (http://invam.caf.wvu.edu)) and Schenck & Perez (1990). Several spore samples from the first harvest were lost, especially from the plots with *Leucaena diversfolia* trees. In total 43 spore were analysed from the first harvest (wet season) and 96 from the last harvest (dry season) (G. Shrestha Vaidya *et al.* 2007). The potential to find effects of the different organic material on spore composition is therefore smaller for the first harvest compared to the second harvest.

Results

The eroded soil that was used in the mesh bags had extremely low levels of C, N, P, K, Ca, and Mg and high levels of Fe and Al. The dried leaf material from the three agro forestry species that was used as organic amendments in the mesh bags differed somewhat in chemical content (Table 1). *Eupatorium* and *Tithonia* appeared to be more similar while *Lantana* had lower N and P content. The compost had considerably higher P content than the dried leaf material (Table 1).

Table 1. Chemical composition mg gm^{-1} of the eroded soil and the different organic amendments used in the mesh bags. Values are one measurement of a pooled and well mixed sample of each substrate.

Sample type	C	N	P	C:N	C:P	K	Ca	Mg	S	Al	Fe
Thitonia diversifolia	441	33.2	2.8	13.2	158	34.1	13.4	3.1	1.8	0.2	0.2
Lantana camara	414	28.6	1.7	14.5	243	12.2	26.3	2.9	2.6	0.7	0.6
Eupatorium adenephorum	464	36.7	2.6	12.6	178	22.6	14.8	2.2	2.0	0.5	0.6
Farmers compost	247	22.3	17.6	11.0	14.0	14.7	15.5	11.8	4.1	2.2	2.8
Eroded soil	1.4	0.11	0.3	12.4	0.4	1.0	1.1	0.6	0.03	12.5	42.3

The compost contained much more P than the leaves from the agro forestry plants AM fungi produces significantly lower amounts of spores in the mesh bags with compost compared to the other treatments, which may indicate that spore formation was inhibited by the high P level in the compost. We found no effect of triple-superphosphate addition and the effect of triple-superphosphate addition on spore formation cannot be evaluated since spore formation in mesh bags without organic matter addition was almost absent. More spores were found in wet season than dry season but in dry season one species *Scutellospora nigra* was found but not found in wet season. In both seasons number of spores is more *in Lantana camara* than other organic matter (Fig. 1 & Fig. 2). The different number of AM spores in different organic matter was found different in number (Fig. 3 to Fig. 11). The addition of compost or green manure is an important way to improve the soil in degraded areas since nitrogen and other nutrients, as well as organic matter which improves soil structure, is added with the organic material (Caravaca *et al.* 2002; Muthukurnar & Udaiyan 2000, Nziguheba *et al.* 2000).

Analysis of Endomycorrhizal fungi (AM spores) in different seasons

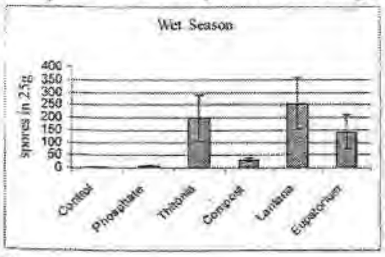


Fig. 1.

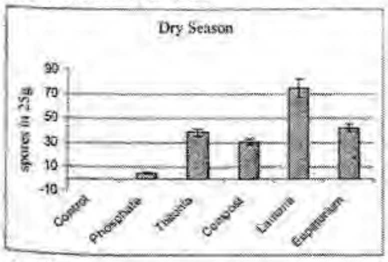


Fig. 2.

Analysis of different Endomycorrhizal fungi (AM spores) in different organic matter

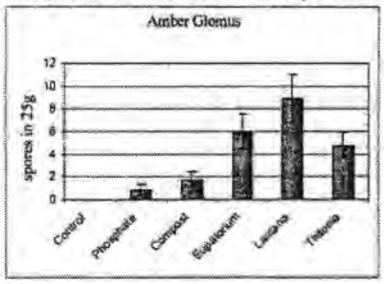


Fig. 3

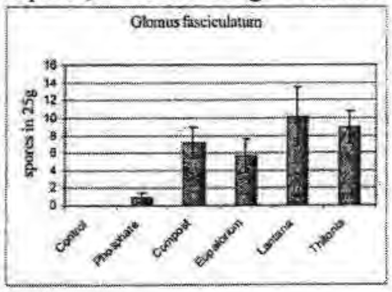


Fig. 4

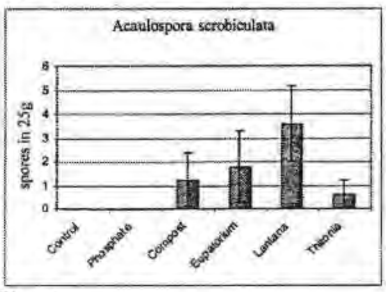


Fig. 5

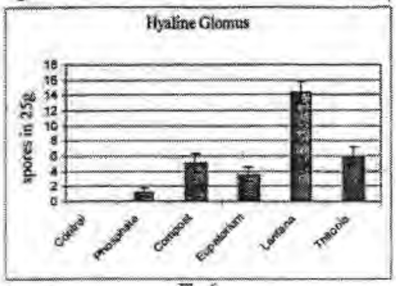


Fig. 6

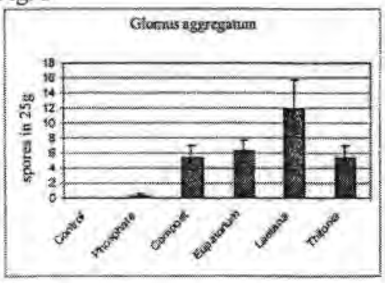
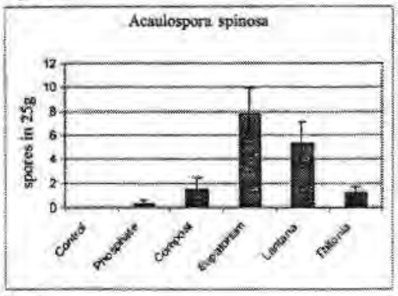


Fig. 7



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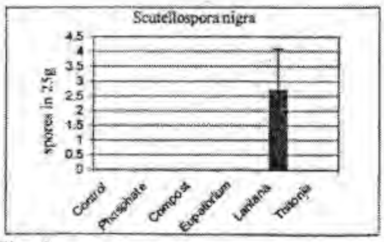

Fig. 9

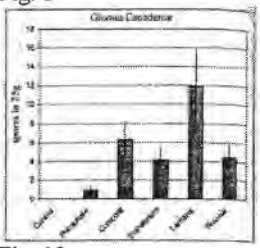

Fig. 10

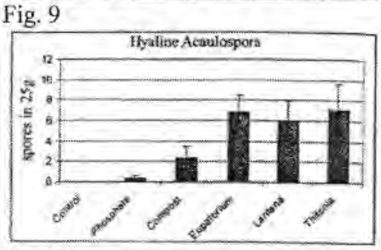

Fig. 11

Discussion

The use of ingrowth mesh bags was found to be a successful way of measuring recently produced AM fungal biomass and spores in eroded slopes of Nepal vegetated with *Bauhinia purpurea* and *Leucaena diversifolia*. Other methods for estimating biomass of AM fungi in soil such as extraction of hyphae or spores (Boddington & Dodd 2000) or estimates of biochemical markers directly in soil samples (Olsson *et al.* 1999) includes an unknown fraction of dead or inactive AM biomass. The production of recently formed extra radical AM mycelia is an important parameter since it can be directly related to the capacity of the plants to take up nutrients and to improvements of the soil structure and stability in degraded soils.

Similar results were found by adding compost made of Acacia cyanophylla leaves to eroded soil in Acacia tortilis savanna in pre-Saharan areas in Tunisia (Labidi *et al.* 2006). The positive effect of organic matter addition on AM growth could be an effect of higher humidity in mesh bags with organic amendments, since the addition of organic matter has a beneficial effect on soil structure and waterholding capacity (Ryan *et al.* 1994). The added organic matter could also increase the soil porosity and decrease the mechanical soil resistance to the growth of AM hyphae (Joner & Jakobsen 1995).

Improved nutrient and water uptake by the planted trees can be expected in response to better AM growth and the positive effect on the growth of AM fungi is in good and found that organic matter addition increased AM fungal hyphal growth (Labidi *et al.* 200x; Nicolson 1959; Koske *et al.* 1975; Joner & Jakobsen 1995) and AM spore formation (Douds *et al.* 1997; Baby & Marnbhushanrao 1996; Muthukumar & Udaiyan, 2000; Gryndler *et al.* 2002; Harinikumar & Bagyaraj 1989; Jamil Mohammed *et al.* 2003; Jeffries & Barea (2001). In addition, St John *et al.* (1983), Frey & Ellis (1997) and Friberg (2001) found that AM fungal hyphae grew best in soils with a high amount of organic matter. The highest VAM fungal population was recorded in November and decreased thereafter. The low VAM fungal population in January and February could be due to low soil moisture (Khadge 1988). Same way in our study also we got more spores in wet season than dry season.

The present study provides the first information on a stimulating effect of organic material addition on extra-radical growth of AM fungi in eroded slopes in Nepal (G. Shrestha Vaidya *et al.* 2007). These results show that organic matter addition can improve AM spores as well as plant survival in such areas.

Acknowledgement

We would like to thank the Swedish International Development Cooperation Agency, the National Science Foundation (DEB 0316136), and we are grateful to Dr. D.P. Serchan, Chief of Soil science Department, NARC for providing *Lithonia* samples and also thanks to Dr. Pariyar chief of fodder Department, NARC . Our special thanks goes to Mr. Purna Bahadur Tamang, Lab assistant of NAST.

Some Higher Fungi from Sagarmatha National Park (SNP) and its Adjoining Areas, Nepal

Anjana Giri and Prabina Rana

Abstract

The present paper includes a list of 69 species of wild mushrooms collected from Sagarmatha National Park (SNP) and its adjoining areas, which are recorded for the first time from this area.

Introduction

The investigation and study on mushrooms of Nepal started since the period of J.D Hooker (1848-1850), from which Berkeley (1854) described 44 species of higher fungi. Since then many authors like Balfour and Browne (1955 & 68); Imazeki *et al.* (1966); Kreisel (1969 & 1976); Singh *et al.* (1977/78); Pandey (1976); Adhikari (1976, 1984, 1987, 1988abc, 1990, 1991, 1996ab, 1999ab, 2000, 2001); Adhikari and Manandar (1986, 1996, 1997, 1998, 2004ab); Ryvarden(1977); Adhikari 7 Parajuli (1993, 1996); Bhandary (1984 &1991); Otani (1982); Hjortstam *et al.* (1984); Cotter (1987); Pandey (1976); Pandey & Budathoki (2003) and Devkota *et al. (2005)* have contributed their knowledge on Nepalese mycoflora.

Study Area

The study area lies in Solu-Khumbu district of the northeastern region of Nepal. It encompasses the Sagarmatha National Park (1148 sq. km) and its Buffer Zone area (275 sq. km) (Fig. 1) .The Park includes the upper catchments areas of the Dudh Kosi and Bhote Kosi Rivers. Due to its altitudinal diversity, various type of vegetation is found in different climatic zones (temperate, subalpine, alpine, nival and permanent snow zones). The dominant vegetation at the lower elevation of the park below 3000 meter above sea level (m asl) is composed mostly of blue pine and Hemlock forest. The lower subalpine region above 3000 m asl comprises *Pinus wallichiana, Abies spectabilis* and *Juniperus recurva* forests. The upper subalpine, above 3600 m asl consists of Birch - Rhododendron forest *(Betula utilis, Rhododendron campanulatum* and *R. campylocarpum)* and the lower alpine region above the timber-line at 3800-4000 m asl houses scrubs *(Juniperus* spp, *R. anthopogan & R. lepidotum)*.

Fig. 1. Map showing the study area

Materials and Methods

The mushroom specimens were collected from Lukla (2,800 m asl) to Pangboche (4,000 m asl) during the month of August to September 2004. The mushroom specimens were collected by carefully digging them with the help of a sharp knife. Data on habit and habitat such as ecological parameters such as altitude forest type, etc. were recorded in the field. The collected mushroom specimens were photographed in their natural habitat before they were picked up. Each collection was placed in butter paper bags and tag numbers were assigned to them. The morphological characters such as spore print and colour, habitat, forest type, pileus and tube colour, colour change induced after bruising or cutting and exposing to chemicals such as potassium hydroxide (KOH), Ferrous sulphate ($FeSO_4$) were noted. The specimens were either sun dried or dried by placing them on tin foil over a local oven. Dried specimens were placed in butter paper bags. Naphthalene balls were used as insect repellents. The dried specimens were identified in the laboratory with the help of standard literatures (Adhikari 2000; Imazeki, Otani & Hongo 1979; Purukayastha & Chandra 1985; Svreck 1975; McKenny 1971; McKnight & McKnight 1987) and studying macro and microscopic characters. The specimens are housed in Nepal Academy of Science and Technology (NAST), Khumaltar.

Enumeration of species

A total of 150 mushrooms specimens belonging to 37 families and 65 genera were collected from Lukla to Pangboche. Out of the collected specimens expect five species, all were identified upto the generic level and 69 were identified upto species level. The identified 69 species are enumerated as follows.

Agariaceae

1. *Agaricus xanthoderma* Genevier

Growing on soil in shady places, under *Pinus* forest, Phungi tenga, 3255m, 26 .8.2004;

Rana, Giri & Shrestha, no. 583, NAST.

Distribution: Ireland, Africa, Nepal.

Amanitaceae

2. *Amanita hemibapha* (Berk. & Br.) Sacc.

On moist soil, under mixed forest *of Rhododendron* and *Tsuga*, Muse Forest, Lukla, 2783m, 13 .8.2004;

Rana, Giri & Shrestha, no. 20, NAST.

Previously recorded from Manichur; Suryabinayak, Dakshinkali, Kakani, Nagarkot; Brajayogini and Kathmandu valley ((Adhikari 1976-1 996a) and amongst grasses under tree, Sundarijal (Singh & Nisha 1976a) (Pandey 1976).

Distribution: Japan, Malaysia, Singapore, Borneo, Java, Nepal.

3. *Amanita gemmata* (Fr.) Gill.

[= *Amanita junquillea* Quel.; *Amanitopsis adnata* (W.G. Smith) Sacc.]

Growing on dry soil in open area, Namche, 3065m, 16.8.2004;

Giri, Rana & Shrestha, no. 129, NAST.

Distribution: Japan, Europe, America, Nepal.

4. *Amanita vaginata* (Bull.: Fr.) Vitt.

[= *Amanitopsis vaginata* (Bull) Roxb.]

Growing on moist soil, under *Tsuga* and *Rhododendron* forest, Muse Forest, Lukla, 2783m, 13.8.2004; Rana, Giri & Shrestha, no. 21, NAST.

Previously recorded from Manichur; Suryabinayak; Dakshinkali; Kakani; Nagarkot; Brajayogini; Kathmandu valley (Adhikari 1976-1996a) and amongst grasses under tree, Sudarijal (Singh & Nisha 1976a) (Pandey 1976).

Distribution: Australia, Europe, NorthAmerica, Japan, India, China, Nepal.

5. *Amanita pachycolea* (Bull.: Fr.) Vitt.

On moist soil, under *Rhododendron* Forest, Debuche, 3700 m 24.S.2004;

Rana, Giri & Shrestha, no.450, NAST.

Distribution: Japan, Europe, Nepal.

Auriculariaceae

6. *Auricularia polytricha* (Mont.) Sacc.

Growing on moist soil under *Pine* forest, Khumjung, 3800m, 22.8.2004;

Rana, Giri & Shrestha, no. 258, NAST.

On tree branch, Tamrang khola (Balfour-Browne, 1968).

Distribution: World wide.

Boletaceae

7. *Boletus auripes* Peck

Growing on moist soil, under *Rhododendron & Quercus* forest, Muse Forest, Lukla, 2783m 13.8. 2004;

Rana, Giri & Shrestha, no. 33, NAST.

Distribution: North America, Japan, Nepal.

8. *Boletus edulis* Bull.: Fr.

Growing on moist soil, under mixed forest of *Pinus* and *Tsuga,* Muse Forest, Lukla, 2783m, 13.8. 2004;

Giri, Rana & Shrestha. no. 101, NAST.

Previously recorded form Kakani (Pandey, 1976); Syabru (Cotter, 1987) and Suryavinayak (Adhikari *et al.* 1996).

Distribution: Worldwide.

9. *Boletus pulverulentus* Opat.

Growing on moist soil, under mixed forest *of Rhododendron* sp. and *Tsuga sp.,* Muse Forest, Lukla, 2783m, 13.8.2004;

Rana, Giri & Shrestha, no. 22, NAST.

Distribution: Taiwan, North America, Europe, Nepal.

10. *Boletinus asiaticus* Sing.

Growing on moist soil, in *Pinus* forest, Tok Tok, 2725m, 15.8.2004;

Rana, Giri & Shrestha, no. 84, NAST.

Distribution: Japan, China, North America, Taiwan, Nepal.

11 .*Boletinus cavipes* (Klotz.: Fr) Kalchbr.

Growing on moist soil associated with mosses, under *Pinus* forest, Phakdin, 2500m, 14.8.2004;

Rana, Giri & Shrestha, no. 70, NAST.

Place not mentioned (Singh, 1966).

Distribution: Europe, Japan, China, North America, India, Nepal.

12. *Gyrodon merulioides* (Schw.) Sing.

Growing on moist soil in open place near to *Rhododendron Quercus* forest, Muse forest, Lukla, 2783m, 13.8.2004;

Giri, Rana & Shrestha, *no.* 32, NAST.

Distribution: Europe, North America, Australia, South Africa, Nepal.

13. *Phylloporus bellus* (Mass.) Corner

Growing on dry soil at steep wall under Coniferous forest, Lukla/Ghat, 2604m, 14.8.2004;

Giri, Rana & Shrestha, no. 42, NAST.

Distribution: Japan, Malaysia, Singapore, Nepal.

14. *Pulverboletus ravenelli (Berkeley &* Curtis) Murrill

Growing on moist soil under Coniferous forest, Lukla/Ghat 2604m, 14.8.2004;

Rana, Giri & Shrestha, no. 43, NAST.

Distribution: Europe, North America, South America, Nepal.

15. *Suillus placidus* (Bonord.) Singer

Growing on moist soil under *Pinus - Daphnea* forest, Namche, 3150m, 16.8.2004;

Rana, Giri & Shrestha, no. 128, NAST.

In pine forest, Chautara, Daman, Nagarkot and Syabru (Cotter, 1987).

16. *Suillus sibiricus* (Singer) Singer

Growing on rocky soil in open area, near to *Pinus* forest, way to Namche, 3150m, 16.8.2004;

Giri, Rana & Shrestha, no. 130, NAST.

In Pine forest, Ghasa, Godavari, Kakani, Larjung, Lete, Syabru (Cotter 1987) and Pisang (Bhandary 1991).

Distribution: Europe, Japan, Nepal.

17. *Tylopilus alboater(Peck)* Sing.

Growing on steep wall on moist soil, under Coniferous forest, between Lukla to Ghat, 2604m, 14.8.2004;

Giri, Rana & Shrestha, no. 50, NAST.

Distribution: East Asia, North America, Europe, Nepal.

18. *Tylopilus eximus* (Peck) Sing.

Growing on moist soil, under pure *Rhododendron* forest, Jamikhiau, 3825m, 22.8.2004;

Giri, Rana & Shrestha, no. 270, NAST.

Distribution: East Asia, Costa Rica, North America, Europe, Nepal.

Cantharellaceae

19. *Cantharellus cibarius* (Fr.: Fr) Fr.

Growing on moist soil, in *Rhododendron* forest, Khunde, 3900m, 20.8.2004;

Rana, Giri & Shrestha, no.237, NAST.

In *Pinus roxburghii* forest, on soil, Bajrajogini (Manichur) (Adhakari 1976); Manang (Bhandari 1991); Sudarijal and Kathmandu market (Adhikari 1996a and Adhikari *et al.* 1996) and on soil in *Pinus roxburghii* forest, Lumle (Devkota 2005).

Distribution: Worldwide.

Clavariaceae

20. *Clavaria acuta* Sch.: Fr.

Growing on moist soil, under *Abies* forest, Phurte, 3585m, 17 8.2004;

Giri, Rana & Shrestha, no. 210, NAST.

On soil in *Castanopsis* forest, Godavari (Adhakari 1988b); in mixed forest, Suryavinayak and Sundarijal (Adhakari 1996; Adhikari *et al.* 1996) and on soil of *Rhododendron arboreurn,* Bhirmuni, Lumle (Devkota 2005).

Distribution: Europe, Australia, North America, USSR, Japan, India, Nepal.

Clavulinaceae

21 . *Clavulina cinerea* (Bull.:Fr.) Schroet.

(= *Clavaria cinerea* Fr.)

Growing on moist soil, Jamikhiau forest, Khumjung, 3825m, 22.8.2004;

Giri, Rana and Shrestha, no.268, NAST.

On ground, Godavari (Pandey 1976) and Kathmandu market (Adhikari 1996; Adhikari 1997; Adhikari *et al.* 1996)).

Distribution: Europe, North America, Japan, India, Nepal.

Coprinaceae

22. *Coprinus micaceus* (Bull.: Fr.) Fr.

(= *Coprinellus micaceus* (Bull: Fr.) Vil., Hop. & Joihn.

Growing on moist soil and dead tree trunk open area near *Rhododendron* and *Abies* forest, Tengboche, 3702m, 24.8.2004;

Rana, Giri & Shrestha, no.410, NAST.

Distribution: Worldwide.

23. *Coprinus niveus* (Pers.) Fr

Growing on moist soil in open area near *Pinus* forest, Tengboche, 3445m, 26.8.2004;

Rana, Giri & Shrestha, no. 579, NAST.

Distribution: America, Europe, Nepal.

24. *Coprinus radians* (Desm.: Fr.) Fr.

Growing on twig of *Rhododendron* sp., Jamikhiau forest, 3860m, 22.8.2004;

Giri, Rana & Shrestha, no.279, NAST.

Distribution: Europe, America, Japan, Nepal.

Cortinariaceae

25. *Cortinarius traganus* (Fr.: Fr.) Fr.

Growing on moist soil, under *Rhododendron* Forest, Omaka Forest (Pangboche), 3890m, 25.8.2004;

Giri, Rana & Shrestha, no. 552, NAST.

Distribution: North America, Europe, Nepal.

26. *Dermocybe phoenicea* (Bull.) Moser

Growing on moist soil, under *Rhododendron & Abies* mixed forest, Khumjung, 3800m, 22.8.2004;

Giri, Rana & Shrestha, no. 261, NAST.

Distribution: Japan, Europe, Nepal.

Fomitopsidaceae

27. *Gloeophyllum sepiarium* (Wolf.: Fr.) Karst.

(= *Lenzites sepiaria* Wolf.: Fr.)

Growing on *Pine* root, *Pinus* forest, Jorsalle, 2690m, 15.8.2004;

Rana, Giri & Shrestha, no. 100, NAST.

Previously recorded from Hetauda (Pandey, 1976).

Distribution: Europe, Japan, China, North America, India, Nepal

Ganodermataceae

28. *Ganoderma applanatum* (Pers.) Pat.

Growing on dead log of *Abies,* Lausasa 3570m, 23.8.2004;

Rana, Giri & Shrestha, no.312, NAST.

On rotten tree, Terai forest, Dharan (Balfour-Browne, 1968); on rotten tree trunk, Bakhri Kharka (North of Pokhara); Taglung (Kaligandaki) (Balfour-Browne, 1968); Hetauda (Pandey, 1976); on *Quercus* sp., Daman (Singh 1976); on stump, Phulchoki (Lalitpur Dist.) (Singh & Nisha, 1976c); on tree trunk, Namrung (Gorkha Dist.) (Adhikari, 1988a) and on wood of *Belula utilis,* Nilgatti Odar (Bajhang Dist.) (Adhikari, 1988a). In tropical to temperate belts (Adhikari, 1996).

Distribution: Worldwide.

Gomphaceae

29. *Gomphus clavatus* (Pers: Fr.) S.F. Gray

(= *Craterellus clavatus* Pers.)

Growing on soil, shady and moist place, under *Abies* forest, Phurte, 3546m, 17.8.2004;

Giri, Rana & Shrestha, no. 206; NAST.

On soil, Kakani (Pandey, 1976)

Distribution: Europe, North America, Japan, Nepal.

30. *Gomph floccosus* (Schw.) Singer *var. floccosus*

Growing on moist soil, under *Abies* forest, Lausasa 3570m, 23 .8.2004;

Giri, Rana & Shrestha, no.320, NAST.

On earth bank in Quercus forest (Balfour-Browne, 1968); in humus in *Abies-Betula* forest, Ratomata, Chakure Lekh (Balfour-Browne, 1955) and on ground, in the mixed forest, Syabru (Adhikari 1996a).

Distribution: Europe, China, North America, Japan, India, Nepal.

Gomphidiceae

31. *Chroogomphus tomentosus* (Muff.) O.K. Miller

Growing on moist soil, in *Rhododendron* forest, Khunde, 3900m, 20.8.2004;

Giri, Rana & Shrestha, no. 241, NAST.

Distribution: Japan, Nepal.

Hydnaceae

32. *Hydnum repandum* L.: Fr.

Growing on moist soil under *Rhododendron* forest, Jamikhiau, 3860m, 22.8.2004;

Giri, Rana & Shrestha, no. 280, NAST.

In *Pinus roxburghii* forest, on soil, Daman, Kakani, (Pandey 1976); Kathmandu Manichur (Adhikari 1976); Suryavinayak (Singh & Nisha 1976a); market (Adhikari 1987) and Tokha (Adhikari 1996a; Adhikari & Adhikari 1997; Adhikari *et al.*1996).

Distribution: Europe, North Amenica, Japan, Nepal.

Hygrophoraceae

33. *Hygrocybe strangulata* Orton

Growing on moist soil under *Rhododendron* forest, Khunde, 3900m, 20.8.2004;

Giri, Rana & Shrestha, No. 227, NAST.

Distribution: Europe, America, Nepal.

34. *Hygrocybe coccinea* (Schaeff.: Fr.) Kummer

Growing on moist soil under *Rhododendron* forest, Khunde, 3900m, 20.8.2004;

Rana, Giri & Shrestha, no. 229, NAST.

On soil, Phulchowki (Singh & Nisha, 1976a) and on moist shady place in *Quercus* forest, Doka Dada, Lumle (Devkota 2005).

Distribution: Europe, Japan, China, N. America, India, Nepal.

Hyxmenochaetaceae

35. *Coltricia perennis* (Fr.) Muff. [= *Polystictus perennis* (L.) Fr.]

Growing on dry and sandy soil in open area, Tok Tok, 2725m, 15.8.2004;

Giri, Rana & Shrestha, no. 81, NAST.

Previously recorded from Thodung, Lamjura, Rigmo, and Dudh Koshi (Ryv., 1977); on moist ground, Phulchowki (Lalitpur Dist.) (Singh & Nisha, 1976a) and on thick humus soil with mosses, Seti khola Agra goan forest (Bajhang Dist.) (Adhikari, 1988a).

Distribution: Europe, North America, India, Japan, Russia, Nepal.

Lycoperdaceae

36. *Lycoperdon perlatum* Pers.: Pers. (*Lycoperdon gemmatum* Batsch.)

Growing on soil, under *Abies spectabilis* tree, Deboche, 3665m, 25.8.2004;

Rana, Giri & Shrestha, no.505, NAST.

Growing on soil in moist shady places among mosses, Kyangjing, Langtang, Rasuwa district (Central Nepal) (Adhikari, 1988d); between Khandhe and Seti Khola bagar, Bajhang district (West Nepal) (Adhikari, 1996); and Lohar (Bhandari, 1991).

Distribution: China, Europe, India, Japan, North America, Tasmania, Africa, Australia, Nepal.

37. *Lycoperdon pyrjforme* Schaeff.: Pers.

(= *Lycoperdon emodense* Berk.)

Growing on moist soil, under *Rhododendron* forest, Phungi tenga, 3590m, 24.8.2004;

Giri, Rana & Shrestha, no.407, NAST.

Growing on rotten dead wood in moist shady places, Suliigadh (West Nepal) (Balfoun- Browne 1968); Thodung, Lamjura and Junbesi (Kreisel 1969); Phulchowki and Sundarijal (Singh & Nisha 1976a); Kakani (Pandey 1976); Kali gad, surmasarowa Lekh, Khuli, Simen, Langtang valley (Kreisel 1976); between Syabru besi and Lama hotel, Langtang National Park (Adhikari 1996); Kakani and Pokhara (Adhikari *et al.* 1996) and on moist ground, under shade of *Alnus nepalensis,* Gauri Khoria, Lumle (Devkota 2005).

Distribution: Europe, Sino- Japan, Central and South East Asia, North America, Australia, Africa, Tasmania, New Zealand, Japan, Australia, Nepal.

Marasmiaceae

38. *Rhodocollybia butyraceae* (Bull.: Fr) Lennox

[= *Collybia butyracea* (Bull. :Fr.) Kummer]

Growing on moist soil, under Coniferous forest, way to Namche, 3150 in, 16.8.2004;

Giri, Rana & Shrestha, no. 141, NAST.

On soil, Godavari (Pandey, 1976).

Distribution: Europe, Japan, North America, Nepal.

39. *Mycena capillaries* (Schum.:Fn.) Kummer

Growing on leaf of *Quercus* sp., under mixed forest *of Quercus-Rhododendron,* Muse forest, Lukla, 2783m, 13.8.2004;

Rana, Giri & Shrestha, no.4, NAST.

Distribution: Australia, Europe, Nepal

Nidulariaceae

40. *Cyathus striatus* (Hud.: Pers.) Will.

Growing on moist twigs *of Abies* sp., Phurte, 3585m, 17.8.2004;

Rana,Giri & Shrestha, no.21 1B, NAST.

On ground and rotten wood in forest, Sundarijal (Singh & Nisha, 1976a).

Distribution: North America, China, Europe India, Japan, Russia, Nepal.

Paxilaceae

41. *Paxillus involutus* (Batsch: Fr.) Fr.

Growing on moist soil under *Rhododendron* forest, Jamikhiau, 3825m, 22.8.2004;

Rana, Giri & Shrestha, no. 271, NAST.

Distribution: North America, Europe, Nepal.

Pezizaceae

42. *Peziza badia* Pers.: Fr.

Growing on dry soil, under mixed forest of *Rhododendron - Abies,* Tengboche, 3445m, 26.8.2004;

Rana, Giri, & Shrestha, no.590, NAST.

On soil, BanThanti (Otani, 1982).

Distribution: Europe, Japan, Nepal, America.

Pleurotaceae

43. *Pleurotus dryinus* (Pers.: Fr.) Kummer
[= *Pleurotus corticatus* Fr.: Fr.) Quel]

Growing on tree trunk *of Abies spectabilis,* Lausasa, 3570m, 23.8.2004;

Giri, Rana & Shrestha, no.306, NAST.

On tree trunk of *Quercus semicarpifolia,* Phulchowki (Singh & Nisha, 1976b).

Distribution: Japan, India, Europe, North America, Nepal.

Polyporaceae

44. *Heterobasidion annosum* (Fr.) Bref.

Growing on cut stump *Pinus* sp. tree, Jorsalle, 2690m, 15.8.2004;

Rana, Giri & Shrestha, no 91, NAST.

On wood of *Rhododendron arboreum,* Manichur (Adhikari, 1988a) and in tropical to temperate belts (Adhikari, 1996a).

Distribution: Europe, Japan, China, North America, India, Nepal.

Pyronemataceae

45. *Aleuria aurantia* (Fr.) Fuck.

(= *Peziza aurantia* Fr.)

Growing on moist places on twigs of *Rhododendron* sp., Deboche, 3665m, 25.8.2004;

Giri, Rana & Shrestha, no. *554*, NAST

On soil, Ghar Khola (Balfour-Browne, 1968; Singh & Nisha, 1976a); Kyumnu (Otani, 1982) and from Maipokhari area, Ilam (Adhlkari, 1998).

Distribution: Europe, Japan, China, India, America, Nepal.

46. *Otidea onotica* (Pers.: Fr.) Fuck.

Growing on moist soil, Phungitenga, 3340m, 21.8 2004;

Giri, Rana & Shrestha, no. 403, NAST.

On soil, Dhunche, Sing Gompa (Otani, 1982).

Distribution: North America, Europe, Japan, Nepal.

47. *Scutellinia scutellata* (L.: Fr.) Lamb

Growing on *Tsuga* tree, Deboche, 3665m, 26.8.2004;

Rana, Giri & Shrestha, no. 572, NAST.

On soil, dead Yucca and twigs, Balaju, Kathmandu (Waraitch & Thind, 1977).

Distribution: North America, Europe, Japan, China, Australia, Nepal.

Ramariaceae

48. *Ramaria flava* (Schaeff.: Fr.) Quel.

On Moist Soil, under *Abies spectabilis* tree, Phurte, 3615m, 17.8.2004;

Giri, Rana & Shrestha, no.207, NAST.

Sold at Kathmandu market (Adhikari, 1996; Adhikari &Adhikari, 1997; Adhikari *et al.* 1996).

Distribution: Europe, North America, Australia, Japan, India, China, Nepal.

Russulaceae

49. *Lactarius deliciosus* (L.: Fr.) S.F. Gray

Growing on moist soil, under *Abies* forest, Lausasa, 3570m, 23.8.2004;

Giri, Rana & Shrestha, no. 303, NAST.

This species is recorded from Manichur (Adhikari, 1976); Daman (Pandey, 1976); Manang (Bhandary, 1991); on ground in *Abies spectabilis, Larix himalaica* and *Rhododendron* sp. forest between Syabrua and Chandanbari (Bill and Cotter, 1989) and in mixed forest, Sundarijal (Adhikari *et al.* 1996).

Distribution: Europe, North America, Japan, Nepal.

50. *Lactarius piperatus* (Fr.) S.F. Gray

Growing on moist soil under Coniferous forest, Muse Forest, Lukla, 2783m, 13.8.2004;

Giri, Rana & Shrestha, no. 1OGH, NAST.

Kathmandu valley (Singh, 1966); Daman (Pandey, 1976); growing on soil in *Pinus roxburghii* forest, Tokha, Nagarkot (Adhikari, 1996; Adhikani *et al.* 1996); Suryavinayak, Lele and Phulchoki (Adhikari, 1996); Hetauda (Poudel, 1997) and on soil in *Rhododendron* forest, Gauri Khonia, Lumle (Devkota, 2005).

Distribution: China, Europe, Russia, North America, Japan, Nepal.

51. *Lactarius subdulcis* (Pers.:Fr.) S.F. Gray

Growing on Moist soil, Muse Forest, Lukla, 2783m, 13.8.2004;

Giri, Rana & Shrestha, no. 10L, NAST.

From Kathmandu valley (Adhikari, 1976; Singh, 1966)

Distribution: Europe, North America, Japan, Nepal.

52. *Lactarius volemus* (Fr.) Fr.

Growing on moist soil, under *Rhododendron* - *Betula* mixed forest, Deboche, 3665m, 25.8.2004;

Giri, Rana & Shrestha, no. 502, NAST.

From Manichur (Adhikari, 1976); Nagarjoon, Kathmandu valley (Pandey 1976); on soil in *Pinus roxburghii* forest, Nagarkot, Lele, Bajrayogini, Dakshinkali, in mixed forest, Matatirtha; Suryavinak and Phulchowki (Adhikari, 1996; Adhikari *et al.* 1996); Mai pokhari (Adhikari, 2000) and on soil in shade of *Rhododendron arboreum,* Mulbani, Lumle (Devkota, 2005).

Distribution: Worldwide.

53. *Russula densi folia* (Secr.) Gill.

Growing on moist soil under *Pinus* forest, Tengboche, 3702m, 24.8.2004;

Giri, Rana & Shrestha, no.427, NAST.

Growing on soil in moist shady place in *Pinus roxlnirghii* forest, Royal Botanical Garden, Godavari (Adhikari, 1984); Lele, Bajrayogini, Lele and in mixed forest, Suryavinayak (Adhikari, 2000).

Distribution: Worldwide.

54. *Russula olivacea (Schaeff.)* Fr.

On moist soil, under mixed forest *of Rhododendron* - *Tsuga,* Muse forest, Lukla, 2783m, 13.8.2004;

Giri, Rana & Shrestha, no. 24, NAST.

Growing on soil in moist shady place in *mixed forest of Pinus wallichiana-Abies* sp., Junbesi-Rachuwa (Adhakari, 1 990b).

Distribution: Europe, North America, Nepal.

55. *Russula metachroa* Hongo

Growing on moist soil in open place, in *Abies* forest, Phurte, 3585m, 17.8.2004;

Giri, Rana & Shrestha, no. 203, NAST.

Distribution: Japan, North America, Europe, Nepal.

56. *Russula paludosa* Britz.

Growing on moist soil under *Rhododendron* and *Abies* tree, Tengboche, 3645m, 24.8.2004;

Giri, Rana & Shrestha, no.413, NAST.

Distribution: North America, Europe, Nepal.

Sclerodermataceae

57. *Scleroderma citrinum* Peris. :Pers.

[=*Scleroderma vulgare* (Horneum) Fr.; *Scleroderma aurantium* Pers.]

Growing on soil in shady places, in *Rhododendron* forest, Khunde, 3900m, 20.8.2004;

Rana, Gini & Shrestha, no. 243, NAST.

Growing on soil in moist shady places, in *Pinus roxburghii* forest, Methand Pokhara (Balfour - Browne, 1968); Nagarjoon (Pandey, 1976); Godavari (Singh & Nisha, 1976a); Manichur, Sybrubesi (Langtang area), (Adhikari, 1987); Sundarijal, Kathmandu valley; sold in Kathmandu market, in mixed forest, Matatirtha and Pokhara, Raniban (Adhikari *et al.* 1996).

Distribution: Europe, North and South America, Australia, Japan, India.

Strobilomycetaceae

58. *Strobilomyces strobilaceus* (Scop.:Fr.) Berk.

[=*Strobilomyces floccopus* (Vahl. : Fr.) Karst.]

Growing on moist soil under Coniferous forest, Lukla/Ghat, 2604m, 14.8.2004;

Rana, Giri & Shrestha, no.41, NAST.

On ground, Phulchowki (Singh & Nisha, 1976a) and Suryavinayak (Adhikari, 1996).

Distribution: Worldwide.

Strophariaceae

59. *Hypholoma capnoides* (Fries) Kumm.

[= *Naematoloma capnoides* (Fr.: Fr.) Kumm.]

Growing on moist soil in bunch, under *Pinus* and *Rhododendron* forest, Phurte 2840m, 17.8.2004,

Rana, Giri & Shrestha, no. 121, NAST.

Distribution: North America, Europe, Japan, China, Nepal.

60. *Stropharia semiglobata* (Batsch.: Fr.) Quel

Growing on Yak dung, Lausasa, 3770m, 23.8.2004;

Rana, Giri & Shrestha, no.302A, NAST.

Distribution: America, Europe, China, Japan, Nepal.

61. *Pholiata aurivella* (Batsch.: Fr.) Kummer

Growing on Abies tree trunk in a bunch, shady moist place, Tengboche, 3702m, 24.8.2004;

Giri, Rana & Shrestha, no.408, NAST.

Manichur (Adhikani, 1976); Market and Landrung (Bhandary, 1984).

Distribution: Worldwide.

62. *Pholiota nameko* (Ito) Ito & Imai

[= *Collybia nameko* T. Ito; *Pholiota gultinosa Kawam.*; *Kuehneromyces nameko* (T. Ito) S. Ito]

Growing on moist soil, under Abies tree, Khumjung, 3800m, 21.8.2004;

Rana, Giri, & Shnestha, no. 251, NAST.

Place not mentioned (Bhandary, 1984).

Distribution: Japan, Nepal.

Tremellaceae

63. *Pseudohydnum gelatinosum* (Scop.: Fr.) Karst.

Growing on dead log of Rhododendron tree, Omaka forest (Pangboche), 3890m, 25.8.2004;

Giri, Rana & Shrestha, no. 550, NAST.

Distribution: America, Japan, Europe, Nepal.

Tricholomataceae

64. *Armillaria mellea* (Vahi.: Fr.) Kummer.

Growing under coniferous forest, Lukla, Muse forest, 2783m, 13.8.2004;

Giri, Rana and Shrestha, no. 1, NAST.

Host not mentioned, Lele (Pandey, 1976); Arun valley (Balfour - Browne, 1968); Phulchoki (Singh & Nisha,1976a); Kutungsang and Kalopani (Cotter,1987) and growing on decayed log in moist shady place in afforested area of pine (exotic), Kakani (Adhikari *et al.* 1996).

Distribution: Worldwide.

65. *Laccaria amethystina* (Huds.) Cooke

Growing on moist soil under coniferous forest, Lukla, Muse forest, 2783m, 13.8.2004;

Rana, Giri and Shrestha, no.l0c, NAST.

On soil, Hetauda (Pandey, 1976); Suryavinayk (Bhandari,1980); in *Pinus* forest, Kakani, Bajrayogini, in mixed forest Suryabinayak, Matatirtha and Sundarijal (Adhikari &Adhikari, 1997).

Distribution: Europe, North America, Japan, Nepal.

66. *Laccaria bicolor* (Maine) P.D. Orton

Growing on moist soil associated with mosses, under Pine forest, Phakdin, 2500m, 14.8.2004;

Rana, Giri & Shrestha, no. 61, NAST.

Distribution: Japan, Nepal.

67. *Laccaria laccata* (Scop.: Fr) Cooke

Growing on moist soil under *Rhododendron* and *Juniperus* forest, Syanboche, 3940m, 20.8.2004;

Giri, Rana & Shrestha, no. 233, NAST.

On soil, Arun Valley (Balfour - Browne, 1968); Bajrabarahi, Nagarjoon (Pande1976); Godavari (Singh & Nisha, 1966c); growing in moist shady place in *Pinus* forest, Kakani, Tokha, Bajrayogini, Lele, Nagarkot, in mixed forest, Sudarijal, Suryavinayak, Matatirtha and Pokhara (Adhikari *et al.* 1996) and on soil, Syaniswara, Lumle (Devkota, 2005).

Distribution: Worldwide.

68. *Lacccaria proxima* (Boudier) Pat

Growing on moist soil, under Pine forest, Jorsalle, 2690m, 15.8.2004;

Giri, Rana & Shrestha, no. 102, NAST.

Distribution: Japan, North America, Bulgaria, Nepal.

69. *Tricholoma saponaceum* (Fr.:Fr.) Kummer

Growing on shady places, on soil, under *Abies* forest, Phurte, 3615m, 17.8.2004;

Rana, Giri & Shrestha, no. 208, NAST.

On soil, Godavari (Pandey, 1976).

Distribution: Europe, Japan, North America, Nepal.

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Organic Matter Stimulates Bacteria and Arbuscular Mycorrhizal Fungi in *Bauhinia purpurea* and *Leucaena diversifolia* Plantations on Eroded Slopes in Nepal

Geeta Shrestha Vaidya, Keshab Shrestha, Buddi R. Khadge, Nancy C. Johnson, and Håkan Wallander

Abstract

Erosion resulting from landslides is a serious problem in mountainous countries such as Nepal. To restore such sites it is essential to establish plant cover that protects the soil and reduces surface erosion. Mycorrhizal fungi growing in symbiosis with plants are essential in this respect because they improve both plant nutrient uptake and soil structure. We investigated the influence of organic matter and P amendment on recently produced biomass of bacteria and arbuscular mycorrhizal (AM) fungi in eroded slopes in Nepal. Eroded soil mixed with different types of organic matter or P was placed in mesh bags, which were buried around trees of *Bauhinia purpurea* and *Leucaena diversifolia* between June 2003 and December 2003 (the wet season) or between December 2003 and June 2004 (the dry season). Signature fatty acids were used to determine bacterial and AM fungal biomass after the 6 month intervals. The amount and composition of AM fungal spores were analyzed in the mesh bags from the dry season. More microbial biomass was produced during the wet season than during the dry season. Furthermore, organic matter addition enhanced the production of AM fungal and bacterial biomass during both periods. The positive influence of organic matter addition on AM fungi could be an important contribution to plant survival in plantations on eroded slopes. Different AM spore communities and bacterial profiles were obtained with different organic amendments and this suggests a possible way of selecting for specific microbial communities in the management of eroded sites.

Introduction

Nepal is prone to soil erosion and is susceptible to sediment disasters caused by slope failure, landslides, debris flows, and bank erosion. The productivity of these soils is deteriorating due to accelerated surface erosion (Jagannath *et al.* 1998). To restore such sites it is essential to establish plant cover that protects the soil and reduces surface erosion. Plants prevent surface erosion through soil binding by roots, reduction of splash erosion through rainfall interception, improving water percolation along root channels, entrapment of material moving down the slope (Lammeranner & Rauch 2005), and by supporting soil microbes important in soil aggregation (see below).

The addition of organic matter such as green manure is a common practice used to improve soil nutrient content and soil structure. Organic residues from plants such as *Tithonia diversifolia* and *Lantana camara* have been found to be especially beneficial because they are reported to have high contents of nitrogen (N) and phosphorus (P), which are mineralized rapidly from the organic material. Nziguheba *et al.* (2000) found that P is released more rapidly from such organic residues than from triple superphosphate.

Most herbaceous and many woody plant species form symbioses with arbuscular mycorrhizal (AM) fungi. These fungi improve water and nutrient uptake in exchange for carbohydrates supplied by the plant. Successful colonization by mycorrhizal fungi is especially important in degraded soils where nutrient availability is low; furthermore, AM fungi improve soil structure because they produce extraradical hyphal networks and their hyphae contain and release glomalin, a putative homolog of heat-shock protein 60 (Gadkar & Rillig 2006). The concentration of glomalin-related protein in soil is usually correlated with aggregate stability (Wright & Upadhyaya 1998; Rillig 2004; Rillig & Mummey in press). Improved soil structure increases *water* infiltration and can reduce soil erosion (Tisdall & Oades 1982).

Many biotic and abiotic factors influence the growth and partitioning of AM fungal biomass into intraradical (inside roots), extraradical hyphae (in soil), and spores. Farming systems (Boddington &

Dodd 2000), soil moisture (Anderson *et al.* 1983), organic matter (Ryan *et al.* 1994; Hodge *et al.* 2001), pH (Porter *et al.* 1987; Van Aarle *et al.* 2002), and temperature (Koske 1987) are all examples of factors that can influence the distribution of AM fungal hyphae and spores. Managing soil could thus be a way of optimizing the proliferation of indigenous AM fungi (Boddington & Dodd 2000). In particular, the addition of organic matter can have a beneficial effect on the growth of indigenous AM fungi in nutrient limited soils (Caravaca *et al.* 2002; Gaur & Adholeva 2002) Organic amendment enhances spore production (Johnson & McGraw 1988; Douds *et al.* 1997) extraradical proliferation of hyphae (St. John *et al.* 1983; Joner & Jakobsen 1995; Hodge *et al.* 2001), and Colonization of roots (Muthukumar & Udaiyan 2000). Giovanetti and Avio (1985) suggested that this beneficial effect might be related to increased pore volume in soil, which has a beneficial effect on AM colonization, the mycorrhizal growth response and AM spore numbers. Furthermore, Ryan *et al.* (1994) attributed increased AM fungal biomass to the beneficial effects of organic matter on soil structure water status, and synergistic microbial activities in the soil. Organic matter addition could thus enhance restoration at eroded sites by increasing the beneficial effects of AM fungi on soil stabilization and plant establishment.

Availability of soil P also mediates the growth and function of AM symbioses. Phosphorus enrichment of extremely P-poor soils will increase AM fungal biomass but once a threshold level is reached, further P enrichment is likely to reduce AM fungal biomass (Johnson 1998; Treseder & Allen 2002). Eroded soils in Nepal are generally impoverished regarding soil organic matter, and soils have low phosphorus availability due to high phosphorus fixing capacity (Brown *et al.* 2000).

The aim of this study was to investigate whether growth and spore formation by indigenous AM fungi in eroded sites in Nepal can be stimulated by the addition of organic matter or P amendment. Responses of AM fungal and bacterial biomass to the addition of triple superphosphate or four different organic supplements was measured by placing root-exclusion mesh bags containing amended soil in the rooting zone of trees planted in eroded forest sites in Nepal. Production of AM fungal and bacterial biomass within the mesh bags as examined after 6 months during the wet season and again after 6 months during the dry season. The use of mesh bags made it possible to directly compare the influence of different litter types on newly formed microbial biomass within the rhizosphere of the same host plant. We hypothesized that organic matter amendment would increase microbial biomass and spore by AM fungi. Furthermore, different amendments were expected to generate different AM fungal and bacterial communities.

Methods

This field experiment was conducted in Baluwa Forest, Kavre district in Central Nepal. This forest is situated 41 km east of Kathmandu on the side of the Arniko highway facing the Tibetan border. The study site was completely eroded as a result of a landslide about 5 years before it was planted with *Bauhinia purpurea* L. at one site and *Leucaena diversifolia* (Schldl.) Benth at another site. Both of these species are important fodder plants in Nepal. Plantation was carried out by the Fodder Department of the Nepal Agriculture Research Council, Khumaltar, Nepal. At the time of our experiment 3 years after planting approximately 30% of the plants had survived. The soil at the experimental site is a red soil (Rhodustalfs, member of the Alfisols soil order). The chemical characteristics of the soil have been examined earlier by the Division of Soil Science, NARC. It was found that the soil was acidic (pH 4.0) and poor in nutrients and organic matter. The total content of N, measured by the Kjeldahl method, was 4.1 mg/g and the organic matter content was 0.73%.

Experimental Design and Methods

Growth of AM fungi under field conditions was estimated with in-growth mesh bags, similar to a design used earlier to estimate the growth of mycorrhizal fungi in forests (Wallander *et al.* 2001) sand dunes (Olsson & Wilhelmsson 2000) and pre Saharan desert shrubland ecosystems (Labidi *et al.* 2007). The mesh bags were made of nylon mesh (50 μm mesh size) to allow fungal colonization but excluded roots because the latter cannot penetrate the mesh. These bags were used to clearly distinguish the effects of soil amendment on new AM fungal production in the field. The mesh bags were filled with eroded soil mixed with different forms of organic matter or triple superphosphate (see below).

The eroded soil was collected from a degraded site at Bisankhu Narayan (Godawari), Lalitpur. This site was completely eroded due to a landslide after heavy rainfall. No vegetation was present on the site when the soil was collected. Forty five grams of eroded soil was placed in the mesh bags and mixed with different types of organic matter or left unmixed as *a* control. We used 5 g of dried leaves or dried compost (10% by weight) as organic matter additions. Fully expanded leaves of three common agroforestry plant species (*Tithonia diversifolia* (Hemsl.) Gray [Asteraceae] *Lantana camara* L. [Verbenaceae], and *Eupatorium adenophorum* L. [Asteraceael]) were collected from border rows from farmer's fields and from roadsides, and the compost was collected from a farmer. The compost was made of disposed vegetable waste, cow dung, straw and husk. We also included treatment with triple superphosphate addition. In this case, 45 mg of triple superphosphate was mixed with 50 g of eroded soil. The mesh bag that were used as controls were filled with 50 g of eroded soil without any amendment. In total, six treatments were included: eroded soil amended with either triple superphosphate, compost, *Tithonia* litter, Lantana litter, *Eupatorium* litter, or eroded soil without amendment (control). The experiment lasted for 1 year, one set of mesh bags was buried from June 2003 to December 2003 (the wet season) and a second set was buried at the same locations from December 2033 to June 2004 (the dry season). Some rain (2-3 days) was recorded at the beginning and the end of the dry season in January and June. Plenty of rain was recorded during the wet season although no estimates of the amounts were made. One replicate mesh bag for each treatment, season, and site were buried in the rhizosphere of one tree (at 10 cm depth and approximately 10 cm from the base). In total, eight replicate mesh bags were buried around eight trees for each tree species. The trees at the sites were approximately 1.5-2 m high. A total of 192 bags were used (2 seasons x 6 treatments x 2 tree species x 8 replicates). The amount of fatty acids in each mesh bag was analyzed to provide at estimate of bacterial and AM fungal biomass (see below). In some cases the mesh bags were damaged by animals in the field so results from six to eight replicates were used in the statistical analysis. At harvest the content of the mesh bags were mixed and subsamples were placed in a freezer (-20 °C) and they were later transported to Sweden for fatty acids analysis. Sub samples from the mesh bags were also kept and used for fungal spore analysis. For spore analysis, a complete dataset was only obtained for the dry season.

Elemental Analysis of Plant and Soil Material

The elemental composition of the eroded soil, compost, and plant material put in the mesh bags was analyzed. Fresh leaves of the three plant species, compost, and eroded soil were air dried at 40 °C and the organic materials were ground to pass through a 0.5 mm sieve. The concentrations of Al, Ca, Fe, K, Mg, P and S of dried plant leaves, dried compost and eroded soil were analyzed with inductively coupled plasma-atomic emission spectrometer after digesting the material in concentrated nitric acid. The carbon and N contents were analyzed with an element analyzer (Modell vario MAX CN; Elementar Analysensystem GmbH, Hanau, Germany). These data are reported in Table 1.

Table 1. Chemical composition (mg/g) of the eroded soil and the different organic amendments used as substrates in the mesh bags.

Substrate Used	C	N	P	C:N Ratio	C.P. Ratio	K	Ca	Mg	S	Al	Fe
Eroded soil	1.4	0.11	0.3	12.4	0.4	1.0	1.1	0.6	0.03	12.5	42.3
Farmers compost	247	22.3	17.6	11.0	14.0	14.7	15.5	11.8	4.1	2.2	2.8
Eupatorium adenophorum	464	36.7	2.6	12.6	178	22.6	14.8	2.2	2.0	0.5	0.6
Tithonia diversifolia	441	33.2	2.8	13.2	158	34.1	13.4	3.1	1.8	0.2	0.2
Lantana camara	414	28.6	1.7	14.5	243	12.2	26.3	2.9	2.6	0.7	0.6

Analysis of Phospholipid and Neutral Lipid Fatty Acids

Lipids were extracted from 3 g of lyophilized soil mixture either triple superphosphate, compost, from each mesh bag using the method of Frostegård *et al.* (1991). Analysis of the same materials that had not been placed in the mesh bags was also performed to estimate the background amounts of bacterial and AM-specific fatty acids in these materials. Minute amounts of bacterial fatty acids were found in the different litter types and in the eroded soil, whereas larger amounts of bacterial fatty acids were found in the compost. Small amounts of AM-specific fatty acids were found in the compost, whereas no AM-specific fatty acids could be detected in the different litter types or eroded soil added to the mesh bags. The background values of bacterial and AM-specific fatty acids were subtracted from the values obtained in the incubated mesh bags to give the production of microbial biomass in the mesh bags during incubation.

Extracted lipids were fractionated into neutral lipids, glycolipids, and polar lipids on silicic acid columns (100-200 mesh, Unisil) by successive eluation with chloroform, acetone, and methanol. The methanol fraction (containing the neutral lipids) were subjected to mild alkaline methanolysis to transform the fatty acids into free methyl esters. These were analyzed on a gas chromatograph with a flame ionization detector and a 50 m HP5 capillary column, according to the method of Frostegård *et al.* (1993). The phospholipid fatty acid (PLFA) 16:1ω5 and the neutral lipid fatty acid (NLFA) 16:1ω5 were used as indicators of AM fungal biomass (Olsson 1999). The PLFA 16:1ω5 is not entirely specific to AM fungi because it can occur in some bacterial groups but most of this PLFA is considered to originate from AM fungi when the ratio of NLFA and PLFA 16: ω5 is above 1 (Olsson 1999). The PLFA 16:1ω6, 9 was used as a general indicator of fungi, and the sum of the PLFAs i15:0, a15:0, 10Me18:0, 18:1ω7, and cy19:0 was used as indicator of bacterial biomass (Frostegård *et al.* 1996).

Spore Analysis

The Am fungal spores in 25 g of the soil and amendment mixtures inside the mesh bags were extracted, identified and quantified. Spores were extracted using wet sieving and sucrose centrifugation (McKenney & Lindsey 1987). Spores were mounted in polyvinyl alcohol on slides and examined using a compound microscope. Specimens were classed into species using taxonomic characteristics described on the INVAM Web site (http://invam.caf.wvu.edu/) and by Schenck and Perez (1990). In total 96 spore samples were analyzed from the dry season.

Statistical Analysis

Three-way analysis of variance (ANOVA) was performed using the general linear model procedure included in the software Statistica 7.1 (Statsoft. Inc., Statsoft Scandinavia AB, Uppsala, Sweden) using site (tree species), treatment (amendments). and season (wet or dry season) as fixed factors. Data were log transformed to obtain a normal distribution of residuals. Dunnet's test was used to determine whether treatments (amendments) were different from the control (no amendment). For spore analysis we only had a complete dataset for the dry season so treatment and site were examined using two way ANOVA and post hoc comparisons were analyzed using Tukey's honestly significant difference test. The composition of signature bacterial PLFA's and the species composition of the AM spore communities in the mesh bags were analyzed using two way analysis of similarity (ANOSIM) of spore counts and fatty acid concentrations followed by post hoc pair wise tests for significant treatment effects. Because this technique is incompatible with data consisting of all zeros in the spore analysis, the entire control treatment was excluded from the analysis because no spores were observed in any of the control samples; however, the control is obviously different from all other treatments because it completely lacks spores. ANOSIM is analogous to a multivariate analysis of variance (MANOVA): it uses multiple response variables and one or more categorical-dependent variables, similar to MANOVA but without the stringent distribution assumptions that are seldom met in community datasets. We used the Bray-Curtis distance with this technique because it does not assume bivariate normality and does not interpret shared zeros as a positive correlation (McCune & Grace 2002). Mantel tests were conducted to assess the correlation (*r*) between spore community composition and the composition of bacterial PLFAs based on the Bray-Curtis distance measure. In both ANOSIM and Mantel tests, the effect size statistic (*r*) is compared to 5,000 random permutations of the data. The probability values are the proportions of random results that yielded a more extreme result than the actual data. ANOSIM was performed with the software Primer v5 (Clark & Gorley 2001). Mantel tests were conducted using the software PC-ORD (McCune & Grace 2002).

Result

The production of fatty acids in the mesh bags did not differ between the two sites (Table 2). However, the addition of organic matter had a strong positive influence on the production of NLFA 16:1ω5 (Fig. 1a), PLFA 16:1ω5 (Fig. 1b), and bacterial PLFAs (Fig. 1c). In addition, significantly more PLFA 16:1ω5, NLFA 16:1ω5. and bacterial fatty acids were produced during the wet season than during the dry season in mesh bags with organic amendments as indicated by a significant effect of season (NLFA 16:1ω5) or a significant interaction in the ANOVA between season and treatment (PLFA 16:1ω5 and bacterial PLFAs; Table 2; Fig. 1). A tendency ($p=0.08$) was found toward a stimulatory effect of P addition on PLFA 16:1ω5 and a significant effect of P was found on the amount of bacterial PLFAs ($p = 0.0003$) using Dunnet's test. The NLFA/PLFA 16:1ω5 ratio was significantly lower in the compost, *Lantana*, and *Eupatorium* treatments compared to the unamended control (Table 3).

Table 2. Three-way ANOVA of the effects of site (tree species) treatment (amendment), and season (wet or dry season) on the amount of fatty acids in the mesh bags.

Parameur	Main Effect	df	F Value	p Value
PLFA 16:1ω5	Si	1	1.6	0.55
	T	5	45.6	<0.0001
	Se	1	2.4	0.12
	Si × T	5	0.5	0.8
	Si × Se	1	1.3	0.3
	T × Se	5	3.6	0.004
	Si × T × Se	5	0.4	0.8
	Error	149		
NLFA 16:1ω5	Si	1	0.1	0.8
	T	5	9.9	<0.0001
	Se	1	10.0	0.002
	Si × T	5	1.4	0.2
	Si>1Se	1	0.01	0.9
	T × Se	5	0.6	0.7
	Si × T × Se	5	1.3	0.3
	Error	154		
Bacteria PLFA	Si	1	3.3	0.07
	T	5	114	<0.0001
	Se	1	1.7	0.19
	Si × T	5	0.6	0.7
	Si>KSe	1	1.0	0.3
	1 × Se	5	6.5	<0.0001
	Si × T × Se	5	0.7	0.6
	Error	140		
NLFA/PLFA	Si	1	0.0	0.99
	T	5	4.5	0.0008
	Se	1	3.1	0.08
	S × T	5	1.3	0.3
	S × H	1	1.8	0.2
	T × H	5	1.1	0.3
	S × T × H	5	1.7	0.1
	Error	143		

Si= site; T=treatment; Se=season.

Table 3. The ratio between NLFA 16:1ω5 and PLFA 16:1ω5 in mesh bags with different amendments.[a]

Treatment	*NLFA/PLFA 16:1ω5*
Control	21.2 ± 4.8
Phosphate	18.3 ± 4.7
Compost	5.3 ± 2.0*
Eupatorium	6.7 ± 1.5*
Tithonia	9.4 ± 2.3
Lantana	6.0 ± 1.0*

[a]Values from both seasons and both sites are included in the $\overline{X} \pm$ SE.
[*]Indicates values statistically different from the control (using Dunnet's test p <0.05).

During both the wet and dry seasons the composition of the bacterial PLFAs was significantly influenced by treatment ($p < 0.0001$) but not site. In the wet season, ANOSIM indicated two groupings of bacterial PLFAs, one comprising the control and triple superphosphate treatment and the other the four organic amendments. In contrast, during the dry season, the bacterial PLFA composition in the control mesh bags was significantly different from the triple superphosphate treatment. Also, bacterial PLFA composition in bags amended with the three different plant litters were different from each other, but not different from the compost treatment.

During the dry season, there was no significant difference in spore abundance in mesh bags at the two sites: however, there was a very significant effect of amendment ($p < 0.0001$). No spores were observed in the unamended (control) treatment. Mesh bags amended with triple superphosphate contained significantly fewer spores than bags containing soil amended with organic matter, and bags amended with *Lantana* leaves contained significantly more spores than the others (Table 4). Spores of nine species of AM fungi were identified in the mesh bags (Table 4). The species composition of AM fungal spores was significantly influenced by the amendments ($p < 0.0001$) and weakly by site ($p = 0.07$). ANOSIM distinguished distinct communities of spores in bags amended with triple superphosphate. Spore communities in mesh bags amended with *Lantana* litter were significantly different from all the others except for those amended with *Tithonia* litter. The species composition of spores recovered from bags amended with compost, *Eupatorium*, or *Tithonia* litter were indistinguishable from each other. Mantel tests show a very significant correlation between the species composition of AM spore communities and bacterial PLFAs (excluding PLFA 16:1ω5) in the dry season ($N = 67$, $r = 0.34$, $p < 0.0001$).

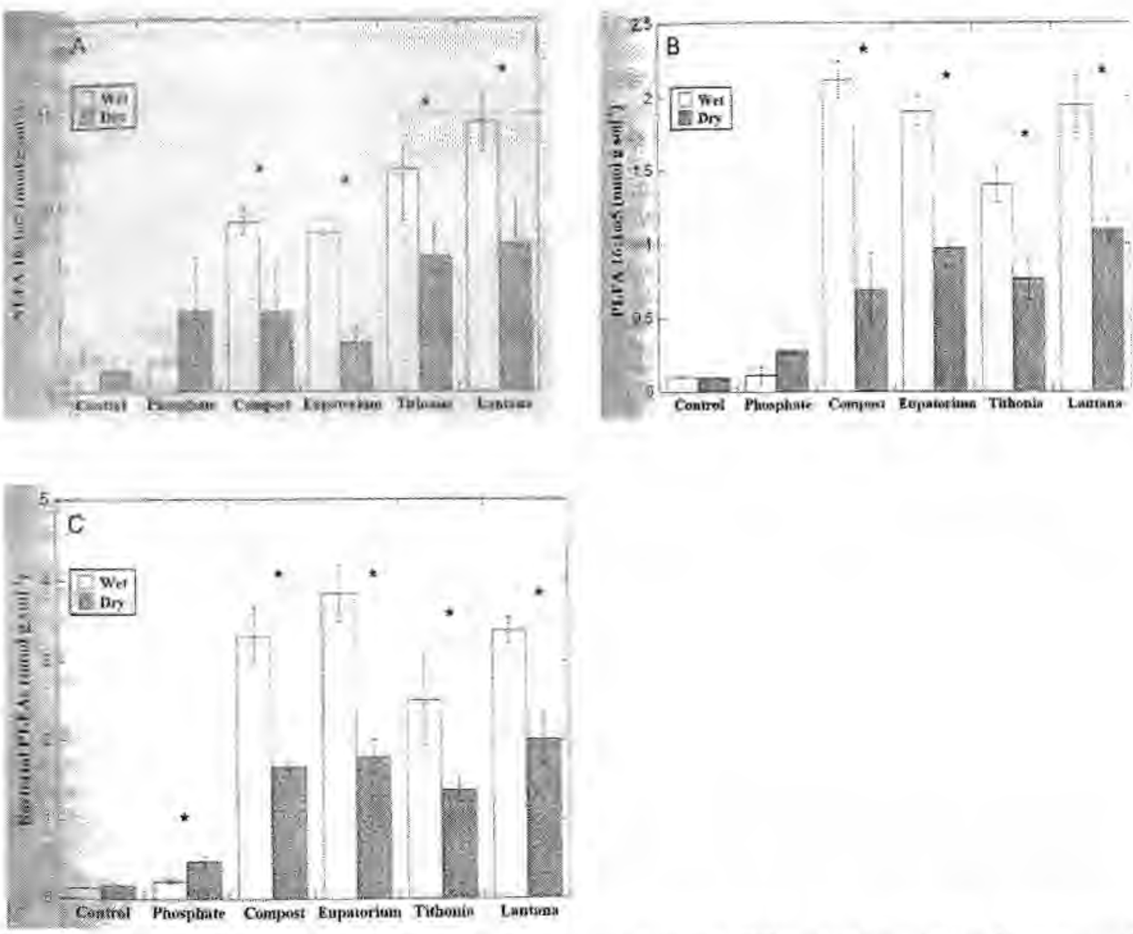

Fig. 1. Content of NLFA 16:1ω5 (a), PLFA 16:1ω5 (b), and bacterial PLFAs (c) in mesh bags buried around *Leucaena diversifolia* and *Bauhinia purpurea* trees during the wet season (between June 2003 and December 2003) and during the dry season (between December 2033 and June 2004). The mesh bags were filled with eroded soil with the following amendments: control (no amendment), triple superphosphate, farmer's compost or litter from *Eupatorium adenophorum, Tithonia diversifolia*, or *Lantana camara*. Bars represent standard errors (SE), and "*" indicates significant main effect of treatment (amendment) compared to the control using Dunnet's test ($p < 0.05$)

Table 4. Mean abundance of Spore morphotypes in mesh bags during the dry season observed in 25 g of soil.

Species	Spores per 25 g soil ($\bar{X}$ ± SE)				
	Phosphate	*Compost*	*Eupatorium*	*Tithonia*	*Lantana*
Acaulospora scrobiculata	0	1.2 (1.2) a	1.8 (1.5) a	0.6 (0.6) a	3.6 (1.6) a
Acaulospora spinosa	0.3 (0.3) a	1.5 (0.9) b	7.7 (2.2) c	1.2 (0.5) h	5.4 (1.7) bc
Acaulospora hyaline	0.3 (0.3) a	2.4 (1.1) ab	6.8 (1.7) b	7.1 (2.3) b	6.0 (2.0) ab
Glomus aggregatum	0.3 (0.3) a	5.4 (1.7) ab	6.3 (1.5) ab	5.4 (1.6) ab	11.9 (3.8) b
Glomus fasciculatum	0.9 (0.5) a	7.1 (1.8) ab	5.7 (1.9) ab	8.9 (1.8) b	10.1 (3.3) b
Glomus amber	0.9 (0.5) a	1.8 (0.6) a	6.0 (1.5) b	4.8 (1.1) b	8.9 (2.1) b
Glomus canadense	0.9 (0.5) a	6.3 (1.8) ab	4.2 (1.2) ab	4.5 (1.3) ab	11.9 (3.9) b
Glomus hyaline	1.2 (0.5) a	5.1 (1.3) b	3.6 (0.9) ab	6.0 (1.2) b	14.3 (1.4) c
Scutellospora nigra	0	0	0	0	2.7 (1.4)
Total spores	4.7 (0.6) a	30.7 (2.5) b	42.0 (3.4) b	38.4 (3.1) b	74.8 (7.3) c

*Means are averaged across the two Sites because site did not significantly influence spore abundance. No spates were observed in the control treatment (no amendment): the other treatments were amendment with triple superphosphate, farmers compost, and leaf titter of *Eupatorium adenophorum Tithonia diversifolia* and *Lantana camara*. Different letters within rows indicate significant differences (p <0.05) according to Tukey's HSD test.

Discussion

Organic amendments significantly increased AM fungal and bacterial biomass in eroded slopes of Nepal revegetated with *Bauhinia purpurea* and *Leucaena diversifolia*. The use of in-growth mesh bags was found to be a successful way of measuring recently produced microbial biomass and AM fungal spores. Other methods of estimating the biomass of AM fungi in soil such as extraction of hyphae or spores (Boddington & Dodd 2000) or estimates of biochemical markers directly in soil samples (Olsson et al. 1999) include an unknown fraction of dead or inactive AM biomass. The production of recently formed extraradical AM mycelia is an important parameter because it can be directly related to the capacity of host plants to acquire nutrients and improve soil structure and stability in degraded soils.

The mesh bag method of estimating the production of AM fungi is especially useful for investigating how growth is influenced by amendment of different substrates because various substrates can be added to adjacent mesh bags placed around a single host plant. Other parameters such as inoculum potential, climatic variables and the activity of the host plant, will be constant and thus confounding effects of differences in plant growth between different treatments can be avoided. Phosphorus addition for instance, usually has an inhibitory effect on the growth of AM fungi (Abott & Robson 1984), but this is because plants with a better P status will allocate less carbon to the fungal symbiont than plants with a lower P status. With our experimental approach it is possible to detect direct effects of P addition on the growth of AM mycelia without indirect effects mediated by host plant physiology. Interestingly, we found a tendency toward a positive influence of P addition on the production of AM fungal biomass and a significant effect on the production of bacterial PLFAs especially during the dry season. This may indicate that microbial growth was limited by P during this period, which is not unlikely because the P-fixing ability of Nepalese red soils can be very strong (Brown *et al.* 2000).

The PLFA 16:1ω5 is not unique to AM fungi, it is also produced by bacteria (Olsson 1999), so the strong effects of the organic amendments on all PLFAs makes the interpretation of PLFA 16:lω5 difficult. However, the equally strong stimulation of NLFA 16:1ω5, which is unique to AM fungi together with the spore densities confirm that AM fungal biomass has increased in response to the organic amendments and varied with season. The ratio of NLFA 16:1ω5 to PLFA 16:1ω5 was significantly lower in mesh bags with than in mesh bags without organic amendments. This indicates that bacterial PLFA 16:lω5 becomes increasingly important in mesh bags treated with organic amendments. However, the overall high ratio of NLFA 16:lω5 to PLFA 16:1ω5 (6 to 22) suggests that the majority of 16:lω5 originates from AM fungi because this ratio is much lower (<1) in bacteria (Olsson 1995).

Production of AM fungi, indicated by the amounts of NLFA and PLFA 16:1ω5 was significantly higher during the wet season between December and June, than in the dry season, between June and December. This is not surprising because soil moisture is reported to stimulate both spore germination and hyphal growth of AM fungi (Hetrick 1984). Furthermore, *Allen et al.* (1989) found that the growth of the AM fungi associated with *Atriplex garneri* was highest during the spring season when the soil moisture was high.

The positive influence of organic amendments on the production of bacterial PLFAs is not surprising because the organic matter content of the ambient eroded soil was extremely low. Similar results were found when compost made of *Acacia cyanophylla* leaves was added to eroded soil in *Acacia tortilis* savanna in pre Saharan areas in Tunisia (Labidi *et al.* 2007). Enhanced bacterial biomass is likely related to the response of C-limited saprobes to increased C availability in the substrate.

The positive effect of the amendments on AM fungi is likely due to more complex mechanisms because these fungi are believed to be obligate biotrophs with no saprobic capability (Smith & Read 1997). Several mechanisms could account for this stimulation: (1) physicochemical alteration of the soil may benefit AM fungi: (2) provision of a substrate that AM fungi can utilize may enhance its growth: and/or (3) nutrient availability in the mesh bags could have been altered in a way that stimulates hyphal growth and sporulation.

In terms of physicochemical alterations, the positive response of AM fungi to organic matter addition could result from higher moisture content in mesh bags with organic amendments because organic matter generally has a beneficial effect on water-holding capacity (Ryan *et al.* 1994). The added organic matter could also increase soil porosity and decrease mechanical resistance to growth of AM hyphae in the soil (Joner & Jakobsen 1995). For example, studies have shown cellulose to have a positive influence on AM growth (Gryndler *et al.* 2002). Improved soil aggregation as a consequence of greater soil organic matter content could also have stimulated AM fungi by providing a more suitable physical growing space (Rilling & Steinberg 2002).

Second, AM fungi have been shown under in vitro conditions to positively respond to simple carbon compounds like the trisaccharide, raffinose, and to form spores and new hyphae as a consequence of signals received from cocoltured bacteria (Hildebrandt *et al.* 2006). It is thus possible that litter and litter associated microbes supported hyphal proliferation in mesh bags in similar ways.

Third, nutrients supplied with the organic material could have removed nutrient limitations of the AM fungi themselves (Treseder & Allen 2002), or increased hyphal growth could result from greater C allocation from the host for nutrient acquisition from the nutrient rich patches. For example, N within the organic amendment may have a beneficial effect on the growth of AM fungi. In laboratory studies it has been found that N-rich organic matter, such as baker's yeast and bovine serum albumin, can stimulate AM growth, whereas organic matter with no N, such as cellulose and starch, can have inhibitory effects (Ravnskov *et al.* 1999).

Species of AM fungi (Douds & Schenck 1990) and other soil microorganisms (McKinley *et al.* 2005) differ in their responses to soil C, N and P availabilities and this could account for the community composition differences that we observed in mesh bags treated with different amendments. For example, mesh bags amended with *Lantana* litter exhibited the highest spore densities and species richness of AM fungi. This may be due to the chemical composition of *Lantana* litter that has a higher C:N ratio and lower N and P contents than to the other organic amendments. Furthermore, it has been shown that decomposition rates and N and P mineralization from litter of these and similar plants are highly correlated to their total P content (Kwabiah *et al.* 2001) that would result in a lower decomposition rate of *Lantana* litter and may influence the composition of the AM fungal community.

The concurrent change in the community composition of bacterial PLFAs and AM fungal spores with amendment treatments is noteworthy. This correlation could occur because bacteria and AM fungi are responding to similar environmental cues. Alternatively, the relationship could indicate direct interactions among the bacteria and AM fungi, Rillig *et al.* (2006) showed that single-species cultures of AM fungi supported distinct PLFA profiles, suggesting that different species of AM fungi support particular bacterial communities. It is likely that both reasons explain the strong correlation between the community composition of bacteria and AM fungi. Future research is needed to evaluate these possibilities and assess the functional significance of different microbial consortia associated with different organic amendments.

Compost contained much more P than leaves from the agroforestry plants, but this did not seem to inhibit the production of AM fungal biomass because all organic amendments had a similar effect on NLFA 16:1ω5 and PLFA 16:1ω5. Also, mesh bags amended with compost did not result in a unique AM community, suggesting that in our study, P availability may have had less influence on the community composition of AM fungal spores than other chemical factors such as N content or C:N ratio, However, a more controlled experiment is necessary to isolate the effects of N and P on AM spore communities, The present study provides the first evidence that organic matter amendments may stimulate extraradical growth of AM fungi and bacterial biomass in eroded slopes in Nepal. Improved AM fungal growth can be expected to result in improved nutrient and water uptake by the plants and this suggests that organic matter addition may improve plant survival in restoration of highly eroded monsoonal ecosystems. The effects of our experimental treatments on plant survival in plantation experiments in these areas will be reported in future studies.

Implications for Practice

- Organic amendments significantly increase the biomass of soil microorganisms, including desirable AM-fungi,
- Organic amendment type is a simple means to control the development of distinct consortia of AM fungi and bacteria.
- Analysis of in-growth mesh bags is a powerful method to monitor the effects of restoration treatments on the biomass and community composition of AM fungi and bacteria in sites that are being restored. This could be especially useful if certain taxa of microorganisms are desirable because they perform particular ecosystems services needed for successful restoration.

Acknowledgments

We are grateful to Dr. Matthew Bowker for his help with the statistical analyses and constructive comments on the manuscript. We are also grateful to Dr. Malthias Rillig and Dr. Pal Axel Olsson for valuable comments on the manuscript and to Dr. D.P. Scherchan for providing the *Tithonia diversifolia* material. Financial support from the Swedish International Development Cooperation Agency. the National Science Foundation (DEB 0316136), and the Fulbright Commission of Sweden is gratefully acknowledged.

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Effect of Plant Residues on AM Fungi

Geeta Shrestha Vaidya, Keshav Shrestha and Hâkan Wallander

Abstract

In this study we have investigated the effect of organic matter on growth of an arbuscular mycorrhizal (AM) fungi in eroded slopes in Nepal such as Forest in Kavre District. Different types of organic matter (leaves of *Tithonia diversifolia, Eupatorium adenophorum, Lantana camara,* farm compost) and tri-superphosphate were mixed with eroded soil. The mesh bags were buried around trees of eroded site. The mesh bags were harvested after 6 months and the AM fungi in the mesh bags was quantified by analyzing the AM spores.

Introduction

Organic farming is the best choice that can help agriculture and environment. The fertility of soil is also influenced by the incorrect use of fertilizers and harmful effects of pesticides. Excessive use of chemical fertilizers (ammonium sulphate) causes acidity in the soil. The quantity of chemical fertilizer used per hectare in Nepal is very low as compared to other countries, but farmers regularly use chemical fertilizer in Kathmandu valley and some of the districts in Terai have started to experience its adverse effects on soil quality.

Soils of Nepal are deficient in N,P,K due to shortage of organic matter in the soils (Shrestha Vaidya *et al.* 2008). Therefore incorporation of organic matter is necessary for improvement and fertility of soil. Organic matter can replace the use of chemical fertilizers as much as possible.

In the early 1990s, however, fertilizer became a target of criticism mainly because of heavy use in developed countries, where it was suspected of having an adverse impact on the environment through nitrate leaching, eutrophication, greenhouse gas emissions and heavy metal uptakes by plants. Plant nutrition in future will require the judicious and integrated management of all sources of nutrients in agricultural fields and forest fields. Prevention of soil erosion is equally important for maintenanace of soil fertility and environment protection. So, there is an urgent need to control erosion prevent potential sediment disaster in Nepal (Shrestha Vaidya *et al.* 2002). Therefore mycorrhizal fungi are essential for the establishment of tree seedlings and for their good growth.

So, addition of organic matter can have a beneficial effect on the growth of indigenous AM fungi in nutrient limited soil (Caravaca *et al.* 2002; Gaur & Adholeya 2002). Organic amendments enhance spore production (Johnson & McGraw 1988, Douds *et al.* 1997, Shrestha Vaidya *et al.* 2008). Organic matter addition to the soil in eroded sites could thus be an appropriate to enhance the beneficial effect of AM fungi on soil stabilization and plant establishment and it also protects environment over the long term and reducing cost of production.

Materials and Methods

The eroded soil was collected from a degraded site at Bisankhunarayan. Forty five gm of eroded soil was put in nylon mesh bags and was mixed with different types of organic matter or left unmixed as control. We used 5 gm of dried leaves and dried compost (10% by weight) as organic matter. Fully expanded leaves of three easy available plant species *(Tithonia diversifolia, Lantana camara* and *Eupatorium adenophorum*) were collected from border rows in farmer's fields and near roadsides and the compost was collected from a local farmer. We also included one treatment of rock phosphate. In this case 45 mg tri-superphosphate were mixed with 50 gm of eroded soil. This represents approximately 40-50kg P per hectare which is an amount usually used by local farmers. The mesh bags that were used as controls were filled with 50 gm of eroded soil without any amendments. In total 6 treatments were included (control, *Tithonia diversifolia, Lantana camera, Eupatorium adenophortim,* compost and rock phosphate). The mesh bags were buried to a depth of about 10 cm where plant roots had the highest density (Shrestha Vaidya *et al.* 2008).

Spore Analysis

The AM fungal spores within 25 gm of the soil and amendment mixtures inside the mesh bags were extracted, identified and quantified. Spores were extracted using wet sieving and sucrose density gradient centrifugation (McKinney and Lindsey 1987). Spores were mounted in polyvinyl alcohol on slides and examined using a compound microscope. Species were identified to species using taxonomic characteristics described in INVAM (2005) and Schenck and Perez (1990).

Weighed 25 gm of soil samples, and mixed in a substantial volume of water and decanted through a series of sieves (750μ, 250μ, 100μ and 50μ) after allowing heavy soil particles to settle for a few seconds. This washing and decanting process was repeated until the water became clear. Roots and coarse debris were collected on a coarse. Then these finely kaolin clay remaining last sieve (50μ) transfer to centrifuge tube then water was added in equal weight in each four tube and then centrifuged it for 3 minutes at 2000 RPM. After this supernatant and floating debris was discarded.

The next step involved resuspending the pellet in 50% sucrose by vigorously shaking tightly stopper tubes. The samples were then centrifuged for 1 minute at 2000 RPM to separate spores from denser soil components. Immediately after centrifugation, spores in the sucrose supernatant were poured onto the finest sieve (50μ) and carefully washed with water to remove the sucrose.

After rinsing the spores, were washed onto a pre-wetted filter paper in a Buchner funnel before vacuum filtration. In this we used Whatman filter paper for spore counting. Semi permanent microscope slide preparations of spores were made using polyvinyl alcohol, lacto-glycerol (PVLG). Spores on microscope slides were squashed to reveal inner wall layers and then were used dissecting microscope for identification (Brundrett *et al.* 1996 and Schenck and vonne Perez 1990).

Results and Discussion

Eroded soil had extremely low levels of, N, P, K, pH and organic matter (Shrestha Vaidya *et al.* 2008). Addition of all types of organic matter had a strong positive influence on AM biomass (Fig. 1). Addition of organic matter had a positive influence on formation of AM spores. The mesh bags with compost, rock phosphate, or eroded soil contained significantly lower spore numbers compared to the three treatments with dried leaves. So in this study *Lantana* has shown better effect than *Tithonia* and *Eupatorium*. *Tithonia* has also shown good effect than *Eupatorium* but compost is better than tri superphosphate.

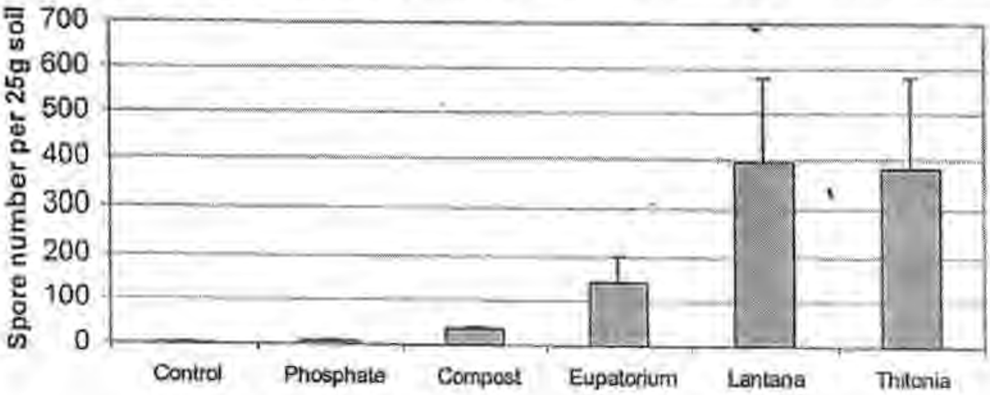

Fig. 1. Analysis of Endomycorrhizal spores (AM spores) in each mesh bag having different organic matter

The mesh bags had the following amendments: control (no amendment), rock phosphate, dried leaves of *Tithonia diversifolia, Lantana camara, Eupatorium adenophorum* and farmers compost.

The higher yields for the first crop with applications of the higher quality *Tithonia* are due to a combination of more P and N added and faster release patterns of P and N from *Tithonia* as compared to *senna*. In this result addition of *Tithonia diversifolia* was found better than the tri supephospate, same result was shown by Jama *et al.* (2000). Increased hyphal growth in the soil with increased organic C content was consistent for the two AM fungi (Joner and Jackboson, 1995).

We have seen that growth of AM in degraded soil is highly stimulated by the presence of organic matter. Different types of organic matter had similar positive influence on AM growth. Addition of rock phosphate on the other hand had no effect on AM growth. Number of AM spores was also positively influenced by organic matter addition. Added leaves of *Lantana camara* and *Tithonia diversifolia* had a stronger positive influence on AM spore formation than other organic materials.

The beneficial role of organic matter may also be related to an improvement of physical properties like increased soil porosity and reduced mechanical resistance to hyphal growth through the soil (Joner *et al.* 1995). Giovanetti and Avio (1985) found that additions of different materials, which increased the pore volume in soil, had a beneficial effect on mycorrhizal growth response, colonization and spore numbers.

Our result has shown that *Lantana* and *tithonia* are better than other treatments. Same type of report was reported by Nziguheba *et al.* (2000). He was reported that the effect of organic materials on resin P was dependent on the quality of the residue. *Tithonia* and *Lantana* with a high P content (>2.5gm kg^{-1}) resulted n high resin P values.

Improved nutrient and water uptake by the planted tree can expected in response to better AM growth and the positive effect on the growth of AM is in good agreement with results obtained by other authors, who also found that organic matter increased AM fungal hyphae growth (Labidi *et al.* 200x, Nicolson 1959; Koske *et al.* 1975; Joner and Jakobsen 1995) and AM spore formation (Douds *et al.* 1997; Baby and Manibhushanrao 1996; Muthukumar and Udaiyan, 2000; Jefflies and Barea 2001). In addition, St. John *et al.* (1983), Frey and Ellis (1997) and Friberg (2001) found that AM fungal hyphae grew best in soils with a high amount of organic matter which was shown in results.

Organic agriculture (OA) causes much less environmental pollution, particularly of ground water supplies. Moreover, nitrate leaching rates per hectare in OA systems are roughly half of those in conventional agriculture systems (Stolze *et al.* 2000 in FAO 2002).

OA techniques improve soil structure and increase soil organic matter, thereby improving the soil's water retention Capacity. It has the additional social benefit of augmenting supplies of drinking water (FAO 2002). The improved soil structure also reduces soil erosion, which is a leading cause of soil degradation worldwide with a negative impact on agricultural yields (FAO 2002). A long term trial study in Switzerland revealed 50 to 80 per cent more earthworms on organically managed farms as compared to conventionally managed ones (Pfiffner and Mader 1997 in FAO 2002). A separate study found that organic soils in Switzerland had up to 90 per cent higher total mass of microorganisms than conventionally managed soils (Fliessbah *et al.* 2001).

Conclusions

The present study provides the first evidence that addition of organic matter amendments may stimulate extraradical growth of AM. It also help for soil quality due to increasing rate endomycorrhiza (AM spores). The application of organic materials reduces the soil bulk density and hence increases total porosity, which has a positive effect on plant growth.

Acknowledgements

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Effect of *Glomus microcarpum* in Relation to the Biomass Production of Wheat Plants

Nirmala Dhungana, Prakash Raut and Anjana Singh

Abstract

Many studies have shown that mycorrhizal inoculations enhance the growth and nutrient uptake by plants. However, in Nepal, the focus of study is being given to the leguminous plants. To study the effect of vesicular-arbuscular mycorrhiza (VAM) in the biomass production of wheat, common VAM fungus *Glomus microcarpum* was inoculated in four varieties of wheat *(Triticum aestivum)* (i.e. WK 810, WK 1320, WK 1357 and WK 2089); the VAM spores being isolated by wet sieving and decanting process. Recording effects were started after one week of seed sowing, then weekly for growth parameters and on 15 days interval for edaphic parameters. In all cultivars, increased root and shoot biomass were recorded in inoculated ones compared to their non-inoculated counterparts. The reaction to the *G. microcarpum* inoculants was not different significantly for all cultivars when comparison was made on them on 89th day after inoculation, where the biomass values were recorded maximum. Also, phosphorus and organic matter contents of soil related inversely to the VAM fungal spore number in the soil and the root infection. This study demonstrates the potential use of *G. microcarpum* as biofertilizer in nutrient poor soils.

Introduction

The vesicular-arbuscular mycorrhizae (VAM) are most commonly reported groups since they occur on a vast taxonomic range of plants, both herbaceous and woody olants (Singh 2004). Roughly 90% of world plant species belonging to various families are thought to form symbiotic mycorrhizal association (Malajezuk *et al.* 1992). Mycorrhizal symbionts increase the efficiency of nutrient acquisition by plants and hence soil fertility Sharma 2001). These fungi build a living bridge between ...... root and bulk soil in most ecosystems (Singh 2004, Sharma 2001). VAM are formed by non-septate fungi. These fungi are obligate symbionts resulting from mutual symbiosis and have not been cultured on nutrient media. They cannot be grown in isolation in pure culture. The mycorrhiza-plant relationship is particularly important for agricultural plants that have large requirements for nutrients and water, basically phosphorus, to achieve optimum yield. These fungi excrete powerful chemicals that dissolve mineral nutrients, absorb water, retard soil pathogens, and glue soil particles together into porous structure In return, they receive sugars and other compounds from plants to fuel their activities. Research has documented improved plant nutrition and water uptake and resistance to a wide range of environmental extremes. Plants establish and yield more abundantly and require less require intensive care. This is one among the major reasons why plants from natural undisturbed areas can thrive without irrigation, fertilizer and pesticides. A major benefit conferred by VAM fungi on their host plants is stimulation of growth from the improved phosphate uptake by inoculated plant roots (Douds *et al.* 2000). Extraradical hyphae of mycorrhizae greatly enlarge the accessible soil volume for phosphorus acquisition (Li *et al.* 1991). In addition, phosphorus taken up is efficiently transported to plant roots (Smith & Smith 1990).

Despite the vast data available on microbiological aspects and their role, there has been no extensive research on VAM fungi and their effect in productivity of the crop plants. However, many fertilizers, are being used in the agricultural field for improved production in our country. Keeping in mind the significance of mycorrhizae in the productivity of crops, present investigation was carried out to observe role of *G. microcarpum* in the productivity of wheat plant *(Triticum aestivum)*. Among the various VAM fungi, this species was selected because of its higher rate of infection and more potentials in terms of productive value.

Methodology

The present study was carried out from January to April, 2006. Pot experiments were established in the garden of Nepal Academy of Science and Technology, Khumaltar, Lalitpur. *G. microcarpum*, a VAM fungus, was employed as root symbiont to develop a symbiotic system with the roots of wheat. Four varieties of wheat namely, WK 810, WK 1320, WK 1357 and WK 2089 were selected for this study.

For the collection of VAM fungal spores, soil samples were collected from the rhizosphere zone of various plants from the depth of 5-7cm from Godawari, Phulchoki and Chandragiri forest areas. VAM fungal spores were extracted from the soil by subjecting the soil samples to wet sieving and decanting (Gerdeman & Nicolson 1963). The spores were observed under stereoscopic binocular and identified according to original species description (Gerdeman & Trappe 1974, Hall & Fish 1979). The spores were picked up with the help of a sharp needle and collected in sterile distilled water.

The soil (red colored) for the experiment was procured from Godawari and sterilized by autoclaving at 121°C for 2 hours for three consecutive days. Earthen pots (23cm diameter) were sterilized with 70% (v/v) ethanol and filled with 5kg of the soil (clay loam- 30.7% sand, 41.4% silt, 27.9% clay, organic matter 0.776%, pH 7.6, nitrogen 0.084%, phosphorus 25.6 kg/ha, potassium 67.2%). The following treatments were imposed in a completely randomized design with five replications for each set of pot with soil: 1. plants inoculated with *G. microcarpum*, and 2. control plants without inoculation, for all four wheat varieties. Wheat seeds (25 seeds per pot) were sown in each pot. Inoculations with the fungal spores were done before

seed sowing (25 spores per pot). The plants were allowed to grow for 92 days of seed sowing. During the experiment, root and shoot fresh weight, root and shoot dry weight (dried in hot air oven at 80°C), % mycorrhizal colonization and spore number in rhizospheric soil were determined weekly after 15 days of seed sowing. For assessment of root colonization, roots were washed with 10% KOH followed by staining with 0.05% trypan blue in lactophenol (Phillips & Hayman 1970). The root pieces (1 cm) were selected at random from the stained samples and mounted on a microscopic slide in groups of 10. Presence of infection was recorded in each of the 10-pieces and was calculated according to Singh (2004)

$$\text{Number of coloniation} = \frac{\text{Number of colonized root segments}}{\text{Total number of segments examined}} \times 100$$

In all, 50 root segments were examined. For estimation of the spores of *G. microcarpum* in the same soil samples used for root colonization, spores were isolated by wet sieving and decanting method (Gerdeman & Nicolson 1963). Number of spores per 20 gm soil was calculated. Organic matter, pH, total nitrogen, free phosphorus and free potassium content of soil were determined at 15 days interval of seed sowing.

Results and Discussion

The oil was collected from rhizospheric areas of different plants of dry and undisturbed land for collection of spores (Fig. a) for inoculum as the mycorrhizal efficiency is more pronounced in such condition. Disturbance of land significantly reduces the inoculum potential of the soil i.e. propagules and/or stability of hyphal network (Reeves *et al.* 1979).

The native VA mycorrhizal population was less in black soil (vertisol) as compared to red soil (Hiremath *et al.* 1990). The black soils with sandy loam texture exhibited higher mycorrhizal activity than those with clay texture. The clay content in soil was also found to influence the VA mycorrhizal population. Owing to this, red colored soil (alfisol) was collected for our experiment.

G. microcarpum inoculations increased root biomass on all the days observed. Higher fresh and dry weight was found in inoculated plants in comparison to control plants. The weights of fresh and dry roots of inoculated plants were higher which might be due to the high number of lateral roots and root hairs, which eventually increased the root dry weight. Readings on all the days were higher in inoculated plants than in noninoculated ones. Similar result has been shown by Hetrick *et al.* (1985) that in well infected root, mycorrhiza constitutes about 10% increment of root biomass.

G. microcarpum inoculation increased the shoot biomass yield over control on all the days observed in this experiment. The increased shoot biomass production by the inoculated plants could be attributed to enhanced inorganic nutrient absorption, and greater rates of photosynthesis in inoculated plants. Similar result has been shown by Cooper (1985). It seems likely that in this study inoculation increased nutrient uptake by shortening the distance that the nutrients had to diffuse from the soil to the roots giving rises to a higher root and shoot biomass increment with a uniform growth. Many authors have reported a significant increment in shoot biomass, after inoculating the plants with mycorrhiza. Kung (2006) has demonstrated that there is significant increase in root and shoot biomass in VAM inoculated plants than in control plants. An increase in nutrient uptake, especially phosphorus in the infertile soil used, could have resulted in relief of nutrients stress and an increase in photosynthetic rate, which obviously could have given rise to an increase in plant growth. As reported by Klironomos *et al.* (2000), the combinations of plants and VAM fungi results in a more efficient extraction of nutrient from soil.

The maximum growth of the plants (root and shoot biomass for all cultivars) was observed on the 89th day after inoculation of *G. microcarpum*. After this the biomass decreased due to aging and drying up of the plants. So, comparison was made for this period among different wheat cultivars to find out better productive variety with inoculation of *G. microcarpum* in pot culture experiment. None of the wheat varieties could be most effective as they were all more or less similar in their root and shoot biomass, the difference was not significant ($P<0.05$), whereas the difference between the inoculated with their noninoculated counterparts was significant (Fig b, Fig. 1, 2, 3, 4). However, *G. microcarpum* showed better influence in terms of fresh root weight in WK 2089, dry root weight, fresh shoot weight and percent colonization in WK 1357, and in dry shoot weight in WK 810 in comparison to other cultivars.

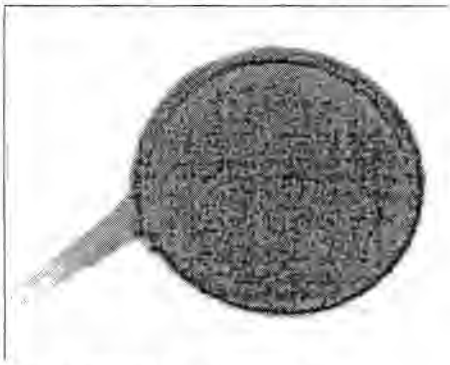

Fig. 1. Spore of *G. microcarpum*

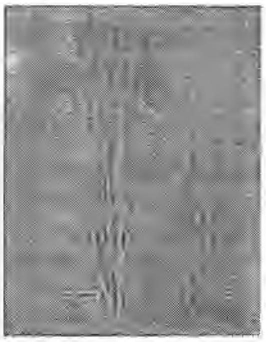

Fig. 2. Effect of *G. microcarpum* inoculants on wheat (WK 1357) on 82nd day after plantation

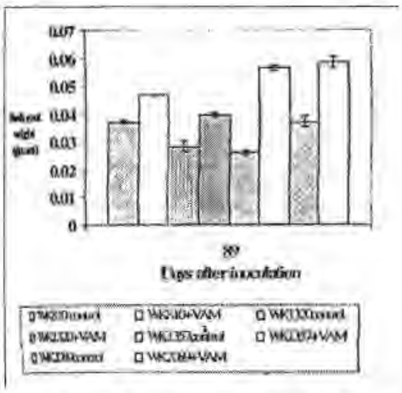

Fig. 3. Effect of VAM inoculation on fresh root weight of four varieties of wheat at 89th day after inoculation

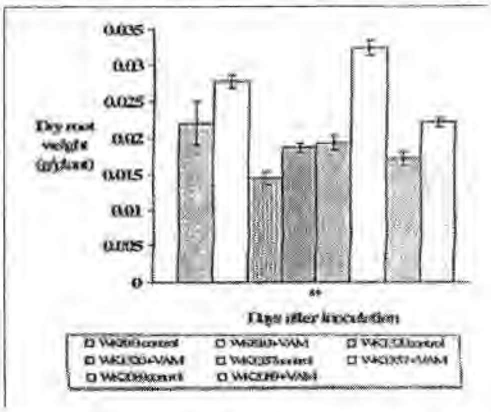

Fig. 4. Effect of VAM inoculation on dry root weight of four varieties of wheat at 89th day after inoculation

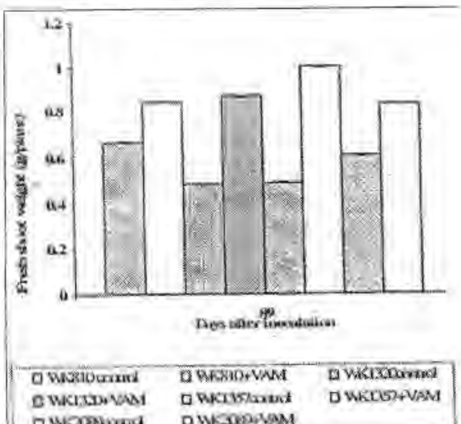

Fig. 5. Effect of VAM inoculation on fresh shoot weight of four varieties of wheat at 89th day after inoculation

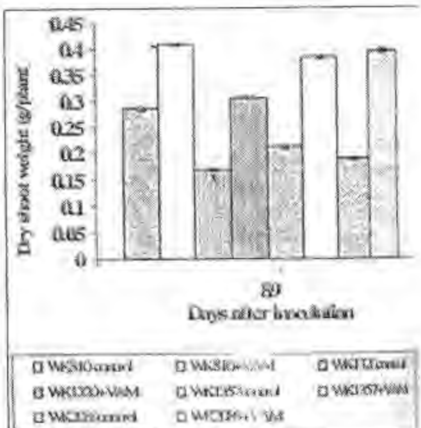

Fig. 6. Effect of VAM inoculation on dry shoot weight of four varieties of wheat at 89th day after inoculation

Many studies have shown that there is a lag phase between mycorrhiza inoculation and the time period when its effect is manifested in the plant (Brandon & Shelton 1993). Similar result has been observed in the present investigation also. This may be the reason that the inoculated plants in our experiment showed better results later on than those of only a few days after inoculation. The experiment was conducted free of contamination of any harmful creatures or chemicals as there was no marked change in pH. Also the plant growth was normal i.e. the root and shoot biomass was in increasing trend except in the last day of observation. The decrease in biomass on the last observation is due to ageing of plants as the consequence of death and fall of leaves and death of old roots.

In this investigation, the increment in percentage colonization of mycorrhiza with time was observed. This could have been because of the reason that the root system infected normally increases with time sigmoidally (Kung 2006). Negligible root colonization with VAM fungi during January (only up to 8% among the cultivars) sampling was observed when the plants were at the 4-to 5-leaf stage in all plots. Once established, the level of root colonization with VAM fungi increased with time, peaking at the heading stage in all plots. Very low levels (2-4% colonization) of VAM fungi in plant roots sampled at the 4-to 5-leaf stage suggests that the infection rate of wheat plants with these fungi was either into basically slow or that suboptimal (e.g., low temperature) soil conditions slowed colonization. Similar results have been found by Mohammad *et al.* (1998). The highest level of colonization by AM fungal inoculation was attained at heading stage of all cultivars (76% for WK 810 and WK1320, 78% for WK 1357, and 74% for WK 2089). The level of colonization declined (up to 70%) during the grain-filling stage. Similar results were reported by Cade-Menun *et al.* (1991), who suggested that as grains ripen, photosynthesis slows down and nutrients are translocated from the leaves to the grains, which reduce the photosynthate supply to roots and results in a decline in colonization observed during grain-filling.

In this investigation, the spore number in the rhizospheric soil of the inoculated plants was affected by the phosphorus and organic matter content and vice versa. Before inoculation as well as in control plants no spore could be observed, whereas in inoculated ones, 4-8 spores (per 20 gm of soil) were found among the cultivars during first reading peaking the values to 52 for WK 810, WK 1320 and WK 2089, and 60 for WK 1357, where the colonization percentage was also found highest. The free available phosphorus increased, in consequence the spore numbers decreased or remained steady. Phosphorus levels during first reading after inoculation were 133.4, 68.8, 164.2, and 141.1 for WK 810, WK 1320, WK 1357 and WK 2089 respectively.

But while taking last reading, values for respective varieties were found to be 25.65, 10.3, 87.2, and 35.9. Similar effect was observed in case of organic matter content also. When the spore numbers were highest, the value of organic matter was 0.676 for WK 810, 0.672 for WK 1320, 0.776 for WK 1357, and 0.724 for WK 2089; the figures were 0.853, 0.724, 1.008 and 1.241 respectively when the spore numbers were very low. Earlier research conducted by Raut (1994) has also observed similar results, where VAM fungi spore number was inversely correlated with the organic matter content and phosphorus status. As per the results obtained, hardly any prediction could be made for the pH, potassium and nitrogen content status and their relation with the VAM fungi; they were not found to show any marked effect that may be due to short period of time to show the effect. However, Raut & Mukerjee (2001) have shown that when acidic level increases, VAM fungi decrease.

On the basis of results of this research, *G. microcarpum* would be an effective biofertilizer if inoculated in WK 1357 cultivar for better production and yield in future.

Plant Growth Promotional Effect of *Azotobacter chroococcum*, *Piriformospora indica* and Vermicompost on Rice Plant

Kamil Prajapati, Kayo D. Yami and Anjana Singh

Abstract

The activities of rhizospheric organisms have been well recognized in nonleguminous plants such as tropical grasses, rice and maize. Such activities include nitrogen fixation, phosphate solubilization and mineralization that are beneficial for the overall growth and development of the plant. An experiment was carried out to study the growth promotion of rice *(Oryza sativa* L.) due to dual inoculation of *Azotobacter chroococcum* and *Piriformospora indica* along with vermicompost. The effects on shoot length, root length, fresh shoot and root weight, dry shoot and root weight, and panicle number on 45th day and 90th day were investigated. In both the stages these parameters of all the treated plants were significantly ($P<0.05$) greater than the uninoculated control. Dual inoculated plants in presence of vermicompost gave better positive effects on both 45th day and 90th day, in comparison to single inoculation of A. *chroococcum, P. indica* and vermicompost. However, *A. chroococcum* treated plants showed significant decrease in dry root weight as compared to control plants on 90th day observation. This suggested that dual inoculation of *A. chroococcum* and *P. indica* had beneficiary response on growth of rice plant.

Introduction

The agriculturally important rhizospheric microorganisms play a remarkable role in nutrient acquisition for plants that ultimately lead improved growth parameters. In pursuit of that goal, various workers have used arbuscular mucorrhizal fungi and nitrogen fixing bacteria, as single inoculants and in combination with each other in various plants (Lauchli & Bieleski 1983).

Azotobacter represents the main group of heterotrophic free living nitrogen-fixing bacteria. They are Gram negative, large ovoid pleomorphic cells of 1.5-20 μm or more in diameter ranging from rods to coccoid cells. They occur singly, in paired or irregular clumps and sometime in chains of varying length. They do not produce endospores but form cysts. They are motile by peritrichous flagella or non motile. *Azotobacter* spp. are most specifically noted for their nitrogen fixing ability but they have also been noted for their ability to produce different growth hormones (IAA and other auxins, such as gibberllins and cytokinins), vitamins and siderophores (Narula *et al.* 1981, Neito & Frankenberger 1989, Tindale *et al.* 2000). *Azotobacter* is capable of converting nitrogen to ammonia (Bishop *et al.* 1980), which in turn is taken up by the plants.

Vesicular arbuscular mycorrhizae (VAM) are beneficial fungi that penetrate and colonize the roots of the plant, then send out filaments (hyphae) into the surrounding soil. The term mycorrhizae literally means fungus-root and VA mycorrhizae (VAM) are considered endomycorrhizal since they colonize the interior parts of the roots. They are associated with the plant in a mutually beneficial relationship. The VAM fungi, nestled inside the roots, send out long filaments or hyphae to explore up to the soil area available to the root alone. The hyphae literally form a bridge that connect the plant root with large areas of soil and serve as a pipeline to funnel nutrients back to the plant. In return, the plant must supply the VAM fungi with carbon for the fungal growth and energy needs.

An axenically cultivable mycorrhiza like fungus was discovered by Varma and his co-workers (Varma *et al.* 1998). The fungus was named *Piriformospora indica*, based on its characteristic pear-shaped spores. Based on the 18S and 28S rRNA analysis and he ultra- structural details of the septal pores, the phylogenetic relationship of this fungus was established within the Hymenomycetes (Basidiomycotina) and closest to Rhizoctonia group and *Sebacina vermifera* (Varma *et al.* 1998, Singh 2004). *P. indica* is the first symbiotic fungus, known in the literature which can be grown on root of a living plant and under axenic culture (Singh 2004). The properties *P. indica* have been patented at the European patent office, Muenchen, Germany (Patent No. 97121440.8-2105, Nov. 1998). It enters the root cortex to form inter and intracellular hyphae. Within cortical cells the fungus often forms dense hyphal coils or branched structures intracellularly. *P. indica* also forms spore or vesicles like structures within or between the cortical cells. Like in AM fungi, hyphae multiply within the host cortical tissues and never traverse through the endodermis. Likewise, they also do not invade the aerial portion of the plant (stem and leaves). Interestingly, the host spectrum of *P. indica* is very much like AMF. The hyphae are highly interwoven, often adhered together and give the appearance of a simple intertwined cord. Hyphae are thin-walled and of different diameters ranging from 0.7 to 3.5 μm. The septate hyphae often show anastomosis and are irregularly septate. They often intertwine and overlap each other. Chlamydospores are formed from thin- walled vesicles at the tips of the hyphae. The chlamydospores appear singly or in clusters and are distinctive due to their pear shaped appearance. The chlamydospores are 16-26 μm in length and 10-17 μm in width. The cytoplasm of the chlamydospores is densely packed with granular material and usually contains 8-25 nuclei. Very young spores have thin, hyaline walls. At maturity, these spores have walls up to 1.5 μm thick, which appear two-layered, smooth and pale yellow. Neither clamp connections nor sexual structures are observed (Varma *et al.* 2002, Singh 2004).

Vermicomposting is largely a biological process in which aerobic and anaerobic microorganisms decompose organic matter and lower the carbon-nitrogen ratio of the substrate. Vermicompost contains major and minor nutrients in plant-available forms, enzymes, vitamins and plant growth hormones (Kerala Agricultural University 2002). It is an excellent soil additive made up of digested and undigested compost Earthworm castings are much higher in nutrient contents than microbial life and have a high value product.

Materials and Methods

The rice variety used in this study was Khumal-4, which was collected from Annapurna Seed Bhandar of Kathmandu valley. A pure culture of *P. indica* was obtained from the Central Department of Microbiology, Kirtipur while *A. chroococcum* was isolated from vermicompost at the Biotechnology Laboratory of Nepal Academy of Science and Technology (NAST), Khumaltar.

A. chroococcum inoculum was prepared in Jensen's broth on a rotary shaker for 5-7 days at 26 °C - 28 °C at 20 rpm. Literature suggests that for plant inoculation, the cultures should reach mid-log phase, which is equivalent to 10-10 cells per mL (Zhang *et al.* 2002). *P. indica* inoculum was prepared on PDA by incubating at 25 °C- 27 °C for about one week. *P. indica* culture was cut into pieces of 4 mm diameter. Vermicompost was obtained from NAST, Khumaltar.

There were five treatments which were control plants without any inoculation (T1), plants inoculated with vermicompost only (T2), plants inoculated with *A. chroococcum* alone (T3), plants inoculated with *P. indica* alone (T4) and plants inoculated with *P. indica, A. chroococcum* and vermicompost (T5). The earthen pots used for the pot culture experiment were filled with sterilized soil. Vermicompost was initially added to the soil. Ten healthy seeds were sown in each pot at a depth of 2 cm of soil and left for germination. After 7 days of seed germination, the *P. indica* inoculum was carefully placed near the root surface. After 5 days of the application of *P. indica,* 1 mL *Azotobacter* broth with inoculum size 1.6 x 10^7 cfu/mL was applied to each plant per pot. After 25 days of seed sowing the seedlings were thinned manually leaving eight healthy seedlings per pot. Growth parameters (shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and panicle number) were measured on 45th (vegetative stage) and 90th (reproductive stage) days of cultivation.

Results

Effect of dual inoculation of *A. chroococcum* and *P. indica* on various growth parameters of rice plant at vegetative (45th day) and reproductive (90th day) stages grown in the soil treated with vermicompost have been presented in Table 1 and 2 respectively. Significant differences were observed in all of the treatments for all the parameters at $p<0.05$. All the growth parameters showed positive response against the treatment over control except dry root weight in plants treated with *A. chroococcum* in reproductive stage where negative response was observed against control.

Table 1. Effects of treatment on growth parameters of rice plant at vegetative stage

Treatment	Shoot length (cm)	Root length (cm)	Fresh shoot wt. (gm)	Dry shoot wt. (gm)	Fresh root wt. (gm)	Dry root wt. (gm)
Control	31.33^a	10.68^a	0.68^a	0.22^a	0.24^a	0.05^a
T1	35.63^b	14.75^b	1.22^b	0.27^a	0.42^a	0.07^a
T2	35.0^b	13.71^b	0.84^a	0.24^a	0.36^a	0.12^b
T3	36.7^b	15.38^b	1.03^a	0.4^b	0.76^b	0.18^b
T4	37.38^b	17.08^a	1.74^c	0.66^c	0.93^b	0.25^b
Mean	35.21	14.32	1.10	0.36	0.54	0.14
LSD at 0.05	2.23	0.95	0.10	0.08	0.21	0.09

Note: In a column followed by a common letter are not significantly different at the 5% level by MRT

Table 2. Effects of treatment on growth parameters of rice plant at reproductive stage

Treatment	Shoot length (cm)	Root length (c)	Fresh shoot wt. (gm)	Dry shoot wt. (gm)	Fresh root wt. (gm)	Dry root wt. (gm)	Panicle Number
Control	100.67^a	35.21^a	23.31^a	16.39^a	19.81^a	9.7^a	0.79^a
T1	101.21^a	35.26^b	24.21^a	17.96^b	26.19^b	10.34^a	2.64^b
T2	100.88^a	35.22^b	24.06^a	16.56^a	20.14^a	8.95^a	2.43^b
T3	101.19^a	37.86^b	29.05^b	18.56^b	23.33^b	10.33^a	2.7^b
T4	107.18^b	39.63^b	32.29^c	23.38^c	38.54^c	16.93^b	3.6^c
Mean	102.23	36.64	26.59	18.57	25.60	11.25	2.41
LSD at 0.05	2.02	2.23	1.56	0.73	2.38	0.97	0.31

Note: In a column followed by a common letter are not significantly different at the 5% level by MRT

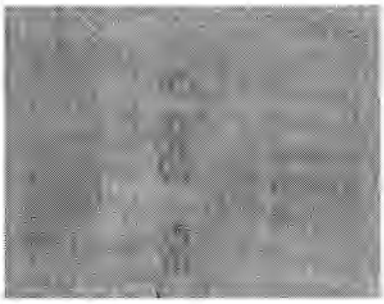

Fig. 1. Chlamydospores of *P. indica* within the cortical cells

Fig. 2. Effect of treatments on growth of rice plant at vegetative stage

Fig. 3. Effect of treatments on growth of rice plant at reproductive stage

Discussion

The experiment was conducted in sterilized soil so that effect of the inoculant could be studied without the interference of normal flora and fauna of the soil. The phytopromotional effect of treatments at vegetative and reproductive stages are shown in Fig. 2 and 3.

A significant positive response was observed with single inoculation of *A. chroococcum* in all growth parameters (viz. shoot length, root length, shoot fresh and dry weight, root fresh and dry weight and panicle number) of both vegetative and reproductive stages of rice plants can be attributed to the ability to fix atmospheric nitrogen. Nitrogen is usually the nutrient that limits plant production in wetlands (Buresh *et al.* 1990) and, under N-limited conditions, plant roots excrete compounds with high C/N ratios, favoring rhizospheric N_2 fixation (Klein *et al.* 1990). Till now, the explanations offered to account for the beneficial action of non-symbiotic microorganisms on plants have been two fold, first is the nitrogen fixing ability of the microorganisms and second is the ability of microorganisms to elaborate growth promoting substances such as vitamins, hormones and amino acids (Azcon *et al.* 1978). Shende *et al.* (1977) attributed the observed beneficial response of crop plants to inoculation with *A. chroococcum* to growth substances produced by the organisms in addition to the fixed nitrogen made available to the plants. In a similar study done by Bank and Goswami (2003), seed inoculation with *A. chroococcum* strains significantly influenced the growth attributes, yield attributes and yield of wheat. So, plant growth promotion in terms of shoot length, root length, shoot fresh and dry weight, fresh root weight and panicle number of both vegetative and reproductive stage over the control by *Azotobacter* inoculation may be due to synergistic effects of several factors. But the decrease in dry root weight in reproductive stage in *Azotobacter* inoculated plants though the length and dry weight of root is superior to the controls, may be due to variable water content that is dependent in plants water status.

The rice plants inoculated with *P. indica* showed significant beneficial effect in all the growth parameters in both vegetative and reproductive stages over the uninoculated control plants. *P. indica* inoculated plants were superior to *A. chroococcum* inoculation and vermicompost treatment in shoot length, root length and dry shoot weight in vegetative stage, and root length, dry shoot weight and panicle number in reproductive stage. In case of fresh root weight in vegetative stage and dry root weight in reproductive stage, the effect of *P. indica* was the same as that of vermicompost. *P. indica,* an endophytic fungus of the Sebacinaceae family, resembles AM fungi in several functional and physiological characteristics. It improves the growth and biomass of wide host range including rice plant and is an efficient phosphate solubilizer and transporter. *P. indica* is able to enhance the absorption of nutrients from the soil which could have moved to the roots principally by mass flow, in addition to those, which could have diffused through the soil slowly. It is envisaged that AM fungal mycelium acts as a key component in a close 'cause and effect interchange' of mineral nutrients carbon compounds, and signal between the plant and rhizosphere population and soil aggregation. Maize another important cereal crop was reported to produce more root and shoot biomass than control plants when inoculated with *P. indica* (Singh 2004). Waller *et al.* (2005) studied the interaction of *P. indica* in monocotyledonous plants by establishing *P. indica* barley system in the laboratory. Infestation of barley roots with *P. indica* led to growth promotion and a modulation of resistance not only in the roots, but also in the leaf.

Beneficial effect on host plant as a result of mycorrhizal infection is usually associated with improved plant nutrition, especially phosphorus by virtue of extensive root system that extend the functional mycelium into surrounding soil, making a greater pool of nutrients available to the plant. This leads to increased plant growth, often as high as several hundred-fold increases in biomass (Menge 1983). *P indica, an* AM like fungi, has been found to mediate phosphorus. uptake from the medium and translocate it to the host in an energy dependent process by producing significant amount of acid phosphates for the mobilization of broad range of insoluble forms of phosphates, enabling the host plant the accessibility of adequate phosphorus from immobilized reserves in the soil (Varma *et al.* 2000).

Plants treated with vermicompost also showed significant improved growth over the untreated control plants in both vegetative and reproductive stages. Vermicompost treated plants were even promoted than *A. chroococcum* in all the above measured parameters except in case of shoot length in reproductive stage themeas its treatment was better than *P. indica* in fresh shoot weight and dry root weight in vegetative stage and shoot length and fresh root weight in reproductive stage.

Vermicompost is an excellent soil additive that contains water soluble colloidal worm cast, nitrogen, phosphorus, and potassium contents along with a large amount of enzymes and growth hormones. When such compost is applied to the plants, due to the water soluble nature of vermicompost, the nutrients they contain become easily available to the plants roots. Basnet (2006) found that vermicompost sample of cow dung contain organic matter (50.25%), nitrogen (1.96%), phosphorus (2.33%) and potassium (4.99%). The vermicompost sample contained several times more nitrogen, phosphorus and potassium than the soil sample. These added mineral nutrients may be the factors responsible for the enhanced growth of the vermicompost treated plants.

In the pot culture trial, plants treated with dual inoculation of *A. chroococcum* and *P. indica* in vermicompost treated soil showed significant beneficial effect on all the measured parameters.

Acknowledgement

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Selective Utilization of Organic Solid Wastes by Earthworm *(Eisenia foetida)*

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Abstract

Rapid growth of population in Kathmandu valley has increased solid wastes generation tremendously. One of the best ways of managing the organic wastes is to recycle domestic wastes at the site of its origin by vermicomposting into valuable organic fertilizers. A laboratory experiment was carried out for proper management of solid wastes of Kathmandu valley, generated from Ayurveda industry, sugar mill (bagasse), wood mill, kitchen, and vegetable and fruit markets. The experiment dealt with the decomposition of solid wastes through the action of red worm *(Eisenia foetida)*. The vermicomposting of mixtures were carried out for 12 weeks. Observations showed that vermicompost obtained from Ayurveda industry wastes was found to be rich in N, P, K and organic matter, and vermicompost from sugarcane bagasse was found best for rapid multiplication of *Eisenia foetida*. Fish scales and sawdust were identified as worst subtrate for this worm.

Introduction

Green revolution of 1960s tremendously enhanced the agricultural production mainly due to the abundant use of chemical fertilizers and pesticides. However, unilateral use of chemical fertilizers and pesticides, devoid of organic sources, has made our soil sick and problematic, putting agricultural productivity at risk. The awareness of organic matter and concept of sustainable agriculture is gaining impetus among farmers in recent years to produce consumable agricultural products. The national average use of organic manure is about 2.5 to 3 ton/ha/yr (TSS 2000). At this rate of manure application, it is not possible to meet the nutrient demand of crops. Incorporation of earthworm in bio-composting process has been considered to be an appropriate technology for bio-waste management for producing nutrient enriched compost. Various investigators have established the viability of the technology using earthworms as a treatment system for different wastes (Harris *et al.* 1990, Logsdon 1994, 2000, Elvira *et al.* 1997, Yami *et al.* (2004).

Growing urbanization and increasing trend of mass migration of population in Kathmandu valley leads to more solid waste generation in urban area, turning the cities very dirty and unhygienic. It has been estimated that 83% of the waste dumps are municipal, 11% agricultural and 6% industrial in Kathmandu valley. On an average one person produces 0.22 to 0.50 kg of wastes in a day. The problem of solid waste management remains unsolved because lack of appropriate technical know how, lack of people's participation and lack of resources. The key management problems are lack of organizational structure, high capital and operating costs and insufficient market development for compost.

In recent years the concept of reuse and recycle is appearing as an acceptable approach to deal with the solid waste problem in community level in Kathmandu valley. However, it needs a systematic handling of the waste and may appear very complicated until a well managed system is established. Perhaps, because of this reason, dumping is often considered as a key solution to solid waste problem in most developing countries including Nepal. Taking the whole volume of solid waste to the dumping site may produce an immediate credit to the responsible authorities but it would not be sustainable way of waste managements.

Incorporation of earthworm in bio-composting process has been considered to be an appropriate technology for bio-waste management for producing nutrient enriched compost especially in urban areas. Earthworm species suitable for vermicomposting are *Eudrillus eugeniae, Eisenia foetida, Lamitt mauritii, Periony excavatas* and *Pheretima elongate*. Among them, *E. foetida* is considered the best for decomposing organic wastes, because of its wide temperature tolerance (Edwards & Baxter 1992). Worm cast contain five times more nitrogen, seven times more phosphorus and eleven times more potassium than ordinary soil, the main minerals needed for plants growth. It also contains a lot of beneficial soil microorganisms, which have at least as much to do with the plant growth and soil fertility. The microorganisms in the worms gut also produce useful compounds like antibiotics, vitamins, plant growth hormones, etc, all of which are also present in the castings.

The main objectives of this research were to study vermicomposting processes in different types of solid wastes as feeding materials to determine the rate of vermicomposting and to analyse chemical composition of vermicompost obtained from various substrate.

Material and Methods

The vermicomposting experiment was conducted at the Biotechnology Laboratory, Nepal Academy of Science and Technology (NAST), Lalitpur Nepal. The process was carried out during summer (June 2005 to September 2005).

Collection of wastes

Agricultural, industrial and kitchen wastes were used as substrate for earthworms. Agricultural wastes included rice straw, cow dung, fruit waste and green vegetable wastes. Kitchen wastes included egg shells, fish scales and vegetable wastes. Industrial wastes included the Ayurveda industry, wastes from Singhdarbar Baidhkhana, saw wastes from wood mill and sugarcane bagasse from juice centres. The newspapers were also collected for this study.

Preparation of bedding

Plastic buckets of 12 liters capacity were taken and four small holes at the bottom and several holes around the sides were made to allow aeration and avoid excessive liquid. The agricultural wastes such as straw were cut into 4 cm long pieces and used as bedding for the earthworm. The bedding in each of thirty buckets was made up of straw, cow dung and vermicompost. Water was sprinkled on the bedding just to keep it moist. Twenty five *earthworms (Eisenia foetida)* were placed in each bucket except in control.

Vermicomposting

Altogether ten types of substrate treatments were done using kitchen, agricultural and industrial waste. The treatments were fruit, vegetable, compost, cocktail sugarcane, wood, ayurveda industry waste I (boiled and non-woody), ayurveda industry waste II (non-boiled and woody), fish scales and control. Each treatment had three replicates (Table 1). The respective substrates were added in each treatment for first two weeks. During vermicomposting, the contents of the bucket were turned carefully by least disturbing the earthworms. The weight of compost contents and number of earthworms in each bucket were recorded in every two weeks. The buckets were kept closed throughout to avoid direct sunlight and rain. After complete decomposition of all substrates (after 12 week), the earthworms were removed from vermicompost and its samples were used for chemical analysis.

Table 1. Details of treatments of the vermicompost experiment (weight in gm)

S N	Substrate type	Rice straw	Cow dung	Fruit	Fish scales	Saw dust	Veg waste	Waste paper	Sugercane bagasse	Pigeon excreta	Ayur Industry Waste I	Ayur Industry Waste II	Total weight	Final weight (after 2 weeks)
1	Fruit	200	500	1000									1700	3000
2	Vegetables	200	500	300			1000						2000	3000
3	Cowdung	300	1000	300									1600	3000
4	Cocktail	200	500	500			450	50		300			2000	3000
5	Sugarcane bagasse	200	500	300					1000				2000	3000
6	Saw dust	200	300	200		300							1000	2000
7	Ayerveda industry waste I	200	500	300							1000		2000	3000
8	Ayerveda industry waste II	200	500	300								1000	2000	3000
9	Fish scales	200	300	200	300								1000	2000
10	Control	500	1000	300									1800	3000

Chemical analysis

Chemical analysis was carried out at Soil Science Division, Nepal Agricultural Research Council (NARC), Khumaltar. The samples were analysed for total nitrogen, available phosphorus and potassium contents, total organic carbon and pH. The parameters were determined in accordance with the procedures of standard methods.

Results and Discussion

Vermicompost samples were blackish moist and highly porous with the smell of earth. During the process of composting, the substrate was added continuously for the first two weeks. Thereafter, the total loss of weight of the wastes were recorded in every two weeks. The decomposition rate of different types of substrates by *E. foetida* was found variable depending upon the waste type (Table 2). The decomposition of fruit waste was found faster. It took six weeks for complete decomposition. The faster decomposition of fruit waste was also recorded by Yami and Shrestha (2005) and Simko (2000). Vegetables and cow dung decomposed in seven weeks, followed by cocktail, which took eight weeks. In cocktail, decomposition of newspaper was very slowly. Similar result was also recorded by Yami and Shrestha (2005), and Pradhan and Tamrakar (1999). A total of ten weeks were required for the complete decomposition of boiled, non-woody, ayurveda industry waste (ayurveda industry waste I) whereas sugarcane and non-boiled and woody ayurveda industry waste (ayurveda industry waste II) decomposed completely in 12 weeks (Table 3). The bucket containing saw dust and fish waste were not decomposed and the earthworms were found dead after a week. The bucket containing fish waste contained larvae of insect and was producing very strong foul smell. Similar observation was also made by Yami and Shrestha (2005) in cooked meat. In the present study, the fruits were found to be the best substrate whereas the saw dust and fish waste were found to be the worst for *E. foetida*.

Table 2. Time taken for complete decomposition of substrates by *E. foetida*

S.N.	Substrate used	Total weight (gm)	Time taken
1	Fruit	3000	6 weeks
2	Vegetable	3000	7 weeks
3	Cowdung	3000	7 weeks
4	Cocktail	3000	8 weeks
5	Sugarcane bagasse	3000	12 weeks
6	Sawdust	2000	No decomposition
7	Ayurveda industry waste I	3000	12 weeks
8	Ayurveda industry waste II	3000	10 weeks
9	Fish scales	2000	No decomposition
10	Control	3000	16 weeks

The death of the earthworm in saw dust treatment was most possibly due to the lack of sufficient aeration whereas in case of fish-waste treatment, it may be due to highly proliferating larvae and egg of insects, which created adverse environment for earthworm growth. Ismail (1994) and Tamrakar (2005) explained that the amendment with sawdust seemed to promote biomass for worms.

The number of earthworms were counted in every two weeks. The biomass potential of the worms is an important aspect for quick biodegradation of organic pastes (Guerro 1981). The number of earthworms was found to be the highest in sugarcane treatment (418), followed by ayurveda industry waste I (372) (Table 3). The ayurveda industry waste-II also contained good number of earthworms (327). The number of juvenile and cocoons were observed much higher in sugarcane. The increment in the number of earthworms was found slow in sugarcane and ayurveda industry wastes at the beginning but it increased rapidly in later observations. This may be due to the lack of sufficient food in the beginning, because of slow decomposition rate of sugarcane and ayurveda industry wastes. The earthworm grown on ayurveda industry wastes (I, II) were found to be healthier than any other treatments. The least number of earthworms was found in the cow dung treatment (135). There was no multiplication of the earthworm in the sawdust and fish waste and the inoculated earthworm were found to be dead (Table 3). This observation reflected the fact that sugarcane and ayurveda industry wastes are good for rapid multiplication of the earthworms whereas saw dust and fish waste were worst for the rapid multiplication of earthworms.

Table 3. Earthworm population in due course of vermin composting

		Mean number of earth worm (weeks)						
		0	2	4	6	8	10	12
1	Fruit	25	35	45	78	132	271	335
2	Vegetable	25	32	39	59	97	167	203
3	Compost	25	28	35	52	92	108	135
4	Cocktail	25	30	40	54	102	152	213
5	Sugarcane bagasse	25	27	34	78	187	278	418
6	Saw dust	25	0	0	0	0	0	0
7	Ayurveda industry wate I	25	26	36	60	158	237	372
8	Ayurveda industry waste II	25	27	38	62	169	303	327
9	Fish scales	25	0	0	0	0	0	0
10	Control	0	0	0	0	0	0	0

The pattern of the weight loss of contents of the vermicompost and the observation of the process during the period of study showed that the fruit waste was utilized most rapidly (highest weight loss 59%), followed by vegetable (54%). Yami and Shrestha (2005) also found the highest weight loss in fruit waste treatment (Table 4). The weight loss was observed very less in ayurveda industry waste I, sugarcane and ayurveda industry waste II i.e. 29%, 37% and 41% respectively. The overall weight loss during composting was observed in the range of 29% to 59.5%. Singh and Kumar (2004) found 25% weight loss in fruit vermicompost during vermicomposting process. In the view of large scale production of vermicompost, the sugarcane and ayurveda industry wastes are most suitable as their weight loss during vermicomposting were observed to be least (Table 4).

Table 4. Mean weight of vermicompost contents at an interval of one or two weeks

	Mean weight loss (kg)						
	Initial	2nd week	4th week	6th week	8th week	10th week	12th week
Fruits	3	2.3	1.9	1.7	1.4	1.3	1.2
Vegetable	3	2.5	2.1	1.9	1.5	1.3	1.3
Cowdung	3	2.4	2.3	2	1.8	1.71	1.71
Cocktail	3	2.7	2.4	2.3	2.1	1.9	1.8
Sugarcane bagasse	2.4	2.26	2.09	1.8	1.6	1.6	1.5
Saw dust	2	0	0	0	0	0	0
Ayurveda industry waste I	3	2.76	2.37	2.25	2.1	2.1	2.1
Ayurveda industry waste II	3	2.7	2.5	2.37	2.13	1.9	1.7
Fish scales	2	0	0	0	0	0	0
Control	3	2.7	2.5	2.3	2	2	1.7

The vermicomposts from different substrates were found rich in N, P, and K contents (Table 5). Nutritional composition of vermicompost varied with substrate used. The total nitrogen content of vermicompost was found higher than the control. The total nitrogen content of the vermicompost increased with time, due to rapid mineralization of organic nitrogenous compounds. Similar observation was also made by Bansal and Kapoor (2000) showing increased nitrogen content as a result of carbon loss in vermicomposting of crop residues and cattle dung. The nitrogen content of the vermicompost depends on the initial nitrogen concentration of the waste. Further enhanced decomposition results in lowering of C:N ratio (Talashilkar *et al.* 1999). Maximum nitrogen content (2.42%) was found in vermicompost prepared from fruits followed by ayurveda industry waste I (2.35). Nitrogen content was found equal in both vermicompost prepared from sugarcane bagasse and vegetable wastes (2.31). The nitrogen content was found to be least (1.99%) in the cocktail. The least nitrogen content was found in control (0.45%). Yami and Shrestha (2005) also found least nitrogen content in control treatment.

Table 5. Chemical analysis of vermicompost

S.N.	Name of sample	pH	OC (%)	N (%)	P (%)	K (%)	Organic matter (%)
1	Fruit	9.1	16.65	2.42	0.54	3.0	28.7
2	Vegetable	9.3	14.7	2.31	0.56	2.15	27.93
3	Compost	9.8	16.2	2.27	0.82	3.1	27.93
4	Cocktail	9.5	14.4	1.99	0.55	2.45	24.86
5	Sugarcane bagasse	8.4	12.15	2.31	0.57	3.05	20.95
6	Ayuerveda I	10.2	15.9	2.35	0.68	3.65	27.41
7	Ayuerveda II	9.3	12.5	2.20	0.64	3.5	20.95
8	Control	7.8	14.65	0.45	0.30	0.25	25.17

Available phosphorus was found to be highest in compost (0.82%) followed by Ayerveda industry waste I (0.68). Potassium was observed highest in ayurveda industry waste I (3.65%). The organic matter was found to be highest in fruit (28.7%) followed by compost and vegetable, i.e. 27.93% respectively. The least organic matter content was found in ayurveda industry waste II and sugarcane (20.95%).

In the present study, the mineral content was found to be higher in vermicompost produced by *E. foetida* than in control (Table 5). Padamaja *et al.*(1995) found that compost produced by *E. foetida* and Perionyx species is better than produced with conventional method. The result was also supported by the finding of Yami and Shrestha (2005) and Tamrakar (2005).

The findings revealed that decomposition process was enhanced by the presence of earthworm and aerobic heterotrophic population of microorganisms. In the present study, it was found that the most utilizable substrates for *E. foetida* in relation to time was fruit. The vermicompost obtained from ayerveda industry waste I was found to be rich in N(2.35), P(0.68), K(3.65%) content and the weight loss during vermicomposting was least for the same. Vermicompost from sugarcane bagasse was also found rich in N(2.31), P(0.57), K(3.05) contents and was best for rapid multiplication of *E. foetida*. Weight loss during vermicomposting was also observed to be lesser in sugarcane. Ayurveda industry waste and sugarcane bagasse are commonly found wastes in bulk form. In this connection, ayurveda industry waste and sugarcane bagasse can be taken as most suitable substrates for mass production of organic fertilizer through vermicomposting. In this study earthworm could not utilize sawdust and fish scales. However, the sawdust mixed with other suitable substrates could be recycled. From the study it could be concluded that vermiculture biotechnology could be widely used for the management and recycling of agricultural wastes, traditional medicinal plant wastes and sugar bagasse, reducing bulk and the level of pollution at the generation site itself.

Ethnomycological Uses of Some Wild Mushrooms in Sagarmatha National Park, Nepal

Prabina Rana and Anjana Giri

Abstract

Ethnomycologieal study was conducted in Sagarmatha National Park and its buffer zone in Solukhumbu region (2500-4000 m asl). A total of 150 species of mushrooms were collected. Among the collected species 26 species were identified as edible mushrooms, two as medicinal and one as decorative by ethnic groups. *Boletus pulverulentus* Opat., *Boletus auripes* Peck, *Chroogamphus tomentosus* (Murr.) O.K. Miller, *Russula metachroa* Hongo, *Tylopilus eximus* (Peck) Singh, and *Hypholoma capnoides* (Fr.) Kumm., *Paxillus involutus* (Batsch: Fr.) Fr. were found new to the list of edible mushrooms of Nepal. The study shows that 90% of the total population of the area consumed wild edible mushrooms collected from nearby forests. The local names of the wild edible mushrooms have been mostly derived on the basis of their morphological features, nature of growth and color.

Introduction

Mushroom collection and consumption have been regular practice since time immemorial by different ethnic groups in Nepal. Various mycophagous groups like Sherpa, Tamang, Gurung, Tharu, Danwar and Newar directly collect and consume mushroom. The wild edible mushrooms serve as important natural seasonal crop which play an important role in the improvement of economic condition of the rural people (Adhikari 2000). Even though Nepal is rich in mycodiversity, very few scientific researches have been conducted (Adhikari 2000).

Study Area

The study area lies in Solukhumbu district of the north eastern region of Nepal. It encompasses the Sagarmatha National Park (1,148 sq krn) and its buffer zone area (Fig. 1). The park includes the upper catchment areas of the Dudh Kosi and Bhote Kosi rivers. The park is largely composed of the rugged terrain and gorges of the high Himalayas ranging from *2,845* m at Monju to the top of the world Sagarmatha (Mount Everest) at 8,848 m above sea level (m asl).

Fig. 1. Map of the study side

Due to its altitudinal diversity, various types of vegetation are found in different climatic zones (temperate, subalpine, alpine, nival and permanent snow zones). The dominant vegetation at the lower elevation of the park below 3000 m asl is composed mostly of blue pine and hemlock forest. The lower sub alpine region above 3000 m asl comprises of forests of *Pinus wallichiana, Abies spectabilis* and *Juniper recurva.* The upper subalpine, above 3600 m asl consists of birch rhododendron forest *(Betula utilis, Rhododendron campanulatum* and *Rhododendron campylocarpum)* and the lower alpine region above the timber-line at 3800 - 4000 m asl houses scrubs *(Juniper* spp, *Rhododendron anthopogon* and *Rhododendron lepidotum*).

The park is mainly populated by Sherpa people. Tamangs and other castes reside in minority in various settlements within the park and buffer zones. The main settlements are Namche Bazar, Khumjung, Khunde, Thame, Tengboche, Pangboche and Phortse. The economy of the Sherpa community has traditionally been agriculture, animal husbandry and trade with Tibet.

Methods

The study was conducted in August-September, 2004 from Lukla to Pangboche (Fig. 1) and mainly focused on Sherpa ethnic group. A total of 150 respondents within the age 10-70 years were interviewed by applying artifact and inventory interviewing methods (Nepal *et al.* 1999) and structured questionnaire. The mushroom specimens were collected carefully by digging them with the help of a sharp knife. Information on habit and habitats were recorded in the field. Each collection was placed in butter paper bags and tag numbers were assigned to them. The morphological characters such as spore print and color, habitat, forest type, pileus and tube color, color change induced after bruising or cutting and exposing to chemicals such as potassium hydroxide (KOH), ferrous sulphate ($FeSO_4$) were noted. The specimens were either sun dried or dried by placing them on tin foil over a local oven. Dried specimens were placed in butter paper bags. Naphthalene balls were used as insect repellents. The dried specimens were identifed in the laboratory with the help of literature (Imazeki *et al.* 1966, McKenny 1971, Svreck 1983, Purukayastha and Chandra 1985, Mcknight and Mcknight 1987, Adhikari 2000) based on macro and microscopic characters. The collected specimens have been housed at Nepal Academy of Science and Technology (NAST).

RESULTS AND DISCUSSION

A total of 150 species of mushroom specimens were collected from different sites of the Solukhumbu region. Out of total collected species, 26 species were identified as edible, two as ethnomedicinal and one as decorative by the ethnic groups (Table 1). Two species of *Boletus (Boletus aures* Peck, *Boletus pulverulentus* Opat.), *Chroogomphus tomentosus* (Murr.) O.K. Miller, *Russula metachroa* Hongo, *Tylopilus eximus* (Peek) Singh, and *Hypholoma capnoides* (Fries) Kumm., *Paxillus involutus* (Batsch: Fr.) Fr. were found new to the list of edible mushrooms of Nepal.

The indexes of similarity of edible mushroom species along different forests range from 40-80%. This analytical result suggests that different forests have got more than 60% similar edible mushroom species. This is because of altitudinal levels which have more or less similar climate, rainfall, temperature and vegetation (Fig. 2).

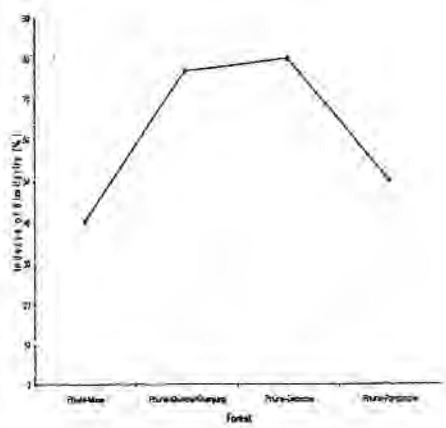


Fig. 2. The indexes of similarity of edible mushrooms of SNP

Table 1. Different types of mushrooms with their scientific names and locality

S.N	Scientific Name	Local Name	Locality (forest)	Altitude (m)	Uses
1.	*Amanita hemibapha* (Berk. And Br.) Sacc.	Rato anda chyau	Muse (Lukla)	2783	Edible
2.	*Amanita vaginata* (Fr.) Vitt.	Seto anda chyau	Muse (Lukla)	2783	Edible
3.	*Amanita* sp.	Sano anda chyau	Muse (Lukla)	2783	Edible
4.	*Armillariella* mellea (Vahk:Fr.)	Chiple chyau	Muse (Lukla), Phurte, Jamikhiau	2783-3800	Edible
5.	*Auricularia polytricha* (Mont.)	Durkho chyau	Chire (Kunde), Deboche, Omakha (Pangboche)	3900-4000	Edible
6.	*Boletus* sp.	Muse chyau (Fhe shyamo)	Chire (Kunde), Jamikhiau (Khumjung) Deboche, and Omakha (Pangboche)	3665-4000	Edible

7.	*Boletus edulis* Bull. ex Fr. Steinpliz	Pani chyau	Muse (Lukla)	2783	Edible
8.	*Boletus* sp.	Rato martip	Muse (Lukla), Chire (Kunde), Jamikiau (Khumjung) Deboche, and Omakha (Pangboche)	2783-4000	Edible
9.	*Boletus* auripes (Peck)	Seto martip	Muse (Lukla), Phurte (Namche)	2783-3615	Edible
10.	*Boletus pulverolentus*	Kalo martip	Muse (Lukla)	2783	Edible
11.	*Cantharella cibarius* (Bull.:Fr.) Kummer	Kyujir pothi	Chire (Kunde), Jamikhiau (Khumjung) Deboche	3665-3900	Edible
12.	*Chroogomphus tomentosus* (Schw.) Singer	Kyujir (keta)	Chire (Kunde), Jamikhiau (Khumjung) Deboche, Omakha (Pangboche)	3665-4000	Edible
13.	*Clavulina cinerea* (Bull.:Fr.) Schroet	Che shyamo	Jamikhiau (Khumjung)	3615	Edible
14.	*Rhodocollybia butyraceae* (Bull.:Fr.) Lennox	Karshya pothi	Monjo way to Namche	3615	Edible
15.	*Gomphus clavatus* (Pers. Ex Fr.) S.F. Gray	Ee-shamu	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung)	3615-3900	Edible
16.	*Gomphus floccosus* (Schw.) Sing.	Khumbhe chyau	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung) Deboche	3615-3900	Edible
17.	*Hydnum repandum* L.: Fr.	Kasturi chyau	Jamikhiau (Khumjung)	3800	Edible
18.	*Hygrophorous* sp.	Dudh chyau	Phurte (Namche)	3615	Edible
19.	*Laccaria laccata* (Scop.: Fr.) Cooke	Chinduk shyamo	Jamikhiau	3800	Edible
20.	*Leccinum* sp.	Petok	Chire (Kunde), Jamikhiau (Khumjung)	3800-3900	Edible
21.	*Ramaria flava* (Sch.:Fr.)	Che shyamo	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung) Deboche	3615-3900	Edible
22.	*Ramaria botrytis* (Pers.:Fr.) Ricken	Che shyamo	Phurte (Namche), Deboche	3615-3665	Edible
23.	*Russula metachroa* Hongo	Pakar shyamo	Jamikhiau (Khumjung)	3615-3800	Edible
24.	*Hypholoma capnoides* (Fr.) Kumm.	Taktale	Phurte (Namche)	3615	Edible
25.	*Paxillus involutus* (batsch:Fr.) Fr.	Dyanba chyau	Jamikhiau (Khumjung)	3800	Edible
26.	*Tylopilus eximus* (Peck) Sing	Kyakti shyamo	Chire (Khunde), Jamikhiau (Khumjung), Deboche	3665-3900	Edible
27.	*Lycoperdon perlatum* Peers.: Pers	-	Deboche	3665	Medicinal
28.	*Lycoperdon pyriforme* Schaeff.: Pers.	-	Phungi tenga	3590	Medicinal
29.	*Ganoderma* sp.	Chale chyau	Muse (Lukla)	2783	Decoration

Popularity of edible mushrooms

According to respondents the most prized edible mushrooms in that region were *Amanita hemibapha, Boletus edulis, Boletus auripes, Cantharella cibarius, Chroogomphus tomentosus, Gomphus clavatus, Gomphus floccosus, Hydnum repandum, Rarnaria flava, Leccinum* sp. and *Hygrophorous* sp. Species like *Amanita vaginata, Auricularia polytricha, Tylophilus eximus, Boletus pulverlentus, Clavulina cinerea, Russula metachroa, Hypholoma capnoides, Rhodocollybia butyraceae, Armillaria mellea, Laccaria laccata* may have been less popular due to their smaller size or less abundance.

The regionwise popularity of these mushrooms is as follows: *Amanita hemibapha, Boletus edulis* and *Boletus pulverlentus* were popular among informants residing from Lukla to Jorsalle. Similarly, *Gomphus clavatus, Gomphus floccosus, Hygrophorus* sp. and *Ramaria flava* were popular among local residents of Namche and surrounding area. *Leccinum* sp., *Boletus* sp., *Paxillus involutus* and *Chroogomphus tomentosus* are popular among people living in Khunde and Khumjung area. *Boletus* spp and *Cantharella cibarius* are popular in Thangboche and Pangoboche.

Collection and marketing of mushrooms

Wild edible mushrooms are regarded as a delicacy food in that region. It was found that even economically sound people hired men and women to collect mushroom occasionally. Labor collectors earned around Rs. 100-500 per day according to the amount and variety of mushroom species. In Khumjung, mushrooms were found to be sold at 50 rupees per "mana" (approximately 100 gm) irrespective of the variety. According to locals the rainy season is the favorable time for collection of wild edible mushroom. Some local people above Namche believe that they should not initiate new work (business) or engage in agricultural work during the month of July-August. So during this period locals were found to be busy collecting and drying wild edible mushrooms from nearby forests. The local who went early gets a good collection of mushrooms. Students were also found to be engaged in the collection of edible mushrooms during off days.

Almost all of the edible mushrooms were found to be growing between July and September. According to respondents the frequency and diversity of fructification of mushrooms depended upon various factors such as temperature, percentage of rainfall, humidity, etc. in a particular season. They even believed that if snow remained for a longer period the frequency of the growth of mushroom was higher. The prolonged snow fall may attribute to the moist conditions of soil which may favor the growth of mushrooms.

Other uses

In addition to food item, mushroom is also used in health treatment by the rural people. According to local informants spores of *Lycoperdon perlatum* and *L. pyriforme* have been used to heal wounds and cure baby rashes. Hobbs (1987) reported that several species of *Lycoperdon* can be used to stop bleeding in a fresh wound. Similar results were reported by Adhikari (1988, 2000), Bhandary (1991) and Ghimire *et al.* (2001) from eastern and western regions of Nepal. Joshi and Joshi (1999) reported that the aerial parts of *L. pyriforme* are used as tonic in case of weakness, by the locals residing in Pokhara. Sharma (2003) reported that spores of *L. pyrjforme* are used by people from Jammu to arrest the flow of blood from the wounds and in the treatment of piles. Some locals used mushrooms like *Ganoderma* sp. for decorative purposes while some polypores were used to make lids of bottle and stop cork. This was also reported by Adhikari (2000). In this study it was observed that mushrooms were not used for religious or ceremonial purposes.

In the Nepalese mycoflora, there are at least forty species of mushrooms which have been known to be toxic and dangerous (Basnet 2004). There is always risk of eating a poisonous species mistaking it to be an edible one. In Nepal the mortality rate due to consumption of poisonous mushrooms have been found to occur around 15- 20 persons annually (Adhikari 2004). As per interviews with local people, hospital staff and traditional healers no mushroom poisoning cases were reported till date in the study area. The local people aged between 10-70 years could easily distinguish the poisonous mushrooms from edible ones on the basis of knowledge handed over from generations. Apart from the edible species, locals recognized the poisonous mushrooms. The poisonous mushrooms were referred to as *"bhoot* (Eng: ghost) *chyau"* in Nepali dialect and *"sindi shyamo"* in Sherpa dialect.

Even though the region is rich in a variety of wild edible mushroom, these edible mushrooms have not found a place as a delicacy in the Hotels. For business purposes hotel and lodge owners bring mushrooms all the way from Kathmandu for culinary uses.

Conclusion

The study contributes to the documentation of ethnomycologically important mushrooms and to safeguard the indigenous knowledge handed down from generations in the study area. In the field survey it is observed that local people collected the mushrooms haphazardly. The edible mushroom was consumed by most of the local inhabitants. It is vital to assess the nutritive value, toxicity and develop cultivation technique of the most prized wild edible mushroom of that region.

Etiology and Control of Citrus Canker Disease in Kavre

Dinesh Dhakal, Chiranjivi Regmi and Sital R. Basnyat

Abstract

Citrus canker, caused by the bacterium *Xanthomonas citri* (Hasse) occurs in large areas of the world's citrus growing countries including Nepal. Though the disease has serious effect in Nepal, this is the first detailed study carried out to isolate the pathogen and confirm it by available biochemical tests and pathogenicity test. Furthermore, the study was intended to find the proper and economical control measure to combat disease in citrus orchards. The causative agent of the disease was isolated from the diseased plants and pure culture was obtained. The isolated pure culture was subjected to Gram staining, catalase test, oxidase test, O-F test, starch hydrolysis, nitrate reduction test, methylred test, Voges-Prauskauer test, indole production test, urease test and carbohydrate utilization test. To reconfirm it, pathogenicity test was conducted on host plant and after the appearance of the typical citrus canker lesion on host, the bacteria was reisolated, thus proving the Koch's postulates. Different controlling chemicals. copperoxychioride (2.5%), copperoxychloride + kasugamycin (1000X), bordeaux mixture 1 % and 2% were sprayed to the plants in citrus orchard at Dhulikhel and the decrease in disease severity after spraying of the chemicals was calculated with reference to the plants that were not sprayed with the chemicals. It was observed that spraying of the chemicals help in decreasing the disease severity. The chemical spray however was not able to eradicate the disease. The study concluded that *Xanthomonas citri* was the causative agent of the disease citrus canker and copper based chemicals when sprayed very early with the appearance of first symptoms of the disease could eliminate it in citrus fruits to minimum level.

Introduction

In the fiscal year 2004/2005, the total area of citrus cultivation was 25,909 ha out of which 14,606 ha was described as productive area with a total production of 15,956 mt (10.75 mt/ha) of fruits. Lime is one of the citrus fruits cultivated in Nepal. In the year 2004-2005 the total production of lime was 19,132 mt (HMG/N 2004/2005).

Of all the agricultural pests and diseases that threaten lime, citrus canker is one of the most devastating. The disease caused by bacterium *Xanthomonas citri (Hasse)* occurs in large areas of the world. The disease is endemic in India, Japan and other South-East Asian countries, from where it has spread to all other citrus producing continents except Europe. Considering the high demand of lime in local markets that is being fulfilled by import from India. Government of Nepal has initiated Citrus (Lime) Mission Programme in Tehrathum, Bhojpur and Dhankuta districts with an objective to substitute the import of this fruit by increasing its production. No detail research has been carried out in Nepal on citrus canker. This study was carried out during 2005 to 2006 in Kavrepalanchowk district as it is representative of most of the hilly districts of Nepal.

Regarding the pathogen of citrus canker, in the late 1980s, strains associated with canker A were proposed as a new species. *Xanthomonas citri*, whereas types B and C as well as other strains causing citrus bacterial spot remained within *X. campestris* as pathovars aurentifolii and citrumelo respectively (Gabriel *et al.* 1989). Schaad *et al.* (2000) proposed a reclassification that places citrus canker and citrus bacterial spot strains within *Xanthomonas* as a species *citri* (A strains), *aurantifolii* (B and C strains) and *citrumelo* (citrus bacterial spot strains). However, other authors rejected this new proposal, citing insufficient data to justify the removal of these strains from the species *axonopodis* (Vauterin *et al.* 2000, Young *et al.* 2001). Most recently, Brunings and Gabriel (2003) proposed the retention of *X. citri* as a species that includes only citrus canker strains (A and B-C).

Materials and Methods

Survey

Survey on canker infestation was carried out in different citrus orchards of Sarada Batase, Khanal Thok and Dhulikhel of Kavrepalanchowk district. In the survey, Citrus plants were checked for the symptoms which include raised, corky, tan lesions with water soaked margins and yellow halos on the leaves.

Disease Status and its severity

Status of the disease was found out by calculating disease frequency and severity. Disease frequency and severity were calculated by using the following formulae. (Johnston& Booth 1983)

$$\text{Disease frequency} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 10$$

Severity of disease of each sampled plant was calculated by counting the number of leaves showing canker lesion out of total number of leaves sampled.

$$\text{Disease severity(\%)} = \frac{\text{No. of leaves with lesion}}{\text{Total no. of leaves}} \times 100$$

Study of pathogen

Laboratory works were conducted at Nepal Academy for Science and Technology (NAST), Khumaltar, Lalitpur. Leaf samples used for isolation of the bacteria were collected from an orchard of Dhulikhel, Using a sterile razor blade, younger portions of the lesion was cut from a recently collected material. Then a leaf with lesion was taken on a clean microscopic slide and a drop of sterile water was added and observed under oil immersion objective to see streaming bacteria from the edge of leaf with this characteristic, the isolation procedure was initiated. The cut portion was surface sterilized using 0.1% mercuric chloride and then washed repeatedly with sterile distilled water, transferred to another sterile plate, minced with a flamed razor in 1 mL of sterile distilled water. It was then allowed to stand for 10-15 mm, and then serially diluted up to 10 dilution. Each diluent (0.2) was then spread on the plates on glucose yeast chalk agar (GYCA). The plates were then incubated at 28 °C for 72 hrs. Then suspected mucoid colonies with typical yellow pigment were subjected to Gram staining followed by biochemical and physiological tests. The biochemical tests performed were catalase test, oxidase test, oxidative-fermentative test, methyl red and Voges-Proskauer tests, indole test, nitrate reduction test and urease test. Similarly carbohydrate utilization test was performed (carried on Dye's medium C) using carbohydrates; arabinose, mannose, galactose, glucose, sucrose, fructose, and sorbitol with incubation of 21 days at 27 °C. For the pathogenicity test, disease free lime saplings were pot cultured. Five plants were assigned for different tests and one plant was assinged for control. Single isolated colony from the 3 day old culture in GYCA was taken and mixed with sterile distilled water to prepare bacterial suspension containing $10^6 - 10^7$ CFU/mL (compared with Mac Ferland's Scale at the suspension at 0.1 to 0.2 mL was inoculated on 3 to 4 points of each leaf. In each test plant 2 to 3 leaves were inoculated with bacterial suspension using 1 mL syringe. Similarly sterile water was inoculated to the plant which served as a control. Inoculated plants were then incubated. Symptoms were observed on leaves after four days of inoculation.

Study of control measures

For the control of the disease, 5 plots i.e. 1st, 2nd, 3rd, 4th and 5th ,with five plants in each plots (P1, P2, P3, P4 and P5) were treated with copper oxychloride (2.5 %), copper oxychloride + Kasugamycin (1000X), Bordeaux mixture (1%), Bordeaux mixture (2%) and water (control) respectively. Altogether, 5 observations were done, out of which 2 were done before application of the treatments and 3 observations after the treatments of plants with respective chemical sprays. Disease severity of the plants before and after each treatment was observed at 15 days interval.

Effectiveness of treatments

Effectiveness of treatments was determined on the basis of their capacity to reduce disease severity, which was calculated as follows (Table 3 and 4).

Effectiveness = Final disease severity (5th observation i.e. after 3 treatment) - Initial disease severity (1st observation).

Data analysis

Effectiveness of treatments was compared by LSD (5%) test.

Results and Discussion

Distribution

In the orchard of Dhulikhel, only *C. aurantifolia* was cultivated where out of total 55 plants, 48 were infected with different level of severity and 7 were found uninfeeted. In Khanalthok, out of 24 citrus fruits cultivated which included *C. aurantifolia* and *C. reticulata*, only 9 of *C. aurantifolia* were found infected, though both the fruits were grown in close vicinity. In Saradha Batase, a total of 97 citrus plants were observed which included *C. reticulata* and *C. sinensis* and none of them were infected with the disease citrus canker (Table 1.)

Table 1. Species of citrus plants infected with citrus canker in the surveyed area

Species of citrus plants	No. of plants with citrus canker/No. of plant observed		
	Dhulikhel	Khanalthok	Saradha Batase
C. aurantifolia (Acid lime)	48/55	9/9	0/0
C. reticulate (Mandarin orange)	0/0	0/15	0/72
C. sinensis (Sweet orange)	0/0	0/0	0/25

Results showed that only *C. aurantifolia* grown in Kavre were susceptible to citrus canker as other species such as *C. reticulata* and *C. sinensis* growing in the vicinity were uninfected. Thus our observation comply with the finding of Civerolo (1984), Graham (1992) and Stall (1993) who reported that acid lime is more susceptible to citrus canker than sweet orange and mandarin.

Confirmation of pathogens

The results of our study based on the morphological, microbiological and physiological characteristics (Table 2.) confirm that the pathogen was *X. citri.*

Table 2. Biochemical tests performed for confirmation of *X.citri*

S.No.	Test performed	Results
1.	Gram staining	Gram negative rod
2.	Catalase test	Positive
3.	Oxidse test	Negative
4.	O-F test	Oxidative
5.	Methyl-Red test	Negative
6.	Voges-Proskeur est	Negative
7.	Indole est	Negative
8.	Urease test	Negative
9.	Strach hydrolysis test	Positive
10.	Nitrate reduction test	Negative
11.	Growth on nutrient agar incorporated with 0.1% triphenyl tetrazolium chloride	No growth observed
12.	Growth at 37 ^{0}C	Positive
13.	Acid production from carbohydrates (arabinose, glucose, galactose, fructose, sorbitol)	Acid production from glucose, sucrose, Mannose and galactose

Dynamics of disease development

Dynamics of the disease development was studied on the basis of increase in disease severity in control plots sprayed with water in every 15 days interval.

Efficiency of treatments

Statistical analysis of data (LSD, at 5% level of significance) (Table 3) showed that chemical sprays satisfactorily reduced the disease severity. Treatments had their effect immediately after use and continuously slowed down the disease development through out the rainy season as described by Koizumi (1985), Leite and Mohan (1990), Stall *et al.* (1980), (1982b) and Graham *et al.* (1992) who recommended copper based bactericides as standard control measures for citrus canker world-wide. It was clearly observed that chemical treatments though effective could not control already existing lesions so the treatment should be started with the early sign of symptoms so that the disease spread can be slowed down or checked.

Copper hydroxide, basic copper chloride, copper oxychloride, and tribasic copper sulfate are the most effective bacterial sprays for protecting leaves and fruit from attack of *X. citri.* These materials can reduce the incidence of the disease, but they will not eliminate established infections. Copperoxychloride and kasugamycin have been used with satisfactory results. However, extensive use of copper may also cause phytotoxicity problems in treated groves (international citrus canker research workshop 2000). The plants treated with Bordeaux mixture 2% showed symptoms of phytotoxicity. Therefore, Bordeaux mixture 2% may not be applicable to control the disease canker.

Table 3. Disease severity on first observation (2063/2/27)

Replicates	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
1	55%	50%	60%	70%	50%
2	60%	70%	60%	65%	70%
3	50%	65%	65%	60%	70%
4	58%	65%	70%	70%	65%
5	65%	75%	40%	60%	30%

(Observation after 3rd spray) (206y3/5/15)

Table 4. Disease severity on 5th observation

Replicates	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
1	53%	51%	53%	72%	75%
2	57%	78%	61%	62%	85%
3	52%	67%	61%	61%	85%
4	64%	69%	73%	74%	82%
5	68%	75%	44%	57%	59%

Table 5. Mean effectiveness of each treatment

Effectiveness = Final disease severity (5th observation i.e. after 3rd treatment) – Initial disease severity (1st observation)

Replicates	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	LSD*
	Copper oxychloride (2.5%)	Copper oxychloride + kasugamycin (1000X)	Bordeaux (1%)	Bordeaux (2%)	Control (water)	
1	-2	1	-7	2	25	
2	-3	8	1	-3	15	
3	2	2	-4	1	15	
4	6	4	3	4	17	
5	3	0	4	-3	29	
Mean effectiveness	1.2^b	3^b	-0.6^b	0.2^b	20.2^a	5.81

*at 5% level of significance

On the basis of statistical analysis (LSD, at 5%), it was found that all the treatments significantly reduced the rate of increase of the disease as compared to control.

The local varieties/cultivars of mandarin and sweet oranges were found resistant to citrus canker. The lime was very susceptible and special care has to be taken to control it with copper based chemical sprays.

Acknowledgement

The authors are highly thankful to Nepal Academy of Science and Technology (NAST) for providing laboratory facilities and Mr. Lok Nath Deoju for providing his orchard for the research work.

Antagonistic Study of *Lantana Camara* (Linn) Against with Pathogenic Bacteria

Geeta Shrestha Vaidya and Nabin Bhattarai

Abstract

A research work of antagonistic effect of water solvent and organic solvent (Ethanol of different concentration 50% and 100%) extracts *of Lantana camera* were studied against with pathogenic fifteen strains of bacteria.

Among fifteen species, of bacteria most of them were inhibited by *L. camara* extracts and only two species such as *Kleibsella oxytoca* and *Kleibsella pneumoniae* did not showed antibacterial activity with same extract with same concentration. Extracts obtained from the organic solvent and water solvent showed the different antagonistic properties with the same bacterial strains. Those bacterial strains which were inhibited their growth by water solvent could not inhibited by organic solvent extracts. This depends on presence of polar and non-polar bioactive compounds in the extracts. It also depends on polar and non-polar solvents used for the plant extract. Organic solvent extracts showed antibacterial effect towards *Pseudomonas aeruginosa, Staphylococcus* sp., *Bacillus cereus, Bacillus subtilis, Bacillus thurengiensis, Escherichia coli, Staphylococcus aureus, Proteus mirabilis* and water solvent extracts showed antibacterial effect towards *Pseudomonas aeruginosa, Staphylococcus* sp., *Citrobacter frundii, Proteus* sp., *Bacillus subtilis, Enterobacter aerogenes, Salmonella paratyphi, Staphylococcus aureus, Shigella dysenteriae.* Both solvent extracts showed high antibacterial effect towards *Staph aureus, Staphylococcus* sp. and *Pseudomonas aeruginosa.*

Both plant extracts showed selective antibacterial effect with different strains of bacteria, which shows that these are confined to cure the same bacterial diseases.

Introduction

Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural drugs, natural pesticides and biofertilizers (Balandrin *et al.* 1985; Hostettman and Wolfender 1997). The use of traditional medicine is widespread in Nepal and much of the population still relying on it. In Nepal each and every plant has their own value such as antibacterial, antiviral, antifungal and biofertilizer properties. This can be explained by such factors as the lack of doctors, aelopathic medicines and expensive fertilizers. The use of plant preparations in this tradition has been well documented (Manandhar, 1985, 1986, 1987, 1 989a, b, c, 1990a, b; Bhattarai, 1993; Shrestha and Joshi, 1993), although only a few species have been screened for biological activity (Bhakuni *et al.* 1969).

During the past decade, potent agents against bacterial, fungal and viral infections have become available. Extracts of plants and phytochemicals are getting more importance as potential sources for inhibiting different diseases during the recent decade. But the increasing clinical use has been associated with the emergence of drug resistant strains (Reusser 1996; Tisdale, 2000). Additionally dose-limiting toxic effects are observed. Plants have a long evolution of resistance against viral agents and lead to alternative directions in drug development. Despite the tremendous progress in human health, diseases caused by bacteria and residual toxicity of chemical pesticides is still a major threat to the public health. *Bacterial* pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drugs. So, there is challenge to us and urgent need to discover new antibiotics, biopesticide and biofertilizer from any new resource. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agent has led to the screening of several medicinal plants for potential antimicrobial activity (Scazzocchia *et al.* 2001).

Lantana Camara L. is perennial shrub, exotic to Nepal, due to its adverse growth it is also called unwanted shrub (Shrestha Vaidya *et al.* 2005). In Nepal, *Lantana camara* extract and its powder widely used to check the plant diseases whether it is bacterial or fungal as well as to increase the fertility of the soil and also used to cure human diseases (Shrestha Vaidya *et al.* 2009, 2006). Extensive studies have shown that medicinal plants of several parts of the world contain compounds active against viruses that cause human diseases (Semple *et al.*1998; Kott *et al.* 1999; Sindambiwe *et al.* 1999). Thus plants used also in Nepalese traditional medicine were analysed (Taylor *et al.* 1996a,b). Thus the present investigation represents a preliminary screening *Lantana camara* against the different pathogenic bacterial strains.

Materials and Methods

Generally plant extracts were extracted with the different kinds of solvents such as organic and water solvents, in present study both solvents extract i.e. water solvent and organic solvent extract were used. For extraction, fresh leaves and current stems of the *Lantana camara* were collected from the road side and dried on the shade condition and then grinded in grinder in powder form (ShresthaVaidya *et al.* 2009). For water solvent extraction, 500 gm of shade dried *Lantana camera* plant powder soaked in 2500 mL distilled water for 3 days, squeezed and filtered with the help of cotton cloth. Water content of this filtrate evaporated till the solution in semisolid form. Semi solid solution poured in petridish and kept in dessicater which contained silica gel for residual water absorption from extract. From this dried extract 50% (0.5 gm/ mL) and 100% (1 gm / mL) concentrated solution were made by using distilled water.

For organic solvent extract, 25 gm shade dried powder of *Lantana camara* was taken and placed in soxhiet glasstube, poured ethanol and run the soxhlet apparatus. This mixture of extract evaporated and dried with the help of Rotary vacuum evaporator and placed in dessicator for residual absorption of water from extract. Generally all the extract made on same solution which is used to extract the plant extract but in this research ethanol was used. From this dried extract 50% (0.5 gm/ mL) and 100% (1 gm/ mL) concentrated solution were made on ethanol (Shrestha Vaidya *et al.* 2008).

Fifteen identified bacterial strain were collected from NIST. They were cultured into Nutrient Broth solution and incubated for 3-4 hours for their maximum growth. The bacterial broth solution compared to the turbidity of the 0.005% solution of the $BaCl_2$ (Barium chloride) solution, the turbidity of the bacterial growth solution not more than the turbidity of the $BaCl_2$ solution and swapped on the MHA (Muller Hinton Agar) media finely with the help of the cotton swap. After completion, bored the swapped media with the help of sterile borer and formed well on the media. 50 μL extract and control solution poured with the help of the micro pipette on each well, left for sometime for diffusion (Shrestha and Piya 2002). All the plates incubated at 37°C for 24-48 hours. A control well was made on each plate by applying distilled water for water solvent and ethanol for organic solvent extract.

Results

All concentrations of *L. camara* extracts showed selective effect towards the bacterial strains. Among the fifteen sp of bacteria most of them are inhibited the growth with *L. camara* extracts and only two sp *Kleibsella oxytoca, Kleibsella pneumoniae* did not showed antibacterial activity with same extract. Extracts obtained from the organic solvent and water solvent showed the different antimicrobial properties with the same bacterial strains. Those bacterial strains which inhibited their growth by water solvent could not inhibited by organic solvent extracts. This depends on presence of polar and non-polar bioactive compounds in the extract. It also depends on polar and non-polar solvents used to extract the plant extract. Organic solvent extract showed antibacterial effect towards *Peudomonas aeruginosa, Staphylococcus* sp, *Bacillus subtilis, Bacillus cereus, E. coli, Staphylococcus aureus, Bacillus thurengiensis, Proteus mirabilis* and water solvent extract showed antibacterial effect towards *Peudomonas aeruginosa, Staphylococcus* sp, *Citrobacter Frundii, Proteus* sp, *Bacillus subtilis, Enterobacter aerogenes, Salmonella paralyphi, Staphylococcus aureus, Shigella dysenteria.* Altogether 13 sp out of 15 are inhibited their growth with *L. camara* extracts. Both solvent extracts showed high antibacterial effect towards *Staphylococcus aureus, Staphylococcus* sp, and *Pseudomonas aeruginosa.*

Data Presentation

Data presentation of the water solvent extract

All output of this research work were presented on tabular form, the following bar diagrams showing the effect of plant extract towards different bacterial strains which is extracted from water solvent (Fig. 1 and Fig. 2).

From the above bar diagrams, it was found that the concentration of the extract played main role to inhibit the bacterial growth. Higher the concentration of the extract showed higher concentration of the bioactive compounds. 100% plant extract inhibited the eight strains of the bacteria while the 50% plant extract inhibited only four strains of the bacteria. The 100% water solvent extract showed high antibacterial effect towards *Staph aureus, Pseudomonas auroginosa, Citrobacter frundii* and *Salmonella paratyphi* while the 50% showed high antibacterial effect towards the *Staph aureus, Pseudomonas auroginosa, Citrobacter frundii* and *Staph* sp. This showed that the inhibitory effect of the extract depends on the concentration. Higher the concentration showed higher inhibitory effect while the lower concentration showed lower inhibitory effect towards bacterial strains.

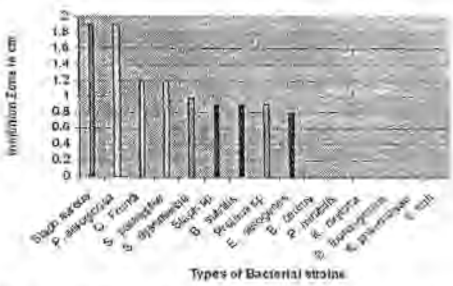


Fig. 1. Effect of 100% *L. camara* water solvent extract towards bacterial strains growth

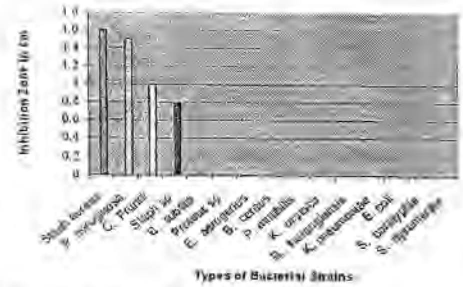


Fig. 2. Effect of 50% *L. camara* water solvent extract towards bacterial strains growth

Data presentation of the organic solvent extract

The following bar diagrams showing the effect of plant extract towards different bacterial strains which is extracted from organic solvent (Fig 3 and Fig. 4).

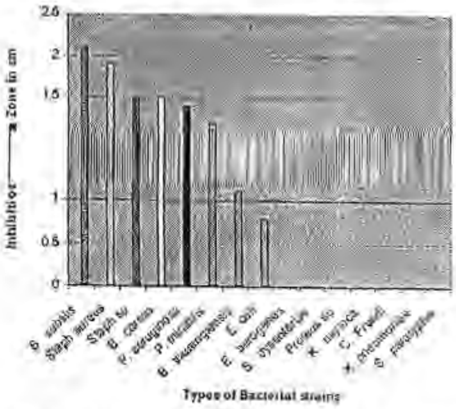


Fig. 3. Effect of 100% *L. camara* organic solvent extract towards bacterial strains growth

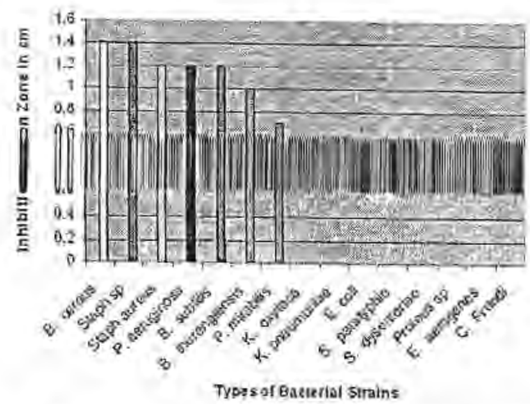


Fig. 4. Effect of 50% *L. camara organic* solvent extract towards bacterial strains growth

From the above bar diagrams, it was found that the concentration of the extract played main role to inhibit the bacterial growth. Higher the concentration of the extract showed higher concentration of the bioactive compounds. 100% plant extract inhibited the eight strains of the bacteria while the 50% plant extract inhibited the same bacterial strains also. Only *E. coli* did not show the antibacterial effect with the 50% plant extract.

The 100% organic solvent extract showed high antibacterial effect towards the *Staph aureus, Bacillus subtilis, Bacillus cereus, Staph sp.* while the 50% showed high antibacterial effect towards the same bacterial strains but less inhibitory zone in comparison to the 100% plant extract. Higher the concentration showed higher inhibitory effect while the lower concentration showed lower inhibitory effect towards bacterial strains.

Following photographs are showing zone of inhibition with different bacteria in different concentration.

Discussion

The major objective of this research was to investigate antagonistic effect of *Lantana camara* with different solvent in different concentration against with different pathogenic bacteria. Generally all the plants show the antimicrobial properties against different microorganisms. The preliminary results obtained from the crude water and organic solvent extracts indicate that further investigation and screening is worthwhile. This antibacterial effect depends on solvents which are used to extract the plant extract. Extract obtained from organic solvents and water solvent shows the different antibacterial properties with same bacterial strains. Those bacterial strains inhibited their growth with organic solvent extract could not inhibited by the water solvent extract. This depends on the presence of polar non-polar bioactive or inhibitory compounds on the extract. Organic solvent extracted more concentration of non-polar bioactive compounds from the plant powder and water solvent extracted more concentration of polar bioactive compounds. So, presence of different concentration of polar and non-polar compounds on the extract showed different inhibitory effect towards same bacterial strains. In other way water solvent extracted more gm of extract that is mainly polar which inhibited the inhibitory effect of non polar compounds and vice versa.

Photo A

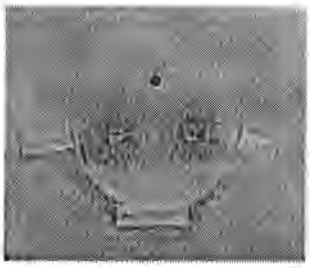


Photo B

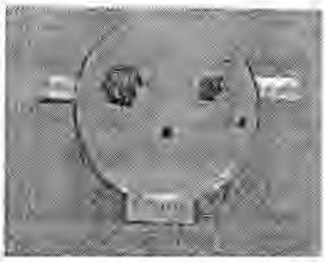


Photo C

Ethanol extract of *Lantana camara* exhibited varying degrees of inhibitory activity against gram positive bacteria but it was inactive against gram negative bacteria. Our report more or less consistent with the report of Basu *et al.* (2005) and Shrestha Vaidya *et al.* (2009). *Lantana camara* extracts have also been found to be a powerful febrifuge (Liogier 1990). CasCassado, 1995 had reported that *Lantana* leaves and their leachates exert allelopathic effects *in vitro* and to a lesser extent in soil on seed germination, root elongation, and plant growth of many species. Shrestha Vaidya *et al.* (2009) had reported that extracts of *L. camara* with different solvent with different pathogenic bacteria had shown antagonistic effects.

Conclusion

In Nepal, generally called *Lantana camara* Linn is an exotic and unwanted shrub. *Lantana* is now a major weed in many regions and it is regarded as one of the most serious weeds in plantation crops. Not only that, it is poisonous to cattle, goat and cow also with direct eating. Inumerable biologically active compounds are found in plants (Alade and Irobi 1993, Clark and Hufford 1993, Samy *et al.* 1999) that possess antibacterial properties (Brantner and Grein 1994, Samy and Ignacimuthu 1998). From these results, it can be concluded that *Lantana Camara* extracts showed effective antibacterial activity towards pathogenic bacterial strains. So it's extract useful and effective towards the control of human, animals and plants bacterial diseases.

Acknowledgements

We thank to the Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur, Nepal for support and providing facilities to complete this research work.

Effects of Arbuscular Mycorrhiza in the Productivity of Maize and Fingermillet Relay Cropping System

Gautam Shrestha, Geeta Shrestha Vaidya and Binayak P. Rajbhandari

Abstract

Field experiments were conducted in farmer field in 2008 to scrutinize the effects of AM fungi in the productivity of maize/fingermillet cropping system in the rainfed dryland area of Kavrepalanchwok district. Following randomized complete block design, six treatments with four replications were maintained. Results showed significant effect of AM inoculation on plant height and yield of maize at 1% level of significance, whereas fingermillet was positive with nonsignificant difference on both parameters. Improvement in available soil phosphorus status was observed distinct in both maize (26.5 1±9.54 kg P_2O_5/ha) and fingermi llet (30.11±15.15 kg P_2O_5/ha) in arbtiscular mycorrhiza treated plots (T3) than control (TI). Arbuscular mycorrhiza spore density was also improved in the mycorrhiza treatments with (T6) and without nitrogen and potash fertilizer(T3) as compared to control (TI) in both maize and fingermillet.

Introduction

In sustainable and organic agricultural systems, the role of beneficial microorganisms in maintaining soil fertility and bio-control of plant pathogens may be more important than in conventional agriculture where their significance has been marginalized by high inputs of agrochemicals. Better understanding of the role of microorganisms with other management practices like fertilizer application is necessary for the development of sustainable management techniques of soil fertility and crop production.

Arbuscular mycorrhiza (AM) fungi are essential components of sustainable soil-plant systems (Schreiner *et al.* 2003) because of their role in increasing plant growth and nutrient uptake (Smith & Read 1997). Effective arbuscular mycorrhizal associations have reduced the amount of phosphorus fertilizer needed to be applid to a plant or crop (Miyasaka *et al.* 2003). An association between mycorrhiza fungus and plant roots is beneficial to the plant when it is grown under low phosphorus or dryland (i.e. low rainfall, non-irrigated) conditions (Fageria 2009) and grow well under relatively harsh mineral stress conditions (Clark & Zeto 2000) prevailed in subsistence agriculture in Nepal.

Phosphorus (P) deficiency is a factor limiting crop production on tropical and sub-tropical soils (Fairhust *et al.* 1999, Mokwuny *et al.* 1986, Sanchez & Salinas 1981). As nearly 49% soils of Nepal are acidic in reaction (Sherchan & Karki 2006) most part of phosphorus is fixed in soil preferentially with iron and aluminum (Kanwar 1976) into unavailable forms. Alternative soil management techniques such as AM biofertiliser application is needed for resource poor farmers and has enormous potential for large scale agricultural systems and can be beneficial in sustainable production of main crops contributing to reduced input of expensive, environmentally harmful, low use efficient and limited phosphate fertilizers and chemical pesticides (Baar 2008, Shrestha 2007).

Materials and Methods

Farmers' field experimentation was conducted at Mathurapati Phulbari VDC of Kavrepalanchwok district. This VDC is at a distance of 6 km from Dhulikhel, headquarter of Kavrepalanchwok district.

Detail of treatments:

T1	Control
T2	FYM only
T3	AM inoculums
T4	NK + FYM
T5	FYM + NPK
T6	FYM + NK + AM inoculum

- Each treatment had four replications. The above mentioned experiments were conducted in maize/ fingermillet - fallow cropping pattern.

- Experimental plot size: 25 sq. m. (each)
- Total experimental field size: 600 sq. m.

Mycorrhiza spores were isolated following wet sieving and decantation method adapted by Gerdemann & Nicolson *(1963)*. Spores were mass produced in 1:1 sand and red soil mixture (autoclaved at 121°C and 15 lbs for 3 h) with low phosphorus content (Table 1). Onion tubers were grown as host crop. Pots were watered regularly to maintain water levels to enhance proper growth of onion roots. Inoculum was harvested at 90 days of inoculation after achieving adequate number of spores and percentage root colonization. Root portions were chopped to make fine pieces of 1 to 2 cm length. Substrate along with spores and roots was mixed properly to make homogenized mixture of AM inoculum.

Table 1. Soil analysis results of inoculum media and farmers' field before planting maize (mean)

Particular	Soil pH	OM%	Total nitrogen (%)	P_2O_5 (kg/ha)	K_2O (Kg/ha)
Soil inoculums media	5.94	2.20	0.15	2.80	39.62
Before planting maize	5.18	4.04	0.13	12.38	310.69

AM inoculum application was done on the basis of 800 varieties of crops used, sowing date, fertilizer doses, g in 1 sq. meter referring to Prabakaran *et al.* (1995). In crop geometry and harvesting dates are as presented in fingermillet, it was mixed during nursery bed preparation, the Table 2. for T3 and T6 with additional application during planting time.

Table 2. Agronomical details of crops

Crop	Variety	Sowing date	Fertiliser N:P:K kg/ha	Crop geometry R X P (cm)	Harvesting date
Maize	Population 45	16th May	60:40:40	75 x 25	29th September
Finger millet	Kavre Kodo	9th July (nursery)	80:40:30	10 x 5	23rd November

Biometric measurements were done in 5 fixed plants per replication. Total 120 plants were taken as sample for data collection. Soil analysis was done before planting maize and after harvest of each crop to determine the soil fertility status following standard procedures.

Results

Plant height

Plant heights in both maize and fingermillet were higher in T3 (AM inoculum) as compared to control (T1) (Table 3). It was higher in T3 even than in T5 in maize which was with the recommended dose of fertilizers and manures (Table 3) with significant difference.

Table 3. Plant height in cm (mean± SE)

Treatments	Maize	Fingermillet
T1	186.25 ± 7.39	48.87 ± 5.32
T2	228.25 ± 3.92	84.10 ± 2.70
T3	241.75 ± 12.00	74.47 ± 6.42
T4	236.50 ± 6.98	71.40 ± 9.73
T5	233.75 ± 4.39	75.73 ± 5.84
T6	239.25 ± 9.29	68.00 ± 9.68

Grain yield

Grain yield is higher in AM inoculum (T3) treatment compare to control (T1) in both crops. Combined effect of farm yard manure, nitrogen and potash fertilizers and AM inoculum was found in grain yield of maize and fingermillet (Fig. 1).

Maize grain yield was statistically significant at 1% level (Table 6) whereas fingermillet yield was non significant.

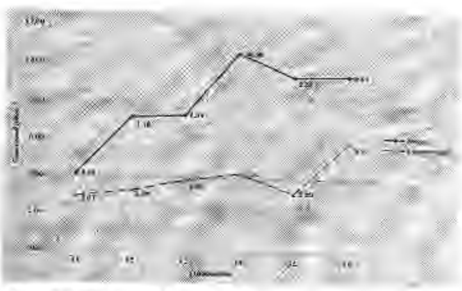


Fig. 1. Grain yield of maize and fingermillet

Soil analysis

Sandy loam type of soil was predominant in the area. First year soil fertility evaluation results showed that phosphorus content of the study area was low with slightly acidic soil pH (Table 1). In maize, available phosphorus content in soil was higher in T3 and T6 where AM inoculum was applied without phosphorus fertilizer compare to T5 treatment with phosphorus fertilizer (Fig. 2).

Phosphorus content was higher in the T3 and T6 treatments in fingermillet as compared to other treatments as a positive impact of AM inoculum application (Fig. 3).

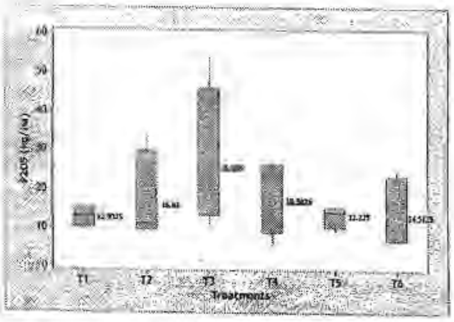


Fig. 2. Available phosphorus status after maize harvest

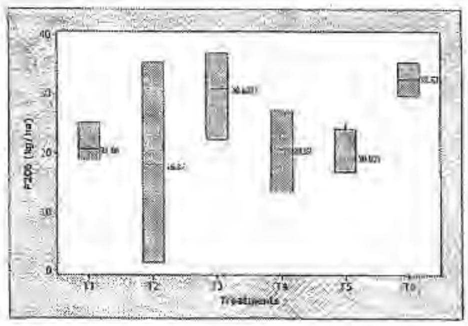


Fig. 3. Available phosphorus status after fingermillet harvest

Mycorrhizal spore density

Mycorrhizal spore density in fingermillet T3 (AM inoculum) treatment was higher than other treatments showing development of mycorrhizal population in dryland situation. In T6 treatment, spore density was higher in maize than that of other treatments. Spore density in Tl treatment in both crops was lowest (Fig. 4).

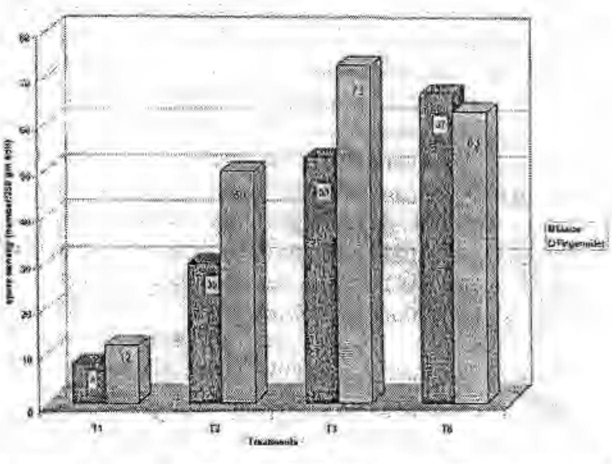


Fig. 4. Mycorrhiza population development in mycorrhiza inoculated soil

Discussion

Confirming results of Stevens *et al.* (2002), our research revealed that total plant height difference was observed higher in phosphorus applied treatments (T5), both in maize (233.75±4.39 cm in T5 than 239.25±9.29 cm in T6) and fingermillet (75.73±5.84 cm in T5 than 68.00±9.68 cm in T6) than nitrogen and potash fertilizers and AM inoculum applied treatment (T6).

In this study, soil analysis after maize showed 26.51±9.54 kg P_2O_5/ha in T3 after maize and 30.11±15.15 P_2O_5 kg/ha after fingermillet as compare to the control treatments (T1). These findings agree with those reported by Vogel-Miku and Regvar (2006) in line with Smith (2000) the contents of mineral nutrients e.g., phosphorus, significantly increased in inoculated plants, indicating functional exchange of nutrients and presumably carbohydrates between the partners due result of increased available phosphorus in soil.

Agreeing with Kahiluoto and Vestberg (1999) findings that low input cropping system including composting had higher spore density (32.2 and 44.2 per 100 ml soil solution in spring and autumn respectively), this study also found higher spore density in FYM treatments (T2) (53 and 73 in maize and fingermillet respectively) compare to control (TI). SSD (2002) found that mycorrhiza spore count per 100 g soil was 22 and 15, respectively in vesicular arbuscular mycorrhiza (VAM) + NPK and NPK only in pot trial. Conceding to SSD research results our study resulted that spores density was increased in the maize/millet relay system from 67 after maize and 63.0 spores per 250 g of soil after fingermillet in treatments with FYM, nitrogen and potash fertilizers and AM inoculum (T6). Acceding to Hameeda *et al.* (2007) in which they found about 15% mycorrhizal colonization in control treatments, our research revealed mycorrhizal population development in control (T1) due to natural. Our findings is also supported by SSD (2003) also findings that VAM population in control plots in maize (2) and millet (1) compared to maize + VAM (12) and millet + VAM (5) in 100g soil sample taken.

In summary, mycorrhizal inoculation has proven to be the better alternative to the chemical fertilizers especially phosphorus to increase and maintain the productivity of crops in the past endeavors and so do in this research. Besides yield, mycorrhizal plants made their survival in drought conditions during dry months after planting. As lands of mid and high hills are slopy except in the valley plains, down flow of rainwater along with mycorrhiza has resulted into mere low growth of mycorrhizal spore density in the soil. Long term fertility trials with efficient strains of mycorrhizal inoculums will better perform in the field conditions.

Acknowledgement

Authors are grateful to the concerned farmers in Mathurapati Phulbari VDC for their cooperation and Sustainable Soil Management Program (SSMP/ Helvetas Nepal) for providing financial support to this research.

Some High Value Medicinal Plants of Khumbu Region Nepal

Deepa Shree Rawal, Jaishree Sijapati, Neesha Rana, Prabina Rana, Anjana Giri and Sangita Shrestha

Abstract

Visualizing the present biodiversity status of Nepal, an integrated approach comprising of biodiversity documentation, bioprospecting, sustainable utilization and conservation has become an urgent need. Realizing the importance of medicinal plants altogether 45 plants were collected from Khumbu region with the altitudinal variation of 2582 m to 4470 m. Among the collected plant species 12 have been characterized as highly valuable. The important biochemical constituents and morphology of these plants have been described in this paper.

Introduction

Medicinal plants are second most valuable bioresources of Nepal after water resources. Many highly demanded and globally important medicinal plants such as *Swertia* spp., *Neopicrorhiza scrophulariiflora, Podophyllum hexandrum, Taxus wallichiana, Podocarpus* sp. etc. are harbored in various geoclimatic region of Nepal (Polunin & Stainton 1984, Press *et al.* 2000). The total number of medicinal and aromatic plants reported in Nepal varies according to various authors (DPR 1970, 1984; Malla & Shakya 1999, Baral & Kurmi 2006). The compilation of Medicinal and Aromatic plant database of Nepal was published in 2000 (Shrestha *et al.* 2000), which listed 1624 species of medicinal plants. Most recently, Ghimire (2008) reported 1950 species of medicinal plants in Nepal.

According to recent estimates by the World Health Organization, more than 3.5 billion people in the developing world rely on plants as components of their primary health care (www. fao.org). High value medicinal plants have been the basis for modern allopathic drug development while the use of indigenous drugs from plant origin form major part of complementary and alternative medicines in the form of herbal drugs and Ayurvedic medicines. Many such high value medicinal plants have been equally used in other forms of alternative medicines such as Chinese, Tibetan and Homoeopathic medicine (Li *et al.* 1997, Dhama & Dhama 1996). Therefore, the production, consumption and international trade in medicinal plants and phytomedicines are growing and expected to grow quite significantly in future.

Himalayan region of Nepal is one of the major store houses of various high value medicinal plant species. Most of such valuable plants grow in wild conditions as natural components of vegetation of particular region. The necessary plant material (roots, leaves, bark, etc) recollected and sold by the local people to the traders, industries and exporters purchase them from traders. Since there is no scientific system of collecting and regenerating these plants, several such high value plants have either been completely lost or have become endangered. In this scenario, handling of Nepalese medicinal biodiversity should involve an integrated approach comprising of well documentation, their sustainable utilization and conservation.

Conventionally, macroscopic (morphological) and microscopic characters including anatomy, cytology and chemical profiling techniques such as thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC), gas chromatography (GC), high performance liquid chromatography (HPLC), etc are being used for characterizing genotypes and chemotypes (Joshi *et al.* 2004). Such studies can produce deep level insights for conservation and sustainable utilization of high value medicinal plant biodiversity of Nepal.

Present study on high value medicinal plants of Khumbu region was formulated for the exploration, documentation and characterization of high value medicinal plants of Khumbu region in order to devise prospects of such plants for long term sustainable utilization and conservation.

Materials and Methods

Field survey was carried out during May, 2007 at Khumbu region of Nepal, for the ecological, anatomical study and for the collection of herbaria for the morphological studies as well as collection of anatomical and DNA samples. Altitude, longitude and latitude readings of the plant collection sites were taken by using geographical positioning system (GPS). The collected plants were properly dried and mounted for further identification.

Herbarium specimens were prepared for the collected high value medicinal plants. Morphological identification of the specimens was carried out consulting taxonomic experts and using various taxonomic

The ratio of flavonol to anthocyanin was found to be 5.6:1, suggesting that the flavonol plays a role as a copigment in the blueing of *Meconopsis* flowers, rhoeadine alkaloid in traces (Kosaku *et al.* 1996).

***Meconopsis peniculata* Pram**

Monocarpic herb 1.8 m tall, tap root dauciform or narrowly elongated. Stems moderately bristly covered with golden yellow soft hairs. Leaves linear-oblong on outline, deeply pinnatifid or pinnatisect at the base densely clothed with same type of hairs like in stems, segments ovate-oblong, base spatulate, apex acute to obtuse margin coarsely serrate. Flowers solitary terminal or axillary, numerous pendulous, borne singly in upper part in 2-6 flowered lateral cymes in lower part. Ovary sub globose, ellipsoid or ovoid densely covered with hairs. Flowering : June - August, Fruiting time September- October. (Plate 6)

Plate 6. *Meconopsis peniculata*

Phytochemical constituents are alkaline phosphatases, Glucose 6-phosphate dehydrogenase and malate dehydrogenase (Irshad *et al.* 1993) Papaverrubin A, B,C, D E, rhoeadine alkoliod in traces (Arnold *et al.* 1986).

***Neopicrorhiza scrophulariiflora* (Pennell) Hong**

A prostrate herb about 10 cm high with perennial woody rhizome covered with old leaves at the base. Leaves sub sessile aggregated at the base, each leaf 2 - 6cm long and 0.5 -1.2 cm wide, acuminate, serrate, stalked and winged, oblanceolate. Flowers dark blue purple, flowering: July - August in a dense terminal spikes arising from a rosette of conspicuously toothed leaves, spikes 5-10 cm long, Fruits capsule; October - November 6-10mm, Rhizome is bitter. (Plate 7)

Phytochemical constituents contain Kutkin (glucosidal), kurrin (a non bitter product), kutkiol (alcohol), kutkisterol.(Watanabe *et al.* 2005), Rhizomes and roots contain picroside I, picroside II, kutkoside, minecoside, phenol glycoside (picein and androsin) and 4-hydroxyacetophenone. (Anonymous 2002) Kutkiol (an alcohol), pocrorhizin, pocrorhizitin, picroside, picroliv, kuthoside, androsine, aucubin, catalpol, valinic acid and kutkisterol (Kunwar 2006).

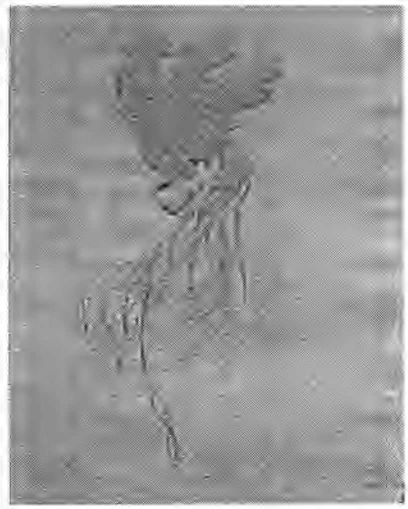


Plate 7. *Neopicrorhiza scrophulariflora*

***Paris polyphylla* Sm**

The herb is about 60 cm high. Leaves stalked, in a whorl at the top of the stem, 5-16 cm long, 1.5-4 cm wide, lanceolate, long-pointed, dark green. Flowers solitary, Flowering time: April-May. (Plate 8)

Rhizome contains pariphyllin A, pariphyllin B, parsterone, pollyphylin D, and trillin (Buckinghain 1994).

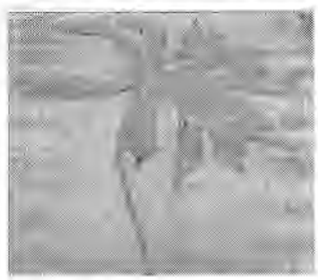


Plate 8. *Paris polyphylla*

***Podophyllum hexandrum* Royle**

An erect perennial herb about 15-40 cm tall. Leaves usually two, deeply three lobed. Long stalked, orbiculate to cordate. Lobes obovate, acuminate, serrate. Flowering time May - June, cup shaped, 2-4 cm across, large white or pale pink. Fruiting time: July - September, ovoid, 2.5-5.0 cm long, scarlet when ripe. (Plate 9)

Plate 9. *Podophyllum hexandrum*

Podophyllotoxin, 4'-dimethyldesoxy podophyllotoxin, α and β pellatin, deoxypodophyllotoxin, podophyllotoxone, isopicropodophyllone, 4'-demethyldesoxy podophyllotoxin, 4'-demethyldesoxypodophylflotixe are the chemical constituents of this plant (Husain *et al.* 1992).

***Potentilla peduncularis* D. Don**

Perennial, herbaceous plant about 30 cm in height, rootstock stout, covered with old leaf bases. Leaves uniformly pinnate about 20 cm long mostly basal covered with silvery hairs appear white at beneath. Flowers yellow, corymbs, flowering and fruiting time July-November. (Plate 10)

Most of the pharmacological effects can be explained by the high amount of tannins and to a lesser extent by triterpenes, present in all plant parts. Polyphenols and flavonoids are also present in this plant (Micha[3] *et al.* 2009).

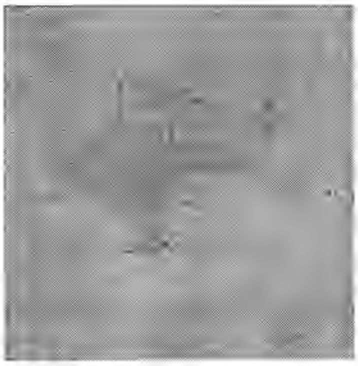


Plate 10. *Potentilla peduncularis*

***Taxus wallichiana* Zucc**

Evergreen much branched coniferous tree nearly 30 m tall. Bark reddish brown, rough, exfoliating in irregular papery scales. Leaves linear, short- stalked, flat, acute, distichous, shiny dark green above, rusty beneath and narrowed towards the base, 2.0-3.5 cm long and 3mm broad. Flowers unisexual, male flowers in short stalk, globose catkins in axils of leaves; female flowers solitary, axillary, green. Cones yellowish, axillary. Fruits or seed cones are red fleshy, 8 mm in diameter. Seeds are olive green.(Plate 11)

Biochemical constituents contain taxine alkaloids and taxol (Joshi & Joshi 2001).

Plate 11. *Taxus wallichiana*

***Zanthoxylum nepalense* Babu**

Shrub with corky bark and numerous straight spines. Leaves stalked pinnatifid, rachis winged, leaflet short stalked 1-3 cm long, ovate, dentate, gland dotted, flower yellowish, fruit a capsule, globose red and wrinked. Flowering time May - June. (Plate 12)

Biochemical constituents : Tannic acid, gallic acid, starch, mineral salts and mucilage from rhizome (Anonymous 1994).

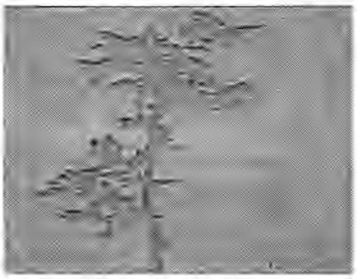


Plate 12. *Zonthoxylum nepalense*

Discussion

Several collected plants were found to have different medicinal uses in different alternative medicines such as Tibetan medicines, herbal drugs have been homoeopathy (Alam *et al.* 2008, Dhama & Dhama, 1996), many others have been mentioned in ethnomedicinal literatures (Ghimire 1999, Rajbhandari 2001, Joshi & Joshi 2001, Bhattarai *et al.* 2006, Rajbhandari *et al.* 2007, Bhattarai *et al.* 2008, Ghimire 2008).

P. hexandrurn, Himalayan May apple, is recognized for its anticancer properties (Alam *et al.* 2008) and has been employed in the treatment of cancer (Prakash *et al.* 2005). It has been extensively exploited in traditional Ayurvedic system of medicine for treatment of a number of ailments like *Condyloma acuminata, Taenia capitis* , monocytoid leukemia, Hodgkins disease, non-Hodgkin's lymphoma, cancer of brain, lung, bladder and venereal warts. Utility of *P. hexandrum* has also been reported against constipation, cold, biliary fever, septic wound, burning sensation, erysipelas insect bite, mental disorders, rheumatism, plague and to provide symptomatic relief in some of the allergic and inflammatory conditions of skin. The radio-protecive effect of hydroalcoholic extracted material from the rhizome of *P. hexandrum* was studied in mice exposed to lethal γ-radiation (10 Gy). The extract was found to restore the hemoglobin content (14.73 0.33) and total leukocyte count (TLC) (4166.66±0.02) in lethally (10 Gy) γ - irradiated mice on the 15th day in comparison to the radiation control mice (Arora *et al.* 2007).

Similarly, various species of *Berberis* are reported to have various medicinal properties. *Berberis aristata* has been reported to have wound healing properties (Biswas & Mukharjee 2003). It produces a bitter tonic, which is anti periodic and diaphoretic. The chief constituents are those of berberis bark, the principle being the alkaloid Berberine (internet visit 2007; www. botanical.com). Aqueous extract of *B.*

literatures (Polunin & Stainton 1984, Press *et al.* 2000). Again, the specimens were compared with previously identified herbaria in National Herbarium and Plant Research Laboratory, Godawari and Plant specialists from Natural History Museum, Swoyambhu.

Description of major vegetation

The vegetation pattern from Lukla (2800) to Jorsalle (2400) to Namche (3400) comprises the canopy vegetation of *Abies spectabilis* D. Don, *Pinus roxburgii Sarg*., *Rhododendron arboretum* Smith, Tsuga demosa D. Don, *Pinus wallichiana* A.B. Jacks etc. In case of the shrubby vegetation plants like Rhododendron triflorum Hook. f, Juniperus indica *Bertol* , Juniperus recurva *Buch. Ham. ex* D. Don, Berberis aristata Hook. F & Thomson, *Berberis erythroclada Ahrendt, Zanthoxylum* nepalense DC, Cotoneaster microphyllus *Wallich.ex Lindley*, Rubus ellepticus Smith, Utrica dioca *L.* were quite common. The ground vegetation consisted mostly of plants like *Euphorbia wallichi Hook. f, Artemisia* sps., *Viola* sp., *Plantago ersoa Wall*, *Primula* sp.

Above Namche along Syangboche and Khunde Khumjung trail (around 3400- 4000). The common vegetation comprised of *Juniperus* sp., *Rhododenron anthopogan* D. Don, *Rhododendron wightii* Hook.f as well as different species of *Berberis*. Around 4000 m, *Juniperus indica* Bertol were present and above 4,000 m, where conditions are drier, along with dwarf rhododendrons and *Cotoneasters*, *Ephedra gerardiana* Wall ex Stapf shrubby *Potentilla* sp, willow *Salix* sp were found.

The shrub layer diminishes as conditions become cooler and above 5000 m, other dwarf shrubs in the dry valley upland, include horsetail *Ephedra gerardiana* Wall ex Stapf, *Juniperous indica* Bertol and *Potentilla* sp. Above this and up to the permanent snow line at about 5,750 m, plant life is restricted to lichens, mosses and dwarf grasses.

Results

Altogether, 45 medicinal plants were collected from the research site. Of them, 12 have been short listed as being high value species for further detail study (Table 1). The selection has been based on IUCN category, CITES list, DPR list, IDRC list and other relevant literature.

Table 1. List of high value medicinal plants collected from the Khumbu Region with geographical positioning

S.N.	Scientific name	Family	Nepali name	Altitude (m)	Longitude/ Latitude
1.	*Artemisia* sp.	Compositeae		3500	N-27°48.132' E-86°42.589'
2.	*Berberis erythroclada* Ahrendt	Berberidacea		3522	N-27°48.229' E-86°42.908'
3	*Berberis aristata* var. micrantha Hook. F. & Thomson	Berberidaceae	Chutro/ Kirmundo	3522	N-27°48.229' E-86°42.908'
4	Ephedra gerardiana Wall ex Stapf	Ephedraceae		4470	N-27°54.21' E-86°42.132'
5	*Meconopsis grandis* Prain	Papaveraceae		3596	N-27°48.405' E-86°42.214'
6	*Meconopsis peniculata* Prain	Papaveraceae	Mulapate	3596	N-27°48.405' E-86°42.214'
7	*Neopicrorhiza scrophulariiflora* (Pennenn) Hong	Scrophulariaceae	Kutki	3634	N-27°48.594' E-86°42.163'
8	*Paris polyphylla* Smith	Liliaceae	Satuwa	2584	N-27°45.145' E-86°43.008
9	*Podophyllum hexandrum* Royle	Berberidaceae	Laghu patra	3984	N-27°49.124' E-86 42.711'
10	Potentilla peduncularis D. Don	Rosaceae		3845	N-27°48.920' E-86°42.845'
11	*Taxus wallichiana* Zucc.	Taxaceae		2783	N-27°41.87' E-86°43.40'
12	Zanthoxylum nepalense DC	Rutaceae	Timur	2717	N-27°45.595' E-86°42.044'

Morphological description and important chemical constituents

Abies spectabilis (D. Don) *Mirb*

Tall evergreen, coniferous tree about 50 m in height. Leaves sessile, linear, flattened, leathery, dark green above and whitish beneath with incurved margins, notched at the apex. Flowers unisexual, female cones solitary, situated a little below the tips of shoots, dark purple when young and brown at maturity with broad fan shaped scales, erect cylindrical, 10-20 cm long and 4-7.5 cm broad. Male cones usually clustered, yellowish and ellipsoid, 5cm long. Cones: April- May. (Plate 1)

Leaves produce essential oil which contains mainly α and β-pinene, camphene, lemonene, bornyl aëetate (DPR 2007).

Plate 1. *Abies spectabilis*

Berberis aristata DC

Spiny shrub about 3 m high. Leaves sub-sessile, clustered, 2-7 cm long, 0.5-2 cm wide, ovate with trifid spines, entire or spinuous dentate, smooth, base tapering. Flower stalked, yellow, in dropping racemes. Fruit ovoid, blue black when ripe. Flowering: March June, Fruit: July-November. (Plate 2).

Root's bark contain berberis aristratin, berberine, berbamine (DPR 2007).

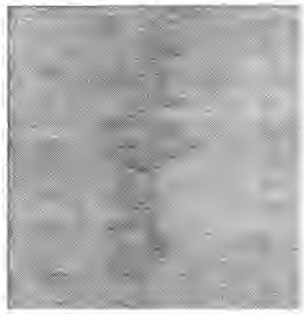


Plate 2. *Berberis aristata* DC

Berberis erythroclada Ahrendt

Much branched spiny shrub, about 1 m high, branches grooved, shiny dark red. Leaf stalks, 1-2 cm long ovate, margin dentate. Flowers yellow, solitary, on a slender stalk. Fruit red. Flowering: June-July. (Plate 3)

Root bark contains berberis aristratin, berberine, berbamine (DPR 2007).

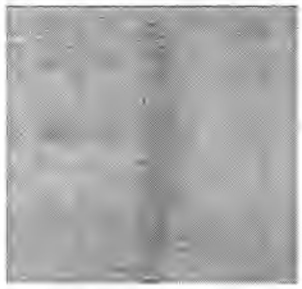


Plate 3. *Berberis erythroclada*

Ephedra gerardiana Wall. ex Stapf

A rigid tufted gymnospermous shrub about 1 m high. Stem much branched whorled spreading. Leaves reduced to 2 toothed sheaths. Branches smooth, slender, green and jointed. Plants are dioceious, male flower ovate, 6-8 mm long with 4-8 flowers. Female cones usually solitary and yellowish, ovules ovoid, 7-10 mm surrounded by fleshy red succulent persistent bracts enclosing two seeds. (Plate 4)

Aerial parts contain ephedrine ephedroxyne, ephedrine and pseudoephedrine. (Husain *et al.* 1992).

Plate 4. *Ephedra gerardiana*

Meconopsis grandis Prain

Polycarpic herb up to 1 m high. Tap root narrowly dauciform or root system fibrous. Stems leafy, basal part covered with apprised bristly, membranous persistant leaf sheaths, clothed with refouse, deflelxed, 3-7 mm long yellow bristle. Leaf elliptic oblanceolate, long petioles, apex acute, base cuneate or shortly attenuate, margins entire or with several broad teeth. Flowers blue, solitary on axils of upper most leaves. Seed reniform. Flowering: June to July. Fruiting: August - September. (Plate 5)

Plate 5. *Meconopsis grandis*

Phytochemical constituents arc cyanidin 3-malonylsambubioside 7-glucoside as the anthocyanin. They also contain large amounts of kaempferol 3-gentiobioside and very small amounts of kaempferol 3-xylosylgentiobioside.

vulgaris has antihistaminic and anticholergenic activity in guinea pig ileum (Shamsa *et al.* 1999). Berbamine, an ingredient of Berberis is widely utilized in Chinese Folk Medicine as a source of leukogenics, anti-arrhythmic and anti-hypertensive, in recent years, the immunosuppressive effect of berbamine has been demonstrated (Luo *et al.* 1998). Therefore, bioprospecting of important biomolecules in Nepalese *Berberis* species can be a prospective research area in future.

N. scrophulariflora (Kutki) is in high demand in and outside of Nepal for its valuable rootstocks. It furnishes the drug, picrorhizin obtained as dried rhizomes and roots; which is used as an adulterant of or as a substitute for Indian gentian *(Gentiana kurroo)*. The rhizomes of this Himalayan perennial herb are used in several traditional medicine systems to treat a wide variety of ailments. Kutki is widely distributed in the Himalayas (3000-5000 m), occurring in Pakistan, India, Nepal, Bhutan and Southern China (Traffic international 1999, Press *et al.* 2000).

Although little is known about biochemical specificities of Himalayan medicinal plants, some evidences suggest that such plants offer great potential for discovery of novel molecules and new sources of active compounds, mainly because of environmental stress to which they are subjected (Ghimire 2008). In mountains, plant secondary compounds exhibit patterns of variation in relation to stress associated with elevation, which relates to plant competition for resources, defense strategies against herbivores and pathogens and harsh climate (Iwashina *et al.* 2004). Concentration of active phytochemical constituents of some Himalayan medicinal plant species have been reported to be high in populations growing at higher altitude as compared to populations growing at the lower altitude (Mikage *et al.* 1987).

Regarding the conservation and sustainable utilization of high value medicinal plant species of Nepal in the changing scenario of environmental changes, an integrated approach needs to be followed. For rational and sustainable utilization of valuable medicinal plant species, conventional plant breeding techniques as well as biotechnological techniques need to be integrated to increase productivity and long term conservation. Considering the present scenario on advancement in biotechnology, in addition to conventional techniques of biodiversity characterization, detail molecular characterization also should be incorporated. So that the technique will further help during intellectual property rights (IPR) protection, devise authentication technique, in revealing taxonomic and evolutionary relationships for bioprospecting.

Antibacterial and Antifungal Effect of *Eupatorium adenophorum* Spreng Against Bacterial and Fungal Isolates

Nabin Bhattarai and Geeta Shrestha Vaidya

Abstract

A research work on antimicrobial effect of water solvent and organic solvent extracts of different concentration of *Eupatorium adenophorum* (Spreng) was conducted at Nepal Academy of Science and Technology (NAST) during August 2008. Fifteen strains of bacteria, six strains of fungi and two concentrations, 50% and 100% of plant extracts were taken for the study. Among the 15 strains of bacteria, most of them were inhibited with E. *adenophorum* extracts and only three species, *Klebsiella oxytoca, K. pneumoniae* and *Shigella dysenteriae* did not show antibacterial activity with the same extract. Extracts obtained from the organic solvent and water solvent showed different antimicrobial properties with the same bacterial strains. Those bacterial strains whose growth was inhibited by water solvent could not inhibited by organic solvent extracts. Organic solvent extract showed antibacterial effect towards *Proteus* spp., *Salmonella* spp., *Staphylococcus* spp., *Bacillus subtilis, B. thurengiensis, B. cereus, Enterobacter aerogenes, Salmonella paratyphi, Staphylococcus aureus, Proteus mirabilis* and water solvent extract showed antibacterial effect towards *Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, Staphylococcus* spp., *Citrobacter frundii, Proteus* spp., *B. subtilis, B. thurengiensis, Enterobacter aerogenes, Salmonella* spp. and *S. paratyphi*. Altogether 12 species out of 15 were inhibited by *E. adenophorum* extracts. Both solvent extracts showed high antibacterial effect towards *Proteus* spp., *Staphylococcus* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Among the six species of the fungi, *Fusarium* spp. were inhibited by plant extract while the *Aspergillus* spp. and *Stenophylum botryosum* did not show any effect of the extracts. The plant extracts showed selective effect with different strains of bacteria and fungi, which indicated that they were confined to cure the same bacterial diseases and fungal diseases.

Introduction

Pharmacological industries have produced large number of new antibiotics throughout the world on one hand but on the other hand resistance to these antibiotics by microorganisms has increased. Microorganisms have the genetic ability to transmit and acquire resistance towards drugs. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for potential antimicrobial activity.

Most of green plants represent a reservoir of effective chemo-therapeutants and can provide valuable sources of natural drugs, natural pesticides and biofertilizers. They have a long evolution of resistance microbial agents which has led to alternative directions in drug development. Therefore, extracts of plants and phytochemicals are getting more importance as potential sources for viral inhibitors during the recent decade. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The use of traditional medicine is widespread in Nepal and much of the population still rely on it. In Nepal each and every plant has its own value such as antibacterial, antiviral, antifungal and biofertilizer properties. The use of plant preparations in this tradition has been well documented (Manandhar 1985, 1986, 1987, 1989a,b,c, 1990a,b, Bhattarai 1993, Shrestha and Joshi 1993, Shrestha Vaidya *et al.* 2009) although only a few species have been screened for biological activity (Bhakuni *et al.* 1969).

Eupatorium adenophorum Spreng is a perennial shrub, exotic to Nepal, due to its adverse growth it is also called Banmara. Systematic screening of such type of unwanted plants may result in the discovery of novel effective compounds. In Nepal its extracts and dried powder are widely used to check plant diseases as well as biofertilizer to increase fertility of the soil. It is also used as fuel wood by biobreqeting process and used in biogas plant. The aim of this work is to study the effect of the plant extract on bacterial and fungal organisms.

Materials and Methods

Generally plant extracts are extracted with different kinds of solvents such as organic and water solvents. For extraction, fresh leaves and stems of the *E. adenophorum* collected and dried on shade condition and grinded in powder form. For water solvent extraction, 500 g of shade dried *E. adenophorum* plant powder was soaked in 2.5 L of distilled water for 3 days then squeezed and filtered with the help of cotton cloth. Water content of this filtrate was evaporated till the solution reduced to semisolid form. This solution was poured in petridish and kept in a dessicator which contained silica gel for residual water absorption from the extract. From this dried extract, 50% (0.5g/mL) and 100% (g/mL) concentrated solution were made by using distilled water. Finally, zone of inhibition of the extract against different bacteria was measured.

Fifteen identified bacterial strain were collected from National Institute of Science and Technology (NIST). They were cultured into nutrient broth solution and incubated for 3-4 h for their maximum growth. The bacterial broth solution compared with the turbidity of 0.005% solution of Barium chloride ($BaCl_2$). The turbidity of the bacterial growth solution was not more than the turbidity of $BaCl_2$ solution and these bacterial solutions were swabbed on the MHA (Muller Hinton Agar) media finely with the help of cotton swab. After completion, bored the swapped media with the help of sterile borer and formed well on the media. Fifty microlitre (50 µL) extract and control solution were poured with the help of the micro pipette on each well, then left for sometime for diffusion. All the plates were incubated at 37 °C for 24-48 h. A control well was made on each plate by applying distilled water for water solvent and ethanol for organic solvent extract (Shrestha & Piya 2002, Shrestha Vaidya *et al.* 2005, 2008).

For organic solvent extract, 25 g shade dried *E. adenophorum* powder was taken, placed in Soxhlet glass tube, poured ethanol and Soxhlet apparatus was run till colorless. This mixture of extract was vaporated and dried with the help of rotary apparatus and placed in a dessicator for residual absorption of water from the extract. Generally all the extracts were made on same solution which were used to extract the plant extract. So in this research ethanol was used. From this dried extract, 50% (0.5 g/mL) and 100% (g/mL) concentrated solution were made in ethanol.

Different species of fungi obtained from Nepal Agricultural Research Council (NARC) were cultured on potato dextrose agar (PDA) media for pure culture and incubated at 27 °C for their growth. Calculated volume of 100%T extract solution was poured in the PDA media before sterilization. Different volumes of 100% extract solution were used for increasing concentration on PDA media. In this way, different concentrated PDA media of the extract (5%-20%) were made. Fungal discs were cut out with the help of the borer and placed on the PDA media which contained plant extract and incubated at 27 °C for their growth. Finally, the growth of fungi was observed from the fungal disc.

Results and Discussion

All concentrations of *E. adenophorum* extract showed selective effect towards the bacterial strains. Among all tested bacterial and fungal strains, this plant extract showed antibacterial and antifungal effect towards bacterial and fungal strains and no effect towards few bacterial and fungal strains.

Among the 15 species of bacteria, most of them had inhibited growth with *E. adenophorum extracts and* only 3 species *Klebsiella oxytoca, K. pneumoniae* and *Shigella dysenteriae* were not affected with same extract. Organic solvent extract showed antibacterial effect towards *Proteus* spp., *Salmonella spp., Staphylococcus* spp., *Bacillus subtilis, B. thurengiensis, Enterobacter aerogenes, Salmonella paratyphi, Staphylococcus aureus, B. cereus, Proteus mirabilis;* and water solvent extract showed antibacterial effect towards *Pseudomonas aeruginosa, E. coli, S. aureus, Staphylococcus* spp., *Citrobacter frundii, Proteus* spp., *Bacillus subtilis, Enteroacter aerogenes, Salmonella* spp., *Salmonella paratyphi, Bacillus thurengensis.* Altogether 12 species had inhibited growth with *E. adenophorum extracts.* Both solvent extracts showed high antibacterial effect towards *Proteus* spp., *Staphylococcus* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Among the fungal strains *Fusarium moniliformae, F. eroliferum, F. proliferatum* and *F. oxysporum* were inhibited by this plant extract while the *Aspergillus niger* and *Stenophyllum botryosum* did not show which is similar to the work of the Tian *et al.* 2007 found that the volatile oil extracted from *E. adenophorum* inhibited four types of fungal pathogens.

E. adenophorum showed antimicrobial properties against different microorganisms. The preliminary results obtained from the crude water and organic solvent extracts indicate that further investigation and screening is worthwhile. From this result extracts obtained from the organic solvent and water solvent showed different antimicrobial properties with the same bacterial strains. Those bacterial strains which inhibited their growth by water solvent could not be inhibited by organic solvent extracts. This antibacterial effect depends on solvents which are used to extract the plant extract. Those bacterial strains which were inhibited with organic solvent extract could not be inhibited by water solvent extract. This depends on the presence of polar and non-polar bioactive or inhibitory compounds on the extract. Organic solvent extracted more concentration of non-polar bioactive compounds from the plant powder and water solvent extracted more concentration of polar bioactive compounds. So, presence of different concentration of polar and non-polar compounds on the extract showed different inhibitory effect towards same bacterial strains. Water solvent, extracted more amount of polar compounds and less amount of non-polar compounds, inhibited the antibacterial effect of non-polar compounds which are present in same extract. Organic solvent, extracted more amount of non-polar compounds and less amount of polar compounds, inhibited the antibacterial effect of polar compounds which are present in same extract.

Higher the concentration of the extract showed higher inhibition zone while the lower concentration showed lower inhibition zone or no effect.

All outputs of this work are presented in Fig. 1 and 2.

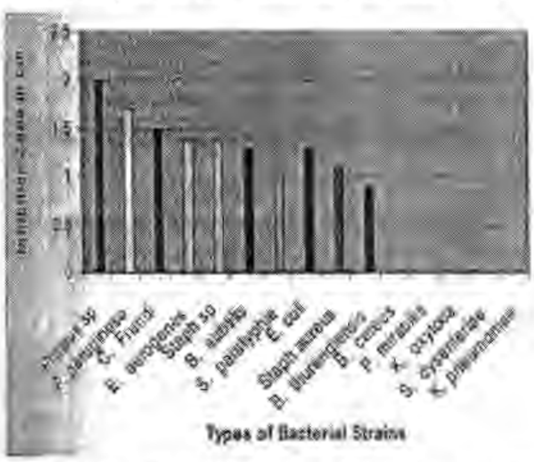


Fig. 1. Effect of 100% *E. adenophorum* water solvent extract towards bacterial strains growth

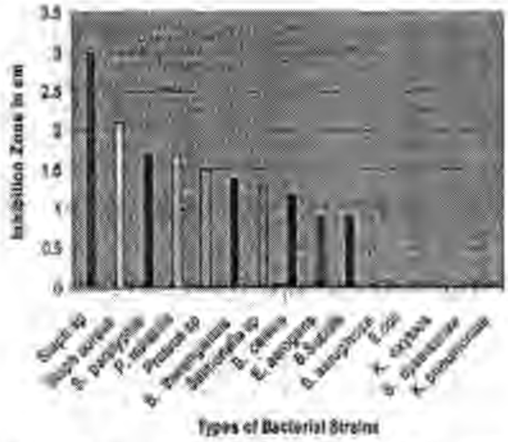


Fig. 2. Effect of 100% *E. adenophorum* organic solvent extract towards bacterial strains growth

The above bar diagrams show the inhibited bacterial strains and their area of inhibition zone by using *E. adenophorum* plant extracts. From these diagrams, it was found that 10 species of bacteria were inhibited by 100% water solvent extract and also 10 species were inhibited by 100% organic solvent extract out of 15 species. These bacterial species were also tested with 50% concentration of same extract solution that inhibited only few species. It was showed that higher the concentration higher is the inhibited number of species and lower the concentration was the lower inhibition number of species.

Salmonella spp. causes typhoid and paratyphoid fever on mammals. *Staphylococcus* spp. causes urinary track infection, wound infection i.e. post operative, food poisoning, boils, abscesses, endocarditis, toxic shock syndrome diseases on human and mastitis on cattle.

Escherihia coli causes gastroenteritis. *E. coli* and *Proteus* spp. are opportunistic human pathogens. They attack at the time of low immunity power and cause UTI. *Citrobactor frundii* and *Enterobacter aerogenes* cause diseases at the time of low immunity power. *B. thurengiensis* is pathogenic to the insects. *Pseudomonas aeruginosa* is also opportunistic human pathogen causes infecting wound, burn and UTI on humans. All the above bacterial strains were inhibited by E. *adenophorum* extracts (Fig. 4 and 5). In present study, S. *aureus* was inhibited this plant extract which is analogous to the work of Tomoko *et al.*, (2002) they found that S. *aureus* inhibited their growth by plants extracts of tropical and sub-tropical plants.

Fig. 3. *Eupatorium adenophorum* in natural habitat

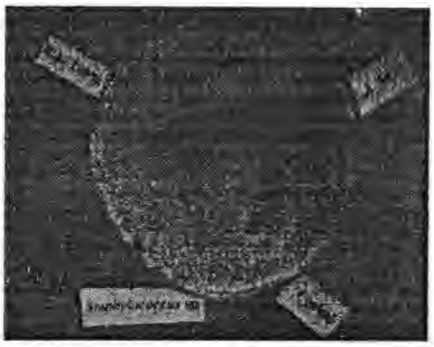


Fig. 4. Inhibitory zone of bacteria

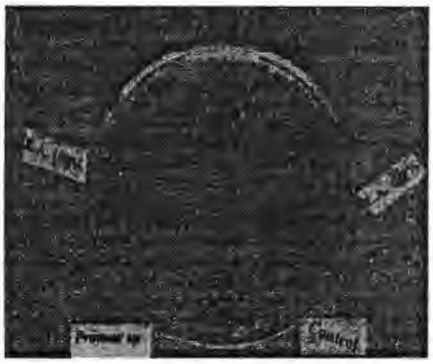


Fig. 5. Inhibitory zone of bacteria

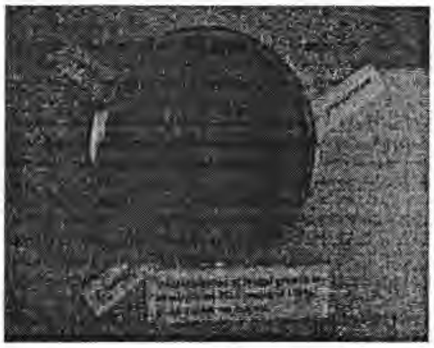


Fig. 6. Total inhibition of fungal grow

Different types of *Fusarium* spp. are the causal organisms of soil born disease of plants and sometimes they may cause seed born disease also. They cause many wilting diseases in several crops. *E. adenophorum* extract inhibited the growth of *F. moniliformae* (Fig. 6) which automatically inhibits the growth of foot rot disease of rice, pith canker of *Pine,* disease of potato tuber and maize seedlings. *F. oxysporum* also causes the wilting disease of lentil, tomato and banana. This plant extract also inhibited the growth of *F. oxysporum* (Fig. 6). So this plant extract can be used to control the wilting disease of such representative plants. Similarly this plant extract inhibited the growth of *F. proliferatum* and *F. eroliferum* which automatically inhibits growth of diseases caused by these organisms.

Acknowledgement

We thank Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur, Nepal for support and providing laboratory facilities to complete this research work.

Function of Organic Matter (Green Manure) and the Effect on Soil Properties

Geeta Shrestha Vaidya, Keshav Shrestha and Hâkan Wallander

Abstract

Trees and shrubs on the lower hillsides in Nepal form symbiosis with arbuscular mycorrhizal (AM) fungi and these fungi are important for the uptake of mineral nutrients from the soil and the mycelia formed by the fungi have an important function in stabilizing the soil (Wright and Upadhyaya 1998, Shrestha 1999 and Shrestha Vaidya *et al.* 2005a). The success of plantations of these eroded slopes is therefore highly dependent on the extent of mycorrhizal colonization of the plants.

In this study we have investigated the role of organic matter on growth of an arbuscular mycorrhizal (AM) fungi in eroded slopes in Nepal such as Chalnakhel, Kathmandu District. Different types of organic matter (leaves of *Thitonia diversifolia, Eupatorium adenophorum* and *Lantana camara)* were collected and were shade dried and finally powedered. Nutrient analysis was done of these organic matter and soil of experimental site before plantation and after harvest. *Lantana camara* was taken for plantation on their nutrient content basis. 100 nursery plants *Leuceania diversifolia* plantation were done in Chalnakhel Among these 50 plants with *Lantana camara* and 50 plants were for control. We investigated the influence of organic matter or P amendments on production of arbuscular mycorrhizal (AM) fungi in eroded slopes in Nepal. Organic matter addition enhanced the production of AM fungal biomass as well as number of AM spores. We suggest that the positive influence of such organic matter additions can make an important contribution to plant survival in plantations of eroded slopes in Nepal, and thus to restoration success.

Introduction

Organic matter is defined as a grouping of carbon compounds which have originated from living beings and deposited on or within the earth's structural components. Lal's (1993) initial definition of soil quality as the capacity of soil to produce economic goods and services and to regulate the environment "Soil quality".

Organic matter is a major source of plant nutrients and is the glue that holds soil particles together and stabilizes the pore structure. It makes soils less vulnerable to wind erosion and functions as a sponge for holding water and slowing down its loss from the root zone by drainage or evaporation. Moreover, nutrients added to soils as organic residues are released more gradually than those from mineral fertilizers and are therefore less prone to leaching, volatization or fixation. A fertile soil should contain from 2.8 percent organic matter, most soils contain less than 2 percent. Organic matter is a good source of phosphorus.

In addition to supplying nutrients, soil organic matter improves soil fertility by imparting favorable chemical and physical attributes to soil. Soil structure is influenced by the association of soil organic matter with minerals to form aggregates. Aggregate formation improves soil structure and water infiltration and improves root growth and provide habitat for a diversity of soil organisms. Soil organic matters (SOM) enhance nutrient cycling, provides habitat for a diversity of soil organisms, and creates a favorable environment for plant growth.

SOM is considered to be a key attribute of soil quality (Larson and Pierce 1991; Gregorich *et al.* 1994) and also environmental quality (Smith *et al.* 2000). So, soil quality is considered a key element of sustainable agriculture (Warkentin 1995).

It is involved in and related to many soil chemical, physical, and biological properties. It has a physical function in that it promotes good soil structure, thereby improving tilth, aeration and moisture movement and retention. Usually the greater the amount of organic matter in the soil the better is the physical properties of the soil. The organic materials also improve the microbial activities of the soil, biological N *fixation,* organic matter decomposition, mineralization, nitrification and antagonism to soil borne pathogens and fermentation are the common features of microbial activities in soil system (S.M. Alam and M.A. Khan 2001).

The addition of organic matter such as green manure is a common practice used to improve soil nutrient content and soil structure. Addition of organic matter such as green manure is a common practice to improve soil nutrient content and soil structure. Organic residues from plants such as *Tithonia diversifolia* and *Lantana camara* have been found to be especially beneficial since they are reported to have a high content of N and P, which is mineralized rapidly from the organic material. Nziguheba *et al.* (2000) found that P is released more rapidly from such organic residues than from triple superphosphate.

Most plants live in symbiosis with mycorrbizal fungi and these fungi improve water and nutrient uptake in exchange for carbohydrates supplied by the plant. Arbuscular mycorrhizal (AM) fungi form symbioses with most herbaceous and many woody plant species. Successful colonization by mycorrhizal fungi is especially important in degraded soils where nutrient availability is low.

Materials and Methods

This field experiment was conducted in Chalnakhel forest in central Nepal. This forest is situated in southern part of Kathmandu valley 12 km. South of Kathmandu City near to Pharping. Study site was newly planted but there was too many spaces for plantation.

Experiment design

The experiment plot size was about 2 m spacing from each other and dug about 1 ft.

Nutrient analysis of the samples

Fresh leaves of *Lantana camara, Tithonia diversifolia, Eupatorium adenophorum* free from disease were collected from agro forestry and also from road side. These leaf materials were then shade dried and were finally powdered. The nutrient analysis were done of these green manures as well as soil of the plantation site also. These nutrient analysis was following Nitrogen, Potassium, Phosphorus, Organic matter and pH and was done in Nepal Agriculture Research Council (NARC), Soil Division, Khumaltar, Lalitpur Table 1. The site was selected in Chalnakhel. The AM spores extraction of the experimental soil was done before plantation and after harvest.

Table 1. Nutrient analysis of organic matter and soil

Sample type	pH	Total N%	Available P kg/ha	Available K kg/ha	Organic matter
Tithonia diversifolia	-	33.2	2.8	34.1	29.87
Lantana camara	-	28.6	1.7	12.2	29.95
Eupatorium adenepborum	-	36.7	2.6	22.6	14.94
Soil before plantation	7.3	0.12	6.0	94.0	1.81
Soil after plantation	8.2	0.5	33.4	188.0	2.93

For plantation only one *Lantana camara* was taken . Total hundred replicates were taken. Among these fifty replicates for control without organic matter and fifty with organic matter such as *Leuceania diversifolia.* Total hundred *Leuceania diversifolia* one year old nursery plants were taken for plantation. These plants were six inches in height. Among these plants , the one year old 50 nursery plant *Leuceania diversifolia* was taken with organic matter *(Lantana camara)* and 50 plants were taken for control. These were planted in Chalnakhel about an one meter to two meter distance on June 2006. 200 gms powedered of *Lantana camara* was added in each plant 50 Plants in the field experiment. These were harvested on May 2007.

Elemental analysis of Plant and Soil Materials

The fresh leaves of the plant species were air dried and ground to pass a 0.5 mm sieve. The Concentrations of K and P of dried plant leaves eroded soil were analyzed with ICP-AES. N was analyzed with an elemental analyzer (Elementar Analysensysteme GmbH. Modell vario MAX CN.). Total nitrogen was determined using Kjeldhal's method. Walkey-Black's method was used for determining organic matter content by wet digestion with K_2CR_207 and concentrated H_2SO_4. Modified Olson's method was used for measuring available phosphorous (with sodium bicarbonate) and P in leaves after digestion in nitric-perchloric acid (5:3) were determined by colorimetr according to Murphy and Riley (1962s). Available Potassium was determined using flame photometry after extraction by neutral and normal ammonium acetate solution. pH of the soil was determined with ratio of 1:1 (soil: water ratio). These data are reported in Table 1.

Spore Analysis

The AM fungal spores within 25 g of the soil of plantation site were extracted before plantation and after harvest and identified them. Spores were extracted using wet sieving and sucrose density gradient centrifugation (McKinney and Lindsey 1987). Spores were mounted in polyvinyl alcohol on slides and examined using a compound microscope. Species were identified to species using taxonomic characteristics described in INVAM (2005) and Schenck and Perez (1990).

Weigh 25 gm of soil samples. Soil is mixed in a substantial volume of water and decanted through a series of sieves (750 Micron, 250 Micron, 100 Micron and 50 Micron) after allowing heavy soil particles to settle for a few minutes. This washing and decanting process is repeated until the water is clear. Roots and coarse debris are collected on a coarse. Then these finely kaolin clay remaining last sieve (50 Micron) transfer to centrifuge tube then was added water in equal weight of each four tube and then centrifuge it for 3 minutes at 2000 RPM. We should ensure that the centrifuge is properly balanced before switching it on. After this supernatant and floating debris was discarded.

The next step involves re-suspending the pellet in 50% sucrose by vigorously shaking tightly stopper tubes. The samples were then centrifuged for 1 minute at 2000 RPM to separate spores from denser soil components. Immediately after centrifugation, spores in the sucrose supernatant were poured onto the finest sieve (50 Micron) and carefully were washed with water to remove the sucrose.

After rinsing the spores, were washed them onto a pre-wetted filter paper in a Buchner funnel before vacuum filtration. In this we used what man filter paper for spore counting.

Semi-permanent microscope slide preparations of spores can be made using polyviny alcohol-lactoglycerol (PVLG). Spores on microscope slides were squashed to reveal inner-wall layers and then were used dissecting microscope for identification (Brundrett M *et al.* 1996 and N.C. Schenck and Yvonne Perez, Third edition 1990). These spore extraction and identification was done in Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur with the help of Dr. Prof. Nancy

Johnson, Environmental and Biological Sciences, Northern Arizona University, USA Fig. 4-7. Spores were counted in each planted soil.

Results and Discussions

After one year all these plantation were harvested. Among control five plants were died due to poor soil quality and low organic matter but with organic matter all the plants were survived. The height of plants were measured control as well as with organic matter.

Before field experiment number of spores per 25 gms, of soil have only 40 spores in average. In this *Glomus* species were more than that of *Acaulospora* species.

After harvest average number of spores in control were 50 and with organic matter number of spores were present 250 per 25 gms. of soil Table 3. In this five species were found such as *Glomus macrcarpon, Glomus constrictum, Acaulospora spinosa* and *Acaulospora scobitulata* (Fig. 4-7). Average height of the plant with organic matter were 2.5 mt. and average height of plant in control were only 0.62 mt. Table 2.

Table 2. Average height of plants

No	Treatment	Average Plant height
1.	Plantation of nursery plant with *Lantana camara*	2.5 mt
2.	Plantation only nursery plant without *Lantana camara*	0.62 mt

In this the plant with organic matter had more height than that of control

Table 3.

No	Treatment	Spores present in 25 gms. of soil
1.	Plantation of nursery plant with *Lantana camara*	250
2.	Plantation only nursery plant without *Lantana camara*	50

In this the soil with organic matters (*Lantanacamara*) had many spores and control had only few.

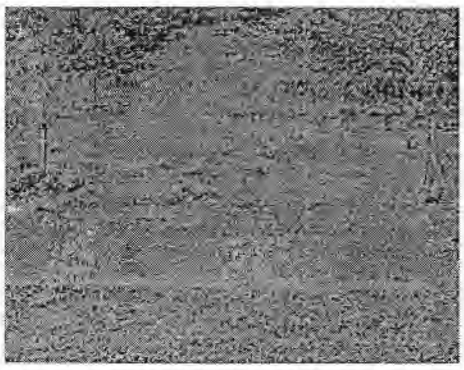


Fig. 1. Before plantation (Chalnakhel forest)

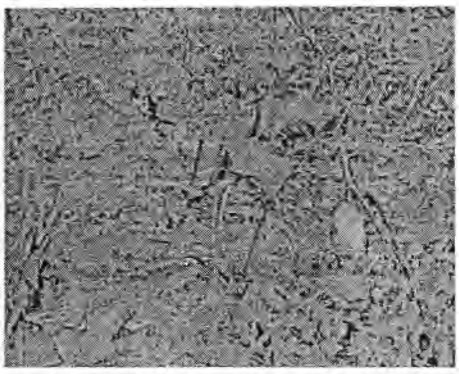


Fig. 2. Plantation after one year (Control)

Fig. 3. Plantation after one year (with *Lantana camara)*

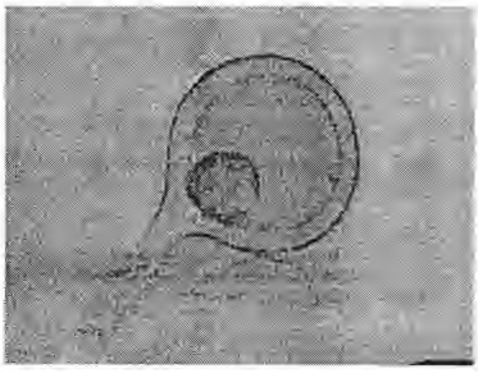


Fig. 4. *Glomus macrcarpon*

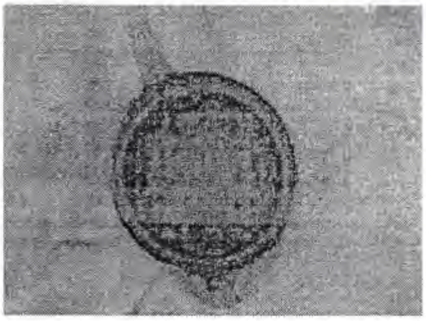


Fig. 5. *Glomus constrictum*

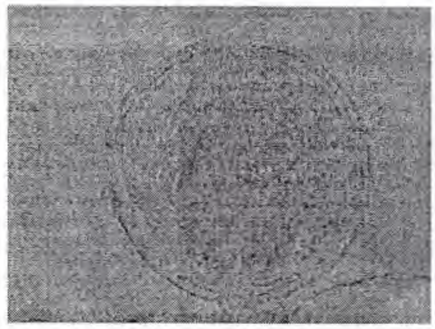


Fig. 6. *Acaulospora spinosa*

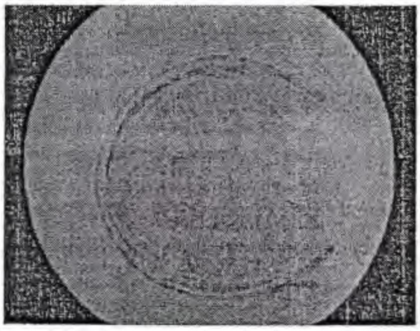


Fig. 7. *Acaulospora scobitulata*

Discussion

The beneficial role of organic matter may also be related to an improvement of physical properties like increased soil porosity and reduced mechanical resistance to hyphal growth through the soil (Ejjoner *et al.* 1995). Giovanetti and Avio (1985) found that additions of different materials, which increased the pore volume in soil, had a beneficial effect on mycorrhizal growth response, colonization and spore numbers. The production of recently formed extraradical AM mycelia is an important parameter since it may be directly related to the capacity of the plants to take up nutrients and to improvements of the soil structure and stability in degraded soils.

It is possible that nutrients such as N added with the organic matter have had a beneficial effect on the growth of AM fungi. In laboratory studies it has been found that N-containing organic matter, such as baker's yeast and bovine serum albumin, can have a stimulating effect on AM growth, while organic matter with higher C:N ratios, such as cellulose and starch, can have inhibitory effects (Ravnskov *et al.* 1999). Geeta Shrestha Vaidya *et al.* 2007b have been found that *Lantana camara* having a higher C:N ratio and lower N and P content than leaves from the other two agroforestry plants *Tithonia diversifolia* and *Eupatorium adenephorum* (green manure).

Furthermore it has been shown that the decomposition rates and N and P mineralization from litter of these and similar plants is highly correlated to total P content (Kwabiah *et al.* 2001). Species of AM fungi (Douds & Schenck 1990) and other soil microorganisms McKinley *et al.* (2005) differ in their responses to soil C, N and P availability.

The addition of compost or green manure is an important way to improve the soil in degraded areas since nitrogen and other nutrients, as well as organic matter which improves soil structure, is added with the organic material (Caravaca *et al.* 2002; Muthukumar & Udaiyan, 2000, Nziguheba *et al.* 2000 and Geeta Shrestha Vaidya *et al.* 2007a.

Improved nutrient and water uptake by the planted trees can be expected in response to better AM growth and the positive effect on the growth of AM fungi is in good agreement with results obtained by other authors (Douds *et al.* 1997; Baby & Manibhushanrao 1996; Muthukumar & Udaiyan, 2000; Gryndler *et al.* 2002; Harinikumar & Bagyaraj 1989; Jamil Mohammed *et al.* 2003; Jeffries & Barea (2001). In addition, St John *et al.* (1983), Frey & Ellis (1997) and Friberg (2001) found that AM fungal hyphae grew best in soils with a high amount of organic matter.

The present study provides the first information on a stimulating effect of organic material addition on extra-radical growth of AM fungi in eroded slopes in Nepal. These results show that organic matter addition can improve plant growth survival in such areas, and it also help for soil quality due to increasing rate endomycorrhiza (AM spores).

Recommendation

The interest in organic agriculture as well as in forestry in developing countries is growing because it requires less financial input and places more reliance on the natural and human resources available. Organic fertilizers offers comparative advantage in soil quality. In this we know that local wastages green manure materials could use for organic fertilizers. So, I want to highly recommended that this type of work should continue in future also. In the context of our country, farmers and growers should be educated to reduce the conventional chemical fertilizers all farmers should know the impact of those chemical fertilizers in the soil. It deteriorated the soil quality. Hopefully, the use of green manure in trial will be beneficial to the farmers to grow organic forest rendering no harm to the environment and the soil.

Conclusion

Organic amendments significantly increased AM fungal biomass in eroded slopes of Nepal. AM fungi is an important parameter because it can be directly related to the capacity of host plants to acquire nutrients and improve soil structure and stability in degraded soils. In this case, the higher forest product with applications of the higher quality *Lantana* are due to a combination of more P and N added and faster release patterns of P and N from *Lantana* as compared to control.

It has been shown that appropriate management of mycorrhizae in forest allows a substantial reduction in the use of chemicals, thus lessening the level of pollution and it keeps the soil in good quality and it has bio-control properties also. The use of green manure in forest and in turn contribute to the development of a healthy and sustainable soil and as well as environment. So, finally it can conclude that organic matter (local wastages green manure) content is usually higher in organically fertility and stability of organic soils as well as moisture retention capacity from which it reduces the risk of erosion and desertification.

Acknowledgement

We would like to thank Dr. B.R. Khadge, Chief of Plant Pathology, Division, Nepal Agriculture Research Centre (NARC), Khumaltar, Lalitpur, Nepal for his valuable suggestion and also thankful to staff of Chalnakhel nursery to their help during the experiment.

Farmers' Field Application of Mycorrhiza in the Maize Based Cropping Systems

Gautam Shrestha, Geeta Shrestha Vaidya and Binayak P. Rajbhandari

Abstract

AM fungi biofertilizer was applied in the farmers' field to measure its effects on the soil fertility improvement, plant yield contribution and to scrutinize mycorrhizal population development. The study was carried out in rainfed dryland area of Kavrepalanchwok district implementing randomized complete block design (RCBD) with total 6 treatments with 4 replications in maize based cropping patterns followed by fingermillet, mustard, wheat and pea which are the staple food crops of the dryland and farming systems prevailed in Nepal. Results show that plant height (cm) was highest in T3 in maize (241.75±12.00) among treatments showing contribution of mycorrhiza. TDM (%) contribution by mycorrhiza was distinct in pea (96.98±1.76) in T6. Concerning yield, maize (10.56 t/ha) and pea (196.60 kg/ha) was high in the T6 (NK+FYM+mycorrhiza). Organic matter (%) contribution by mycorrhiza was observed higher in mustard (3.42±0.07) and wheat (3.83±0.34) in T6. Available phosphorus (kg/ha P_2O_5) status in fingermillet (32.54±5.33), pea (58.30±17.10) and wheat (39.46±3.10) was better in T6. Contribution on nitrogen (%) enhancement by mycorrhiza was distinct in pea (0.61±033) and wheat (0.12±0.063) in T6. Mycorrhizal spore density was higher in T6 treatment of pea (200) and wheat (200) in 200 gm root soil sample.

Introduction

In the last few decades, interest in AM fungi has increased in the name of organic, restoration, and sustainable agriculture. The production and application of these beneficial fungi for agricultural purpose has grown over the last decades worldwide; companies are producing mycorrhizal fungi and their number is rising. The application of AM fungi has enormous potential for large scale agricultural systems and can be beneficial in sustainable production of main crops contributing to reduced input of chemical fertilizers and pesticides (Baar 2008).

Arbuscular mycorrhizal fungi (AMF) form beneficial symbiosis with roots of many plants to allow them to maintain and grow well under relatively harsh mineral stress conditions (Clark and Zeto 2000). The AMF fungi especially benefit plants grown in soils where P limits plant growth (Fageria 2009).

As nearly 49% soils of Nepal are acidic in reaction (Karki and Sherchan 2006) most part of phosphorus is fixed in soil preferentially with iron and aluminium (Kanwar 1976) into unavailable forms (Fagaria 2009). Alternative soil management strategies are needed for resource-poor farmers to improve yields of crops in these soils by adding least amount of inputs such as expensive phosphate fertilizers.

Materials and Methods

Farmers' field experimentation was conducted in Mathurapati Phulbari VDC of Kavrepalanchwok district. This VDC is at a distance of 6 km from Kavrepalanchwok district headquarter Dhulikhel (Joshi and Bhandari 2007).

Details of treatments:

T1 Control

T2 FYM only

T3 Mycorrhiza inoculation

T4 NK+FYM

T5 FYM+NPK

T6 FYM + NK + Mycorrhiza inoculation

- Randomized complete block design (RCBD) was followed with 6 treatments and 4 replications. The above mentioned experiments were conducted in maize/millet - fallow, maize - mustard, maize - pea, maize - wheat cropping patterns.
- Experimental plot size: 25 sq. m.
- Total experimental field size: 3600 sq. m.

Mycorrhiza isolation, inoculum production and field application

Mycorrhiza isolates were obtained from Nepal Academy of Sciences and Technology (NAST) by wet sieving and decanting, adapted from Gerdemann and Nicolson (1963).

Inoculum was cultured in 1:1 sand and red soil mixture which was autoclaved (121 °C and 15 lbs) for 3 hours. The medium used for this was a phosphorus deficient (2.8 kg/ha) with acidic soil pH (5.94) (Table 1). Mother culture containing 20 spores each were added as primary inoculum in each pot (15 kg). Onion tubers, having fast growing habit were used as host plant. Pots were watered regularly to maintain water levels to enhance the proper growth and development.

Table 1. Soil analysis results of the inoculum media

Particular	OM %	Total nitrogen	Soil pH	P_2O_5 (kg/ha)	K_2O (Kg/ha)
Soil Inoculum Media	2.20	0.15	5.94	2.80	39.62

Inoculum was harvested at 65 to 90 days of the inoculation after achieving adequate number of spores and percentage root colonization as per Gerdemann and Nicolson (1963). Plant tops were removed up to ground level with a pair of fine scissors. The root portions were chopped to make fine pieces of 1 to 2 cm length. The whole substrate along with spores and colonized roots was mixed properly to make homogenized mixture of AM inoculum.

Mycorrhiza inoculation was done on the basis of 800 g was added to a 1 sq.m. referring to the Prabakaran *et al.* (1995) with total 20 kg per plot in maize, pea and wheat. Row placement was done in maize planting as it has wider spacing of rows. For fingermillet, during nursery bed preparation, a layer of mycorrhiza inoculum was placed @ 800 g/sq.m. for T3 and T6. Additional 15 kg mycorrhiza inoculum was applied in each plot of mycorrhizal plots.

Crop production

Maize fingermillet, mustard, pea and wheat were selected as crop components of maize based cropping system. Varieties of crops, sowing date, fertilizer doses, crop geometry and harvesting dates as presented in the Table 2.

Table 2. Agronomical details of crops

Crop	Variety	Sowing date	Fertilizer dose N:P:K kg/ha	Crop geometry R x P (cm)	Harvesting date
Maize	Population 45	16th May	60:40:40	75 x 25	29th Sept. '08
Fingermillet	Kavre kodo	9th July (nursery)	80:40:30		23rd Nov. '08
Mustard	Pragati	3rd Oct.	60:40:20	45	22nd Dec. '08
Pea	Sikkime	20th Oct.	15:40:10	30	7th Mar '09
Wheat	Pasang Lhamu	22nd Oct.	50:50:20	20	12nd Mar '09

Field observation and measurement

Biometric measurements were done in 5 fixed plants per replication. Plant height, number of plants per meter square was measured during the harvesting of crops. Plant samples for TDM analysis were taken after harvesting crops.

Soil analysis

Soil analysis was done before planting maize and after harvest of each crop. Following measurements were done to determine the soil fertility status (Table 3).

Table 3. Methods followed for soil analysis

S.N.	Measurements	Methods
1	Texture	Hydrometer
2	pH measurement	pH meter
3	Organic matter	Walkley black
4	Total nitrogen	Modified Kjeldahl
5	Available phosphorus	Modified Olsen's
6	Available potash	Flame photometer

Data analysis

Data analysis was done using MINITAB.

Results and Discussion

Crop parameters

Plant height

Phosphorus is one of the macro nutrients required for proper plant growth viz. increased stalk and stem strength (Griffith 2009). As mycorrhiza enhances the phosphorus availability, plant height is partly contributed by the mycorrhiza symbiosis. In maize highest plant height 241.75±12.00 cm was obtained in the T3 (mycorrhiza), whereas finger millet (84.10±2.70 cm) and pea (134.4±18.3 cm) were more in height in T2 (FYM). Mustard had shown highest height in T4 (38.3±0.9) with NPK and FYM. In contrast, wheat showed distinct plant height in T5 (NK+FYM). Performance of mycorrhiza was more distinct in maize than in other crops (Table 4).

Table 4. Plant height measurements of crops (cm) mean ± SE

Treatments	Maize	Fingermillet	Mustard	Pea	Wheat
T1	186.25±7.39	48.87±5.32	28.1±0.1	81.7±1.7	69.0±9.4
T2	228.25±3.92	84.10±2.70	25.7±4.7	134.4±18.3	82.9±2.9
T3	241.75±12.00	74.47±6.42	26.2±0.2	113.6±14.3	69.9±3.8
T4	236.50±6.98	71.40±9.73	38.3±0.9	108.4±17.7	76.9±7.8
T5	233.75±4.39	75.73±5.84	33.1±2.1	121.1±8.1	84.2±2.7
T6	239.25±9.29	68.00±9.68	29.2±10.8	121.4±27.9	68.8±5.2

TDM

TDM contribution was higher in mycorrhiza inoculated plots in pea (96.98±1.76% in T6). Mycorrhiza treatment (T2) has resulted into 94.98±0.42% in wheat however Covacevich *et al.* 2007 found highest relative wheat shoot dry matter yield at 15.5 mg/kg^{-1} at which average AM colonization was 39%. On a par with Joner and Jakobsen (1995) the results of dry wt. of mycorrhizal and non mycorrhizal plants were not significantly different and was higher in T2 (86.25±3.10%) than mycorrhiza inoculated T3 (80.50±5.58%) in maize (Table 5).

Table 5. Total dry matter (%) (mean ± SE)

Treatments	Maize	Finger millet	Mustard	Pea	Wheat
T1	82.38±2.44	65.57±5.92	93.87±0.35	94.36±1.73	93.93±0.37
T2	86.25±3.10	69.85±3.05	97.30±0.23	95.40±0.10	94.30±0.76
T3	80.50±5.58	67.35±6.57	96.83±0.50	95.04±0.46	94.98±0.42
T4	83.76±2.22	74.51±2.44	96.60±0.14	93.34±0.23	93.30±0.98
T5	82.46±3.91	57.99±3.20	96.28±0.28	92.31±0.60	93.56±0.79
T6	82.46±3.90	58.90±2.52	96.66±0.48	96.98±1.76	94.43±0.82

Yield

Smith and Read (1997) found inoculation increase in crop yield by 37% on an average. Research revealed maize (10.56±0.66 t/ha) and pea (196.60±69.00 kg/ha) yield was highest in T6 treatment whereas T6 followed the T5 yield in the wheat and mustard. FYM treatment yielded highest in fingermillet (3.75 t/ha) followed by T5 (Table 6).

Table 6. Yield (mean ± SE)

Treatments	Maize (t/ha)	Fingermillet (t/ha)	Mustard (kg/ha)	Pea (kg/ha)	Wheat (t/ha)
T1	4.18±0.87	2.21±0.60	412.50±12.50	73.40±1.80	1.96±0.24
T2	9.44±0.78	3.75±2.21	501.00±141.00	116.70±19.20	2.15±0.16
T3	7.18±1.15	2.85±1.40	536.50±16.50	106.00±24.50	2.33±0.51
T4	9.36±0.53	2.56±0.78	457.70±64.30	97.70±18.50	2.12±1.10
T5	7.34±1.31	2.78±0.35	697.99±4.98	116.70±37.40	2.89±0.16
T6	10.56±0.66	3.33±0.51	596.50±85.90	196.60±69.00	2.72±0.81
Grand mean	8.01	2.91	533.2	121.9	2.4
CV%	23.89	60.39	19.03	54.83	49.39

Soil analysis

Soil analysis results have shown that soil pH decrease was seen in T6 (6.25±0.09) in the case of maize after T2 (6.23±0.15). Subsidiary effect of mycorrhiza was observed with 4.50±0.25 (T6) in mustard whereas wheat has 5.12±0.09 in T6. Soil pH was 6.9 in non mycorrhizal plants and difference was found as less as 6.2 in mycorrhizal plants by Li *et al.* (1991). Presence of hyphae may support a higher microbial population and higher rates of nitrification in the hyphal compartment thus contributing to the pH decrease in the hyphal compartments of the mycorrhizal plants in addition to the enhanced net excretion of H^+ due to NH_4^+ uptake by the hyphae (Li *et al.* 1991).

Organic matter status is highest in T6 (3.83±0.34% in wheat) with mycorrhiza, in T3 (2.98±0.22% in pea) with only mycorrhiza among other treatments. As a subsidiary effect of mycorrhiza in maize mustard cropping pattern, T6 in mustard has highest organic matter content (3.42±0.07%) among treatments.

Nitrogen content was higher in pea (0.61±0.33%) and wheat (0.12±0.06) in T6 confirming Powell and Klironomos (2007) that all mycorrhiza are able to transport N and P also confirming legumes are especially prone to mycorrhizal association (Frick 2009). In case of other plants, nitrogen fertilizer and in the case of fingermillet FYM contribution is observed. Studies have revealed that nodulation by indigenous rhizobia is greatly improved by AM fungi (Hayman 1982). Specific root exudates in mycorrhizal legumes may act as chemotactic attractants to rhizobia (Abbot and Robson 1984). Thus, AM fungal colonization and spread within the root somehow predisposes the legume host to form more nodules resulting in higher nitrogen fixation (Goltapeh *et al.* 2008).

Available phosphorus status in the soil after crop harvest was observed distinctly in T6 in fingermillet, wheat and pea. In the case of maize, T3 (26.51±9.54%) better contribution on phosphorus which supports Vogel-Mikus and Regvar (2006) found in line with Smith (2000) the contents of mineral nutrients e.g. phosphorus, significantly increased in inoculated plants, indicating functional exchange of nutrients and presumably carbohydrates between the partners. In the case of mustard, being non responsive to the mycorrhiza, the phosphorus contribution is seen in T5.

Fertilizer treatments (T5 and T4) were higher in potash status among treatments. In pea, available potash content was higher in T6 (66.84±7.69 kg/ha) distinctly and is supported by findings of Das *et al.* (1999) that mycorrhiza inoculation has positive relation with K uptake by legumes.

Table 7. Soil analysis results (mean±SE)

Treatments	pH	Soil OM (%)	Total nitrogen %	P_2O_5 (kg/ha)	K_2O (kg/ha)
			Before planting maize		
	5.18±1.42	4.04±0.24	0.13±0.03	12.38±0.92	310.69±5.60
			Maize		
T1	5.94±0.19	2.99±0.34	0.05±0.02	12.90±1.38	354.57±69.40
T2	6.23±0.15	8.60±0.32	0.15±0.07	16.83±6.29	405.18±102.90
T3	6.03±0.07	2.53±0.19	0.15±0.06	26.51±9.54	485.31±37.80
T4	6.08±0.06	8.65±0.03	0.19±0.08	18.58±4.73	497.20±79.72
T5	5.87±0.14	8.60±0.00	0.10±0.02	13.33±1.41	433.15±143.60
T6	6.25±0.09	8.60±0.00	0.17±0.06	14.51±4.50	459.56±38.68
			Fingermillet		
T1	5.78±0.20	2.32±0.32	0.07±0.05	21.69±6.06	256.19±26.80
T2	5.96±0.14	2.24±0.13	0.24±0.00	18.35±42.92	130.74±19.81
T3	5.57±0.31	2.48±0.33	0.22±0.06	30.11±15.15	279.96±65.07
T4	5.85±0.12	3.48±0.61	0.11±0.02	20.57±14.48	232.42±25.19
T5	5.89±0.36	2.24±0.07	0.11±0.05	20.02±9.03	309.01±92.51
T6	5.61±0.20	3.03±0.84	0.11±0.07	32.54±5.33	298.45±57.93
			Mustard		
T1	5.43±0.30	2.30±0.35	0.01±0.00	37.59±0.85	31.71±7.92
T2	4.59±0.18	3.00±0.07	0.07±0.02	30.38±0.25	47.60±15.80
T3	5.72±0.05	2.75±0.10	0.10±0.02	43.98±0.50	48.06±0.50
T4	4.57±0.19	2.32±0.09	0.14±0.06	43.94±3.14	58.10±11.50
T5	4.75±0.17	2.46±0.31	0.25±0.04	49.55±4.66	89.80±18.50
T6	4.50±0.25	3.42±0.07	0.21±0.07	43.98±2.00	55.48±0.00
			Pea		
T1	5.13±0.03	1.74±0.12	0.04±0.02	26.79±3.23	17.44±0.00
T2	5.65±0.04	2.02±0.24	0.04±0.01	33.28±2.72	46.50±15.40
T3	5.54±0.04	2.98±0.22	0.07±0.03	43.66±4.57	40.69±7.69
T4	5.69±0.18	2.77±0.91	0.10±0.00	41.98±0.89	58.12±7.69
T5	5.45±0.13	2.49±0.00	0.19±0.01	44.69±3.52	43.5[illegible]±5.03
T6	5.96±0.10	2.95±0.63	0.61±0.33	58.30±17.10	66.84±7.69
			Wheat		
T1	5.05±0.06	2.08±0.03	0.01±0.00	23.84±3.65	49.30±0.45
T2	5.80±0.08	3.58±0.03	0.12±0.00	30.81±0.60	105.93±5.01
T3	5.70±0.10	3.46±0.20	0.04±0.01	32.07±0.37	87.20±15.80
T4	5.19±0.04	3.08±0.32	0.10±0.03	31.51±0.52	66.00±4.58
T5	5.33±0.01	2.78±0.28	0.11±0.02	30.95±2.16	55.46±11.50
T6	5.12±0.09	3.83±0.34	0.12±0.06	39.46±3.10	71.30±16.50

Mycorrhizal spore density

Mycorrhizal spore counting has shown better effect on pea (200 spores per 200 gm soil sample in T6) followed by wheat (200), fingermillet (63) and maize (67.2) amending SSD (2002) results of mycorrhiza spore count per 100 gm soil was 15 and 22, respectively in VAM + NPK and NPK only. Mustard being nonresponsive crop, subsidiary effect of maize inoculums was seen (Figure 1). Conferring Hameeda *et al.* (2007) in which they found about 15% mycorrhizal colonization in control treatments, in this research we found mycorrhizal population development in control (8, 12.5, 6, 12 and 3 respectively in maize, fingermillet, pea, wheat and mustard) and FYM (30, 50, 50, 64, 20 respectively) plots might be due to natural infection. Amending the previous findings that inhibition of mycorrhiza development by mineral fertilization (Gryndler *et al.* 2006, Hayman 1982, Thomson *et al.* 1992, Olsson *et al.* 1997), use of N and K fertilizers only avoiding P fertilization can contribute positively. Confirming Harinikumar and Bagyaraj (1998) mustard being non-host plant has reduced the spore density than in preceding crop, maize more than 13% as they found. In the research conducted by Saini *et al.* (2004) with integrated nutrient management found 26.9% VAM infection after 45 DAS in 10 t FYM treatment compare to treatment of 10 t FYM+ *Azospirillum brasilense/Rhizobium* + VAM (47.7% VAM infection after 45 DAS) and this research results are also in par with mycorrhizal population was higher in FYM treatments in wheat (64) with compare to FYM + mycorrhiza treatment (52).

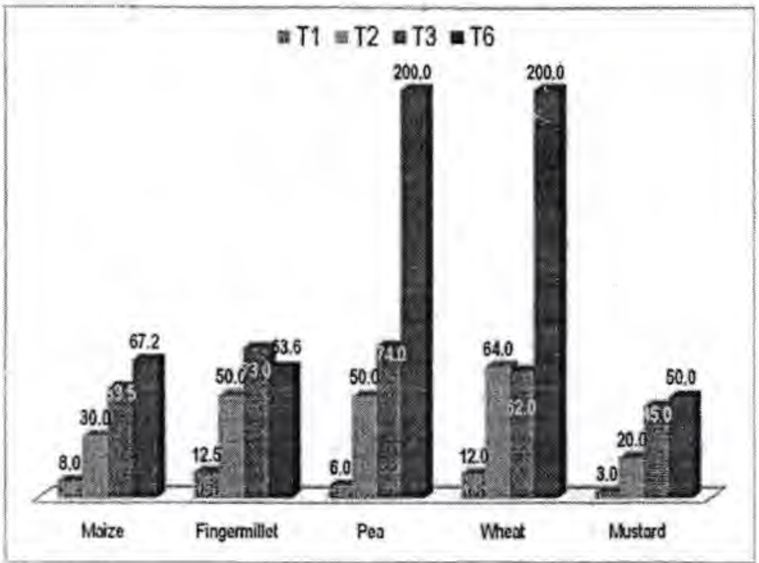


Fig. 1. Mycorrhizal spore density

Conclusion

Mycorrhiza is promising biofertilizer in the soils where phosphorus is present in nonlabile form to make it available. Mycorrhiza helps in yield contribution while applied with FYM, N and K fertilizers. Besides yield phosphorus also enhances TDM, plant height. Legume crop (pea) is more pronounced in the mycorrhizal effect than cereals whereas non mycorrhizal crop mustard could only show the subsidiary effects. Concerning mycorrhizal spore density, wheat and pea performed higher population.

Effect of Mycorrhiza on Crop Yields in the Maize-Based Cropping System

Gautam Shrestha, Geeta Shrestha Vaidya and Binayak P. Rajbhandari

Abstract

Series of field-based experiments were conducted in 2008 and 2009 to measure the effect of AM fungi on crop yields in the maize-based cropping patterns in rainfed dryland area of Kavrepalanchwok district. The experiments with 6 treatments and 4 replications were laid out employing RCBD. The crops included in the pattern were maize, fingermillet, mustard, wheat and pea. The results showed positive effect of AM inoculation on maize, fingermillet, pea and wheat while mustard was found irresponsive to AM inoculation. The average crop yield increment due to AM inoculation was 38%. Pea was found to have the highest number of AM spores followed by wheat and then maize and finger millet. These crops showed positive yield growth trend in association with increasing AM spore density. AM spore density was highest in all the crops when AM inoculum was applied with N, K and organic manure. Estimation of the coefficient of multiple determinations (R^2) revealed higher degrees of dependence of kernel yield variation on AM spore density.

Introduction

In the last few decades, interest in arbuscular mycorrhiza (AM) fungi has increased in the name of organic, restoration, and sustainable agriculture. The production and application of these beneficial fungi for agricultural purpose has grown over the last decades worldwide. The application of AM fungi has enormous potential for large scale agricultural systems, and can be beneficial in sustainable production of main crops contributing to reduced input of chemical fertilizers and pesticides. Arbuscular mycorrhizal fungi (AMF) form beneficial symbiosis with roots of many plants to allow them to maintain and grow well under relatively harsh mineral stress conditions (Clark and Zeto 2000). The AMF fungi especially benefit plants grown in soils where P limits plant growth (Fageria 2009).

As nearly 49 percent of soils of Nepal are acidic in reaction (Karki and Sherchan 2006) most part of phosphorus is fixed in soil preferentially with iron and aluminium (Kanwar 1976) into unavailable forms (Fageria 2009). Alternative soil management strategies are therefore needed for resource poor farmers to improve yields of crops in these soils by adding least amount of inputs such as expensive phosphate fertilizers. This paper presents the effect of AM on yield of various crops grown in maize based cropping system in the hill agroecosystem towards exploring alternatives inputs for sustainable agriculture.

Materials and Methods

Series of field experiments were conducted in farmer's fields at Mathurapati Phulbari VDC of Kavrepalanchwok district in 2008/09 in cooperation with local farmers. In this study, randomized complete block design (RCBD) with 6 treatments (Table 1) and 4 replications was followed. Individual experimental plot size was 25 sq. m. and the total experimental field size was 3600 sq. m.

Table 1. Details of experimental treatments

Treatment number	Treatments
T1	O-level control (without NKP, OM and AM inoculums)
T2	Organic Maure (OM) only
T3	AM inoculation only
T4	NK + OM
T5	OM + NPK (recommended practice)
T6	OM + NK + AM inoculation

Note:

- OM (FYM) was applied @ 10 MT/ha under maize and in the treatment requiring OM.
- NK was applied variously under different crops (maize: 60:40; finger millet: 80:30; mustard: 60:20; pea: 15:10; and wheat: 50:20 kg a.i./ha) in the treatments (T4, T5 and T6) requiring these elements.
- P_2O_5 was applied in the treatment (T5) requiring it @ 40 kg a.i./ha.

The experiments were conducted in hill eco-system with following cropping patterns:

- maize/millet - fallow;
- maize - mustard;
- maize - pea;
- maize - wheat.

Mycorrhiza isolation, inoculum production and field application

Mycorrhiza isolates were by wet sieving and decanting method adapted from Gerdemann and Nicolson (1963). Inoculum was cultured in 1:1 sterile sand and red soil mixture for 3 hours. Phosphorus deficient (2.8 kg/ha) red soil with acidic pH (5.94) (Table 2) was used as medium for culture. Mother culture containing 20 spores each were added as primary inoculum in each pot (15 kg). Onion tubers were used as host plant. Pots were watered regularly to maintain water levels to enhance the proper growth and development. Inoculum was harvested at 65 to 90 days of the inoculation after achieving adequate number of spores and percentage root colonization as per Gerdemann and Nicolson (1963). Plant tops were removed up to ground level with a pair of fine scissors. The root portions were chopped to make fine pieces of 1 to 2 cm length. The whole substrate along with spores and colonized roots was mixed properly to make homogenized mixture of AM inoculum. Inoculum production was done at HICAST. Routine analysis of inoculum media and experimental plot soil before maize planting was done at Soil Lab, HICAST. Results of that analysis are presented in Table 2.

Table 2. Soil routine analysis results of the inoculum media

Particular	Inoculum Media	Experimental field before maize
OM%	2.20	4.04±0.24
Total nitrogen	0.15	0.13±0.03
Soil pH	5.94	5.18±1.42
P_2O_5 (kg/ha)	2.80	12.38±0.92
K_2O (kg/ha)	39.62	310.69±5.60

Mycorrhiza inoculation was done @ 800 g per 1 sq. m. of plot following Prabakaran *et al.* (1995). It was applied in rows in maize, mustard, pea and wheat while in finger millet a layer of mycorrhiza inoculum was placed @ 800 g/sq. m. during nursery bed preparation. Additional mycorrhiza inoculum in the same rate was also applied in each experimental plot of finger millet with AM treatment, i.e. T3 and T6.

Crop production and measurement

Varieties of crops, sowing date, fertilizer doses, crop geometry and harvesting dates were as presented in the Table 3. Crops were grown under rainfed management. Biometric measurements were done in 20 fixed plants per treatment. Plant samples for yield analysis were taken during harvesting. Total sample size was 120 plants per crop species.

Table 3. Agrononical details of crop husbandry

Crop	Variety	Sowing date	Fertilizer dose N:P:K kg/ha	Crop geometry R x P (cm)	Harvesting date
Maize	Population 45	16th May	60:40:40	60 x 25	29th Sept '08
Finger millet	Kavre kodo	9th July (Nursery)	80:40:30	205 x 10	23rd Nov '08
Mustard	Pragati	3rd October	60:40:20	45 x 10	22nd Dec '08
Pea	Sikkime	20th October	15:40:10	30 x 20	7th March '09
Wheat	Pasang Lhamu	22nd October	50:40:20	20 x 5	12th March '09

Soil and data analysis

Soil analysis was done before planting maize and after harvest of each crop. Following measurements were done employing standard methods to determine the soil fertility status (Table 4). After crop harvest, AM population (spore density) in the soil collected from all treatments was done. Data analysis was done using MINITAB and Excel softwares.

Table 4. Methods followed for soil analysis

S.N.	Measurements	Methods
1.	Texture	Hydrometer
2.	pH measurement	pH meter
3.	Organic matter	Walkley black
4.	Total nitrogen	Modified Kjeldahl
5.	Available phosphorus	Modified Olsen's
6.	Available potash	Flame photometer

Yield

In this study, maize (10.56±0.66 MT/ha), finger millet (3.33± 0.51), pea (196.60±69.00 kg/ha) and wheat (2.99±016) yields were the highest in T6 whereas mustard had the highest yield in T5 (Table 5). Highest level of variability (CV %) in yield was found in finger millet and pea.

Table 5. Crop yields as affected by various treatments (mean±SE)

Treatments	Maize (MT/ha)	Fingermillet (MT/ha)	Mustard (kg/ha)	Pea (kg/ha)	Wheat (MT/ha)
T1	4.18±0.87	2.21±0.60	412.50±12.50	73.40±1.80	1.96±0.24
T2	9.44±0.78	3.15±2.21	501.00±141.00	116.70±19.20	2.15±0.16
T3	7.18±1.15	2.85±1.40	536.50±16.50	106.00±24.50	2.33±0.51
T4	9.36±0.53	2.56±0.78	457.70±64.30	97.70±18.50	2.12±1.10
T5	7.34±1.31	2.78±0.35	6974.99±4.98	116.70±37.40	2.40±0.81
T6	10.56±0.66	3.33±0.51	59o6.50±85.90	196.60±69.00	2.99±0.16
Grand mean	8.01	2.91	533.2	121.9	2.4
CV%	23.89	60.39	19.03	54.83	49.39

Findings of this study agree with the results of Smith and Read (1997) who reported that AM inoculation increases crop yield by 37% on an average. In this study, yield increment in T6 as compared to recommended practices (T5) was 43.86% in maize, 19.78% in finger millet, 68.47% in pea, and 21.69% in wheat (Table 6). Thus the average yield increment in these crops was 38.45 percent. In case of mustard, which is irresponsive to AM inoculum, yield declined by 14% in T6 as compared to T5.

Table 6. Effect of AM inoculation (T6) on yield increment as compared to recommended practice (T5).

Crop species	Yield in T5	Yield in T6	Yield increment, %
Maize (MT/ha)	7.34	10.56	43.86
Finger millet (MT/ha)	2.78	3.33	19.78
Mustard (kg/ha)	694.99	596.50	-(14.17)
Pea (kg/ha)	116.70	196.60	68.47
Wheat (MT/ha)	2.40	2.99	21.69

Mycorrhizal spore density

AM spore density computed in T1, T2, T3 and T6 treatments after harvest of the crops revealed variations reflecting the biological nature of the association between the AM fungus and crop species (Figure 1). Pea was found to have the highest number of AM spores followed by wheat and then maize and finger millet (Figure 1). Conferring Hameeda *et al.* (2007) report in which they stated about 15% mycorrhizal colonization in control treatments, this research revealed varying mycorrhizal population development even in control treatment (Tl) and FYM treatment (T2), which might be due to natural proliferation of AM fungi in adjoining small plots.

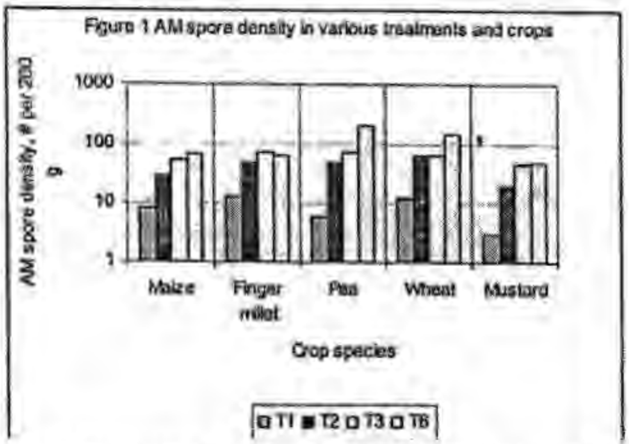
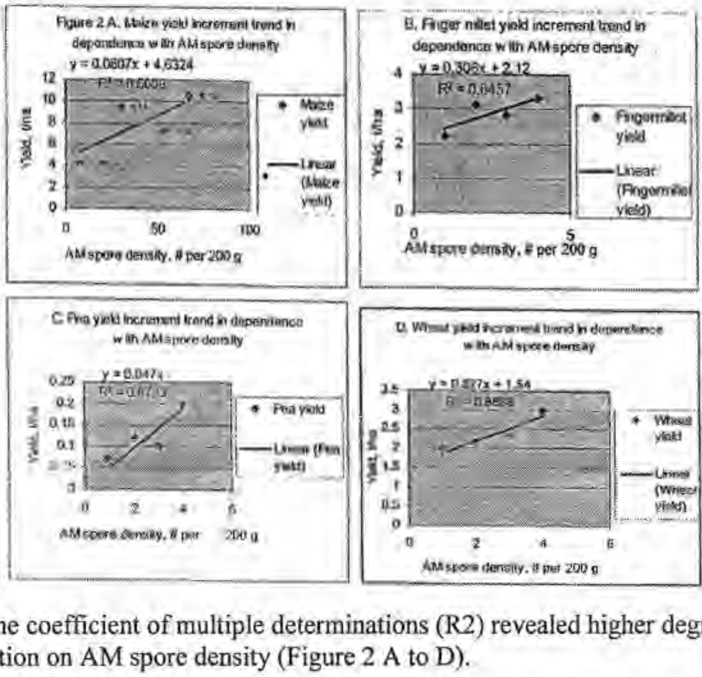


Fig. 1. Mycorrhizal spore density in the soil of experimental plots

It is interesting to note that AM spore density was highest in all the crops when AM inoculum was applied with NK and OM (T6). Thus this result has amended the previous findings that mineral fertilization inhibits mycorrhiza development (Gryndler *et al.* 2006; Hayman 1982; Thomson *et al.* 1992; Olsson *et al.* 1997). Obviously, inhibitory effect of mineral fertilizers like N and K is eliminated if mineral (synthetic) fertilizer is applied with organic manure. Comparing the results of T3 and T6, it is clear that organic manure promotes AM fungus growth and development if integrated synthetic fertilizers and organic manure are used in the rainfed farming system.

Relationship between crop yield and AM spore density

This research has revealed positive relationship between crop yield and AM spore density (Figure 2 A, B, C, D). Maize, fingermillet, pea and wheat were responsive to AM inoculation while mustard was found irresponsive. These crops showed positive yield growth trend in association with increasing AM spore density.



Estimation of the coefficient of multiple determinations (R2) revealed higher degrees of dependence of kernel yield variation on AM spore density (Figure 2 A to D).

Conclusion

Mycorrhiza is promising biofertilizer for the phosphorus deficient soils. AM inoculation along with integrated use of N, K and organic manure helps in increasing crop yield in maize-based cropping system under rainfed management in the hill ecosystem. In regard to AM inoculums responsive crops like cereals (maize, fingermillet, and wheat) and legumes (pea), the crop yield had direct positive relationship with AM spore density.

Enumeration of Herbaceous Plants in Imja Valley, Sagarmatha National Park, Nepal

E.N. Paudel, K.K. Shrestha and D.R. Bhuju

Abstract

A study was carried out in Imja valley, Sagarmatha National Park in Nepal Himalaya to document the flowering plant species. The study covered the area from Phungitanga (3400 m asl) to Chhukung (4,650 m asl). Beside the plot samplings, plant specimens were collected from throughout the route. Altogether 180 species of herbaceous angiosperms under 93 genera and 35 families were recorded. Asteraceae was observed as the largest family with 26 species. Similarly, *Saxifraga* was the largest genus comprising 9 species. Nineteen species represented new locality in eastern Nepal and 43 species with different altitudinal ranges, which were not recorded in previous studies.

Introduction

Of the Earth's surface, the alpine zone alone (above the tree line) makes up 3% land surface, but contains at least 4% of all vascular plant species (ICIMOD 2008). Therefore, mountains have attracted the attention of the researchers for the study of plants and their distribution pattern. The Himalaya comprising high mountain range is considered as one of the unique ecosystems on earth. Nepal (area: 147,181 sq. km) is centrally located in the Himalayan range and is typically a mountainous country with over 70% hills and mountains including alpine and nival zones. Shrestha and Joshi (1996) estimated occurrence of 225 endemic plant species above 3,000 m asl out of 246 such plants recorded from Nepal. Details, however, are lacking as most of the botanical explorations in Nepal have been done in the lower and middle parts of Nepal (Rajbhandari 2001) and so many areas are yet to be explored (Shrestha 2001).

The Khumbu area, which encompasses the Sagarmatha National Park and its adjoining areas are explored by different explorers *viz*. Stainton *et al.* in 1964 and 1969 (cited in Stainton 1972), Tabata *et al.* in 1978 (cited in Rajbhandari 2002), Miehe and Miehe in 1982 (cited in Miehe 1987). Similarly, Joshi (2005) described 123 species of wild flowers from Sagarmatha National Park and its adjoining area. The Darwin Initiative Project made expeditions in 2004 and 2005 representing collection of 344 and 413 species, respectively with 21 new records of flowering plants for Nepal from Sagarmatha National Park (Rajbhandari *et al.* 2004, 2008; Pendry *et al.* 2009).

The present study aimed at exploring and documenting the herbaceous plant species occurring in Imja valley as a complimentary works to prepare Flora of Sagarmatha area.

Materials and Methods

Location

Sagarmatha National Park is located in the north-eastern region of Nepal in Solukhumbu District of Sagamatha Zone. It has a roughly triangular geographical unit of 1,148 km^2 and is one of the most attractive parks in the world and includes a number of well-known high peaks of the Himalayas the most significant being Sagarmatha (Mount Everest 8,848 m).

Imja river valley is located in the north-eastern part of the Sagarmatha National Park. The present study was carried out from Phungitanga (3,400 m asl) to Chhukung (4,650 m). It lies between 27°49'58.08" to 27°54'18.48" North latitudes and 86°30'57.06" to 86°99'15.96" East longitudes. The vegetation up to timber line (3,800-4,050 m) is dominated by *Abies spectabilis, Betula utilis, Rhododendron campanulatum, R. campylocarpum, Acer campbelli, Lyonia ovalifolia, Juniperus recurva, Sorbus* spp., etc. The vegetation above the timber-line to 4,500 m is comparatively drier and the dominant species are *Juniperus indica, Rhododendron anthopogon* and *R. lepidotum* along with dwarf rhododendrons and cotoneasters, shrubby cinquefoil (*Potentilla fructicosa* var. *rigida*), willow (*Salix sikkimensis*) and *Cassiope fastigiata*. Above 4,500 m, grassland and dwarf shrubs like *Hippophae tibetana, Ephedra gerardiana, Juniperus indica* are predominant along with a variety of herbs such as *Gentiana prolata, G. stellata, Leontopodium stracheyi, Codonopsis thalictrifolia, Thalictrum chelidonii.*

Lilium nepalense, *Fritillaria cirrhosa*, *Primula denticulata*, *P. atrodentata*, *P. wollastonii* and *P. sikkimensis*. The park area is characterized by a semi-arid climate. Climatic data recorded at Pyramid Observatory Laboratory, Lobuche (5,050 m) shows that the monthly average temperature is -9.1 °C in February and 4.1 °C in July. While Namche Bazar (about 3,400 m) records -0.4 °C as monthly average temperature in winter (January) and 12.1 °C in July. Considerable variation in precipitation is recorded. Average precipitation is less than 1,000 mm per year (DNPWC 2004). About 80% precipitation falls during June to September and about 10% falls from May to October (Miehe 1989).

Collection and Identification of plant specimens

With emphasis on herbaceous flora, the field visit was made in post monsoon season (September-October) in 2007. Plant specimens were collected from sampling plots as well as enroute. For each species, at least three specimens were collected as far as possible. The voucher specimens were properly tagged during collection with appropriate field notes. The identification of collected specimens was done by comparing herbarium specimens deposited at Tribhuvan University Central Herbarium, Kirtipur (TUCH), and National Herbarium and Plant Laboratories, Godavari (KATH). The plants were also identified consulting relevant literature such as: Polunin and Stainton (1984), Stainton (1997), Grierson and Long (1983-2001), Noltie (1994, 2000, 2002), etc. The nomenclature of species was validated using latest taxonomic literature (Hara *et al.* 1978, 1979 and 1982; Press *et al.* 2000). The herbarium specimens are deposited at Tribhuvan University Central Herbarium (TUCH). The Engler system of Melchior (1964) has been followed in the arrangement of families. Species are arranged in alphabetical orders within each family and is provided with the place of the collection with altitude, latitude and longitude; date of collection and collection number.

Results

Altogether 180 species, including 2 subspecies and 3 varieties of herbaceous angiosperms under 93 genera and 35 families were recorded from the study area. Among them, 146 species were Dicotyledoneae belonging to 76 genera and 30 families; and 34 species were of Monocotyledoneae belonging to 17 genera and 5 families. Out of 180 species, 5 species were identified up to generic level whereas 15 species up to only family level.

The study area was found to be dominated by Asteraceae with 26 species and it was followed by Poaceae and Polygonaceae consisting of 24 species and 13 species respectively. Other larger families were Saxifragaceae (10 species); Gentianaceae, Rosaceae and Apiaceae consisted 9 species each; Ranunculaceae and Scrophulariaceae with 8 species each; Crassulaceae (6 speceis); Boraginaceae and Campanulaceae with 5 species each.

The largest genus recorded was *Saxifraga* comprising 9 species and it was followed by *Potentilla* with 7 species; *Anaphalis*, *Bistorta* and *Pedicularis* each consisting of 5 species. The other larger genera were *Cyananthus*, *Epilobium*, *Poa* and *Rhodiola* each with 4 species.

Table 1. Floristic composition of the study area

Category	Dicotyledoneae		Monocotyledoneae		Total
	Number	Percentage	Number	Percentage	
Family	30	85.71	5	14.29	35
Genus	76	81.72	17	18.28	93
Species	146	81.11	34	18.89	180

Enumeration of flowering plants recoderd from Imja Valley, SNP

POLYGONACEAE

Aconogonum sibiricum (Laxm.) H. Hara

Pangboche surrounding, 4000m, 27°51.39'N, 86°47.57'E, October 1, 2007, SNP53.

Aconogonum tortuosum (D. Don.) H. Hara

Pangboche surrounding, 4000m, 27°51.39'N, 86°47.57'E, October 1, 2007, SNP54.

Bistorta affinis (D. Don) Greene

Above Yaren forest, Pangboche, 4220m, 27°50.48'N, 86°47.63'E, October 5, 2007, SNP130.

Bistorta amplexicaulis (D. Don) Greene

Deboche forest, 3930m, 27°50.18'N, 86°46.37'E, October 10, 2007, SNP207.

Bistorta milletii Lev.

Pangboche surrounding, 4020m, 27°51.40'N, 86°47.39'E, October 1, 2007, SNP65.

Bistorta vaccinifolia (Wall. ex Meisn.) Greene

Yaren, Pangboche, 4010m, 27°51.29'N, 86°48.02'E, October 2, 2007, SNP81.

Bistorta vivipara (L.) Gray

Yaren, Pangboche, 3950m, 27°51.38'N, 86°47.78'E, October 2, 2007, SNP105.

Fagopyrum esculentum Moemch

Between Pangboche & Somare, 4030m, 27°52.00'N, 86°48.20'E, October 7, 2007, SNP152.

Koenigia deliculata (Meisn.) H. Hara

Yaren, Pangboche, 4010m, 27°51.29'N, 86°48.02'E, October 2, 2007, SNP80.

Oxyria digyna (L.) Hill

Dingboche, 4370m, 27°53.79'N, 86°50.35'E, October 7, 2007, SNP164.

Persicaria nepalensis (Meisn.) H. Gross

Near Tenboche, 3880m, 27°50.09'N, 86°45.92'E, October 16, 2007, SNP266.

Rheum acuminatum Hook. f. & Thomson ex Hook.

Between Pangboche and Deboche, 3930m, 27°50.18'N, 86°46.37'E, October 10, 2007.

CARYOPHYLLACEAE

Arenaria depauperata (Edgew.) H. Hara.

Near Chhukum, 4610m, 27°54.31'N, 86°52.26'E, October 8, 2007, SNP181.

Silene indica Roxb. Ex Otth

Between Pangboche and Somare, 3430m, 27°52.00'N, 86°48.20'E, October 7, 2007, SNP149.

Silene khasuaba Rohrb.

Phungitanga, 3430m, 27°49.97'N, 86°44.95'E, 30th Sep. 2007, SNP41.

Silene stracheyi Edgew.

Phungitanga, 3430m, 27°49.97'N, 86°44.95'E, 30th Sep. 2007, SNP40.

RANUNCULACEAE

Aconitum spicatum Stapf

Yaren forest, Pangboche, 4030m, 27°51.32'N, 86°47.99'E, October 2, 2007, SNP84.

Clematis montana Buch.-Ham. ex DC

Between Pangboche and Somare, 4130m, 27°51.82'N, 86°48.03'E, October 7, 2007, SNP147.

Delphinium stapeliosum Bruhl ex Huth.

Deboche surroundings, 3720m, 27°50.33'N, 86°45.58'E, October 12, 2007, SNP220.

Ranunculus brotherusii Freyn

Pangboche surroundings, 4020m, 27°51.42'N, 86°47.37'E, October 1, 2007, SNP60.

Ranunculus tricuspis Maxim.

Near Chukkum, 4320m, 27°54.31'N, 86°52.27'E, October 8, 2007, SNP172.

Thalictrum alpinum L.

Pangboche surroundings, 4020m, 27°51.42'N, 86°47.37'E, October 1, 2007, SNP56.

Thalictrum reniforme Wall. Ex Royle

North part of Dingboche, 4040m, 27°53.58'N, 86°49.33'E, October 07, 2007, SNP168.

Thalictrum reniforme Wall.

Phungitanga, 3430m, 27°49.97'N, 86°44.95'E, September 30, 2007, SNP38.

DROSERACEAE

Drosera peltata var. **lunata** (Buch.-Ham. ex DC.) C.B. Clarke

Deboche surroundings, 3670m, 27°50.29'N, 86°45.58'E, October 12, 2007, SNP233.

PAPAVERACEAE

Corydalis casimiriana Prain

Yaren, Pangboche, 4015m, 27°51.33'N, 86°47.79'E, October 2, 2007, SNP92.

Meconopsis paniculata Prain

Yaren, Pangboche, 3750m, 27°50.75'N, 86°46.46'E, 6October 14, 2007, SNP249.

BRASSICACEAE (CRUCIFERAE)

Capsella bursa-pastoris (L.) Medik.

Dingboche, 4320m, 27°53.49'N, 86°49.49'E, October 7, 2007, SNP161.

CRASSULACEAE

Rhodiola bupleuroides (Wall. ex Hook. f. & Thomson) S.H. Fu

Yaren, Pangboche, 4100m, 27°50.92'N, 86°47.61'E, October 3, 2007, SNP111.

Rhodiola chrysanthemifolia (Leveille) Fu

Deboche surroundings, 3660m, 27°50.36'N, 86°45.41'E, October 12, 2007, SNP225.

Rhodiola crenulata (Hook. f. & Thomson) H. Ohba

Chalunche, 4070m, 27°51.53'N, 86°48.08'E, October 6, 2007, SNP142a.

Rhodiola wallichiana (Hook.) S.H. Fu.

Yaren, Pangboche, 4020m, 27°51.33'N, 86°47.78'E, October 2, 2007, SNP96.

Sedum triactina Berger

Deboche surroundings, 3690m, 27°50.38'N, 86°45.52'E, October 12, 2007, SNP224.

Sedum trullipetalum Hook. & Thomson

Yaren forest, Pangboche, 3930m, $27^{0}51.34$'N, $86^{0}47.75$'E, October 2, 2007, SNP73.

SAXIFRAGACEAE

Bergenia purpurascens (Hook. f. & Thomson) Engl.

Between Pangboche and Deboche, 3940m, $27^{0}50.10$'N, $86^{0}46.28$'E, October 10, 2007, SNP209.

Saxifraga andersonii Engl.

Near Chhukum, 4610m, 27°54.31'N, 86°52.26'E, October 8, 2007, SNP180.

Saxifraga aristulata Hook. f. & Thomson

Pangboche surrounding, 4020m, 27°51.40'N, 86°47.39'E, October 1, 2007, SNP64.

Saxifraga brachypoda D. Don

Pangboche surrounding, 4000m, 27°51.39'N, 86°47.57'E, October 1, 2007, SNP51.

Saxifraga filicaulis Wall. ex ser.

Above Yaren forest, Pangboche, 4220m, 27°50.48'N, 86°47.63'E, October 5, 2007, SNP127.

Saxifraga hispidula D. Don

Above Yaren forest, Pangboche, 4220m, 27°50.48'N, 86°47.63'E, October 5, 2007, SNP128.

Saxifraga montana H. Sm.

Near Chhukum, 4610m, 27°54.31'N, 86°52.26'E, October 8, 2007, SNP171.

Saxifraga parnassifolia D. Don

Pangboche surrounding, 4000m, 27°51.39'N, 86°47.57'E, October 1, 2007, SNP55.

Saxifraga saginoides Hook. f. & Thomson

Near Chhukum, 4610m, 27°54.31'N, 86°52.26'E, October 8, 2007, SNP182.

Saxifraga strigosa Wall. ex Ser.

Yaren, Pangboche, 4000m, 27°51.29'N, 86°47.93'E, October 2, 2007, SNP83.

PARNASSIACEAE

Parnassia nubicola Wall. ex Royle

Yaren forest, Pangboche, 4000m, $27^{0}51.28'$N, $86^{0}47.93'$E, October 2, 2007, SNP76.

ROSACEAE

Fragaria nubicola Lindl. ex Lacaita

Lato Goth, Near Imja, 3600m, $27^{0}50.45'$N, $86^{0}45.71'$E, October 14, 2007, SNP256.

Potentilla coriandrifolia D. Don

Milingo forest, deboche, 3990m, 27°50.33'N, 86°46.80'E, October 11, 2007, SNP217.

Potentilla cuneata Wall. ex Lehm.

Yaren, Pangboche, 4020m, 27°51.33'N, 86°47.78'E, October 2, 2007, SNP98.

Potentilla josephiana H. Ikeda & H. Ohaba

Yaren, Pangboche, 4060m, 27°51.32'N, 86°48.01'E, October 2, 2007, SNP88.

Potentilla peduncularis D. Don

Above Yaren forest, Pangboche, 4220m, 27°50.48'N, 86°47.63'E, October 5, 2007, SNP126.

Potentilla polyphyla Wall. ex Lehm.

Pangbocche-Tengboche, 3840m, 27°50.62'N, 86°46.71'E, October 13, 2007, SNP235.

Potentilla saundersiana Royle

Between Pangboche & Somare, 4130m, 27°51.80'N, 86°48.03'E, October 7, 2007, SNP146.

Potentilla sp.

Above Yaren forest, Pangboche, 4220m, 27°50.48'N, 86°47.63'E, October 5, 2007, SNP129.

FABACEAE (LEGUMINOSAE)

Astragalus himalayanus Klotzsch

Deboche surroundings, 3790m, 27°50.60'N, 86°46.25'E, October 14, 2007, SNP241a.

Astragalus strictus Graham ex Benth.,

Deboche surroundings, 3790m, 27°50.60'N, 86°46.25'E, October 14, 2007, SNP241b.

Hedysarum sikkimense Benth. ex Baker

Near Chhukum, 4610m, 27°54.31'N, 86°52.26'E, October 8, 2007, SNP175.

Parochetus communis Buch.-Ham. ex D. Don

Deboche surroundings, 3610m, 27°50.46'N, 86°45.72'E, October 14, 2007, SNP257.

Thermopsis barbata Royle

Yaren, Pangboche, 4060m, 27°51.32'N, 86°48.01'E, October 2, 2007, SNP87.

GERANIACEAE

Geranium donianum Sweet

Yaren, Pangboche, 4000m, 27°50.91'N, 86°47.18'E, October 4, 2007, SNP118.

BALSAMINACEAE

Impatiens edgeworthii Hook. f.

Deboche surroundings, 3720m, 27°50.33'N, 86°45.57'E, October 12, 2007, SNP221.

VIOLACEAE

Viola biflora L.

Deboche surroundings, 3610m, 27°50.46'N, 86°45.72'E, October 14, 2007, SNP262.

ONAGRACEAE

Epilobium brevifolium subsp. **trichoneurum** (Hausskn.) P.H. Raven

Yaren, Pangboche, 4020m, 27°51.33'N, 86°47.78'E, October 2, 2007, SNP94a.

Epilobium latifolium subsp. **speciosum** (Decne.) P.H. Raven

Yaren, Pangboche, 4020m, 27°51.33'N, 86°47.78'E, October 2, 2007, SNP94c.

Epilobium royleanum Hausskn.
Deboche surroundings, 3740m, 27°50.71'N, 86°46.72'E, October 14, 2007, SNP238.
Epilobium wallichianum Hausskn.
Yaren, Pangboche, 4020m, 27°51.33'N, 86°47.78'E, October 2, 2007, SNP94b.

APIACEAE (UMBELLIFERAE)

Acronema johrianum Babu
Pangboche, 3980m, 27^{0}51.36'N, 86^{0}47.45'E, October 1, 2007, SNP69.
Cortia depressa (D. Don) C. Norman
North part of Dingboche, 4040m, 27^{0}53.58'N, 86^{0}49.33'E, October 07, 2007, SNP167.
Heracleum nepalense D. Don
Yaren forest, Pangboche, 3990m, 27^{0}50.60'N, 86^{0}47.31'E, October 4, 2007, SNP114.
Pleurospermum apiolens C. B. Clarke,
Near Chhukum, 4610m, 27°54.31'N, 86°52.26'E, October 8, 2007, SNP177.
Pleurospermum brunois (DC.) C.B. Clarke
Yaren, Pangboche, 3930m, 27^{0}51.34'N, 86°47.75'E, October 2, 2007, SNP72.
Pleurospermum hookeri C. B. Clarke
Yaren, Pangboche, 4000m, 27^{0}51.29'N, 86°47.93'E, October 2, 2007, SNP79.
Physospermopsis obtusiuscula (DC.) C. Norman
Above Yaren forest, Pangboche, 4200m, 27°50.67'N, 86°47.66'E, October 5, 2007, SNP133.
Selinum candollei DC.
Phungitanga, 3430m, 27^{0}49.97'N, 86°44.95'E, September 30, 2007, SNP34a.
Selinum wallichianum (DC.) Raizada & Saxena
Phungitanga, 3430m, 27^{0}49.97'N, 86°44.95'E, September 30, 2007, SNP34b.

ERICACEAE

Cassiope fastigiata (Wall.) D. Don
Yaren, Pangboche, 3950m, 27°51.38'N, 86°47.78'E, October 2, 2007, SNP104.

PRIMULACEAE

Androsace sarmentosa Wall.
Deboche surroundings, 3790m, 27°50.60'N, 86°46.25'E, October 14, 2007, SNP242a.
Primula atrodentata W.W. Sm.
Pangboche surrounding, 4000m, 27^{0}51.39'N, 86°47.57'E, October 1, 2007, SNP52.
Primula capitata Hook. f.
Deboche surroundings, 3750m, 27°50.75'N, 86°46.96'E, October 14, 2007, SNP250.
Primula sp.
Deboche surroundings, 3770m, 27°50.60'N, 86°46.25'E, October 14, 2007, 242b.

GENTIANACEAE

Comastoma pedunculatum (Royle ex D. Don) Holub
Chalunche, 3990m, 27°51.53'N, 86°47.84'E, October 6, 2007, SNP138.
Gentiana depressa D. Don.
Phungitanga, 3430m, 27^{0}49.97'N, 86°44.95'E, September 30, 2007, SNP42.
Gentiana ornata (G. Don) Griseb.
Yaren, Pangboche, 4000m, 27^{0}51.29'N, 86°47.93'E, October 2, 2007, SNP75.
Gentiana tubiflora (G. Don) Griseb.
Phungitanga-Tenboche, 3450m, 27^{0}50.01'N, 86°45.05'E, September 30, 2007, SNP45.

Halenia elliptica D. Don
Deboche surroundings, 3670m, 27°50.29'N, 86°45.58'E, October 12, 2007, SNP232.
Lomatogonium chumbicum (Burkill.) H. Sm.
Yaren, Pangboche, 4020m, 27°51.33'N, 86°47.78'E, October 2, 2007, SNP95.
Lomatogonium sikkimense (Burkill) H. Sm.
Yaren, Pangboche, 3900m, 27°51.00'N, 86°47.39'E, October 4, 2007, SNP113.
Swertia ciliata (D. Don ex G. Don) B.L. Burtt
Deboche surroundings, 3670m, 27°50.29'N, 86°45.58'E, October 12, 2007, SNP228.
Swertia racemosa (Griseb.) C. B. Clarke
Malinga forest, Panboche, 3770m, 27°50.305'N/ 86°46.116'E, October 12, 2007, SNP211.

RUBIACEAE

Gallium acutum Edgew.
Deboche surroundings, 3670m, 27°50.29'N, 86°45.58'E, October 12, 2007, SNP227.
Galium aparine L.
Dingboche, 4320m, 27°53.49'N, 86°49.49'E, October 7, 2007, SNP162.
Gallium asperifolium Wall.
Phungitanga, 3430m, 27°49.97'N, 86°44.95'E, September 30, 2007, SNP43.

BORAGINACEAE

Cynoglossum zeylanicum (Vahl ex Hornem.) Thunb. ex Lehm.
Between Pangboche & Somare, 4030m, 27°52.00'N, 86°48.20'E, October 7, 2007, SNP148.
Hackelia obtusiloba R.R. Mill
Pangboche surrounding, 4020m, 27°51.42'N, 86°47.37'E, October 1, 2007, SNP61.
Hackelia uncinata (Benth. in Royle) C.E.C. Fisch.
Between Pangboche & Somare, 4030m, 27°52.00'N, 86°48.20'E, October 7, 2007, SNP151.
Microula sikkimensis (C.B. Clarke) Hemsl.
Between Pangboche & Somare, 4030m, 27°52.00'N, 86°48.20'E, October 7, 2007, SNP150.

LAMIACEAE (LABIATAE)

Clinopodium umbrosum (M. Bieb.) K. Koch
Deboche surroundings, 3750m, 27°50.75'N, 86°46.96'E, October 14, 2007, SNP251.
Elsholtzia eriostachya (Benth.) Benth.
Yaren, Pangboche, 3900m, 27°51.00'N, 86°47.39'E, October 4, 2007, SNP109.
Phlomis bracteosa Royle ex Benth.
Deboche surroundings, 3750m, 27°50.75'N, 86°46.96'E, October 14, 2007, SNP247.

SOLANACEAE

Anisodus luridus Link & Otto
Deboche surroundings, 3610m, 27°50.46'N, 86°45.72'E, October 14, 2007, SNP260.

SCROPHULARIACEAE

Hemiphragma heterophyllum Wall.
Deboche surroundings, 3670m, 27°50.29'N, 86°45.58'E, October 12, 2007, SNP230.
Pedicularis gracilis Wall. ex Benth.
Yaren forest, Pangboche, 4050m, 27°51.43'N, 86°47.78'E, October 9, 2007, SNP99b.
Pedicularis oederi Prain
Chalunche, 3990m, 27°51.53'N, 86°47.84'E, October 6, 2007, SNP139.
Pedicularis oxyrhyncha T. Yamaz.
Deboche surroundings, 3670m, 27°50.29'N, 86°45.58'E, October 12, 2007, SNP229.

Pedicularis siphonantha D. Don.
Yaren, Pangboche, 4000m, 27°50.90'N, 86°47.13'E, October 4, 2007, SNP116.
Pedicularis trichoglossa Hook. f.
Yaren forest, Pangboche, 4050m, 27°51.43'N, 86°47.78'E, October 9, 2007, SNP99a.
Veronica ciliata var. **cephaloides** (Pennell) Hong
Churo, near Dingboche bridge, 4220m, 27°52.87'N, 86°49.15'E, October 7, 2007, SNP157.
Veronica sp.
Churo, near Dingboche bridge, 4220m, 27°52.87'N, 86°49.15'E, October 7, 2007, SNP156.

OROBANCHACEAE

Boschniakia himalaica Hook. & Thomson ex Hook. f.
Yaren, Pangboche, 4050m, 27° 51.01'N, 86°47.74'E, October 3, 2007, SNP106.

VALERIANACEAE

Nardostachys grandiflora DC.,
Above Yaren forest, Pangboche, 4250m, 27°50.67'N, 86°47.65'E, October 5, 2007, SNP134.
Valeriana hardwikii Wall.
Deboche surroundings, 3610m, 27°50.46'N, 86°45.72'E, October 14, 2007, SNP258.

MORINACEAE

Morina nepalensis D. Don
Chalunche, 4070m, 27°51.53'N, 86°48.08'E, October 6, 2007, SNP142b.

CAMPANULACEAE

Campanula pallida Wall.
Pangboche, 3730m, 27°50.34'N 86°45.58'E, October 12, 2007, SNP231.
Cyananthus incanus Hook. f. & Thomson
Chalunche forest, 4049m, 27°51.53'N, 86°48.30'E, October 6, 2007, SNP141.
Cyananthus inflatus Hook. f. & Thomson
Above Pangboche, 4200m, 27°50.58'N, 86°47.53'E, October 5, 2007, SNP125.
Cyananthus microphyllus Edgew.
Malinga forest, Debuche, 3850m, 27°50.16'N, 86°46.12'E, October 11, 2007, SNP216.
Cyananthus pedunculatus C.B. Clarke
Above Pangboche, 4220m, 27°50.84'N, 86°47.80'E, October 5, 2007, SNP137.

ASTERACEAE (COMPOSITAE)

Adenocaulon himalaicum Edgew.
Yaren forest, Pangboche, 3950m, 27°51.38'N, 86°47.78'E, October 2, 2007, SNP102b.
Adenocaulon nepalense M. Bittmann
Yaren forest, Pangboche, 3950m, 27°51.381'N, 86°47.781'E, 2.10.2007, SNP102a.
Ainsliea aptera DC.
Yaren forest, Pangboche, 3670m, 27°50.29'N, 86°45.40'E, October 12, 2007, SNP226b.
Ainsliea latifolia (D. Don) Sch. Bip.
Yaren forest, Pangboche, 3670m, 27°50.299'N, 86°45.407'E, October 12, 2007, SNP226a.
Anaphalis contorta (D. Don) Hook. f.
Deboche surrounding, 3750m, 27°50.74'N, 86°46.54'E, October 14, 2007, SNP248.
Anaphalis margaritacea (L.) Benth.
Pangboche, 3980m, 27°51.36'N, 86°47.45'E, October 1, 2007, SNP70.
Anaphalis subumbellata C.B. Clarke
Malinga forest, Panboche, 3770m, 27°50.305'N, 86°46.116'E, 12.10.2007, SNP212.

Anaphalis triplinervis var. **intermedia** (DC.) Airy Shaw
Yaren forest, Pangboche, 4130m, 27°50.68'N, 86°47.46'E, October 5, 2007, SNP122.
Anaphalis xylorhiza Sch.-Bip. ex Hook. f.
Dingboche surrounding, 4340m, 27°53.58'N, 86°49.33'E, October 7, 2007, SNP170.
Artemisia dubia Wall. ex Besser
On the way of Dingboche-Chukkum, 4130m, 27°54.30'N, 86°52.26'E, October 8, 2007, SNP183.
Artemisia stricta Edgew.
Pangboche surrounding, 4020m, 27°51.40'N, 86°47.38'E, October 5, 2007, SNP62.
Cremanthodium oblongatum C.B. Clarke
Yaren forest, Pangboche, 4000m, 27°51.28'N, 86°47.93'E, October 2, 2007, SNP77.
Cremanthodium reniformae (DC.) Benth.
Yaren forest, Pangboche, 4010m, 27°51.29'N, 86°48.01'E, October 2, 2007, SNP82.
Dubyaea hispida DC.
Yaren forest, Pangboche, 3930m, 27°51.34'N, 86°47.75'E, October 2, 2007, SNP72.
Erigeron multiradiatus (Lindl. ex DC.) C.B. Clarke
Yaren forest, Pangboche, 3990m, 27°50.60'N, 86°47.31'E, October 4, 2007, SNP115.
Leontopodium jacotianum Beauverd
Yaren forest, Pangboche, 4130m, 27°50.68'N, 86°47.46'E, 5.10.2007, SNP123a.
Leontopodium stracheyi (Hook. f.) C.B. Clarke ex Hemsl.,
Yaren forest, Pangboche, 4130m, 27°50.68'N, 86°47.464'E, October 5, 2007, SNP123b.
Ligularia amplexicaulis DC.
Pangboche, 3980m, 27°51.36'N, 86°47.45'E, October 1, 2007, SNP68.
Saussurea auriculata (DC.) Sch. Bip.
Lato Goth, Near Imja, 3600m, 27°50.45'N, 86°45.71'E, October 14, 2007, SNP255.
Saussurea nepalensis Spreng.
Lato Goth, Near Imja, 3850m, 27°50.15'N, 86°46.17'E, October 9, 2007, SNP200.
Senecio alatus Wall. ex DC.
Panboche-Tenboche, 3780m, 27°50.27'N, 86°46.10'E, October 13, 2007, SNP236.
Senecio chrysanthemoides DC.
Yaren forest, Pangboche, 4050m, 27°51.43'N, 86°47.78'E, October 9, 2007, SNP101.
Waldheimia glabra (Decne.) Regel
Somare, 4180m, 27°52.85'N, 86°49.06'E, October 8, 2007, SNP184.

LILIACEAE

Aletris pauciflora (Klotzsch) Hand.-Mazz.
Above Yaren forest, Pangboche, 4250m, 27°50.67'N, 86°47.65'E, October 5, 2007, SNP135.
Polygonatum oppositifolium (Wall.) Royle
Deboche surroundings, 3750m, 27°50.75'N, 86°46.46'E, October 14, 2007, SNP254.

JUNCACEAE

Juncus concinnus D. Don
Pangboche surrounding, 4020m, 27°51.42'N, 86°47.37'E, October 1, 2007, SNP57.
Juncus thomsonii Buchenau
Near Chhukum, 4610m, 27°54.31'N, 86°52.26'E, October 8, 2007, SNP179.

POACEAE

Agrostis pilosula Trin.
Yaren, Pangboche, 4000m, 27°51.29'N, 86°47.93'E, October 2, 2007, SNP78.
Anthoxanthum hookeri (Griseb.) Rendle
Deboche surroundings, 3760m, 27°50.58'N, 86°46.22'E, October 14, 2007, SNP246.
Bromus himalaicus Stapf.
Chalunche, 3990m, 27° 51.53'N, 86°47.84'E, October 6, 2007, SNP140.

Calamogrostis sp.

Above Yaren forest, Pangboche, 4250m, 27°50.67'N, 86°47.65'E, October 5, 2007, SNP136 .

Danthonia comminsii Hook. f.

Dingboche, 4320m, 27°53.49'N, 86°49.49'E, October 7, 2007, SNP163.

Deyeuxia scabrescence (Griseb.) Munro ex Duthie

Deboche forest, 3840m, 27°50.94'N, 86°46.79'E, October 10, 2007, SNP202.

Elymus nutans Griseb.

Dingboche, 4320m, 27°53.49'N, 86°49.49'E, October 7, 2007, SNP159.

Elymus schrenkianus (Fisch. & C.A. Mey.) Tzvelev

Pangboche surrounding, 4020m, 27°51.42'N, 86°47.37'E, October 1, 2007, SNP58.

Elymus sikkimensis (Melderis) Melderis

Churo near Dingboche bridge, 4220m, 27°52.87'N, 86°49.15'E, October 7, 2007, SNP158.

Festuca leptopogon Stapf

Deboche forest, 3870m, 27°50.29'N, 86°46.47'E, October 10, 2007, SNP204.

Festuca polycolea Stapf

Deboche forest, 3840m, 27°50.94'N, 86°46.79'E, October 10, 2007, SNP203.

Helictrotricon virescens (Nees ex Steud.) Henrard

Yaren, Pangboche, 4060m, 27°51.32'N, 86°48.01'E, October 2, 2007, SNP90.

Oryzopsis munori Stapf

Deboche surroundings, 3760m, 27°50.58'N, 86°46.22'E, October 14, 2007, SNP245.

Poa annua L.

Between Pangboche & Somare, 4030m, 27°52.00'N, 86°48.20'E, October 7, 2007, SNP169.

Poa hirtiglumis Hook. f.

Pangboche surrounding, 4020m, 27°51.42'N, 86°47.37'E, October 1, 2007, SNP58.

Poa nemoralis L.

Churo, 4220m, 27°52.87N, 86°49.15'E, October 7, 2007, SNP144.

Poa pagophila Bor

Near Chhukum, 4610m, 27°54.31'N, 86°52.26'E, October 8, 2007, SNP174.

CYPERACEAE

Carex daltonii Boott.

Milingo forest, Deboche, 3990m, 27°50.33'N, 86°46.80'E, October 11, 2007, SNP219.

Carex sp.

Milingo forest, deboche, 3990m, 27°50.33'N, 86°46.80'E, October 11, 2007, SNP218.

Kobressia fragilis C.B. Clarke

Churo, 4220m, 27°52.87'N, 86°49.15'E, October 7, 2007, SNP145.

Kobressia nepalensis (Nees) Kuk.

Yaren, Pangboche, 4100m, 27° 50.92'N, 86°47.61'E, October 3, 2007, SNP110.

ORCHIDACEAE

Herminium macrophyllum (D. Don) Dandy

Deboche surrounding, 3750m, 27°50.75'N, 86°46.46'E, October 14, 2007, SNP252.

The explorations of flowering plants in Nepal at higher altitudes are very few due to inaccessibility and remoteness. Thus, the present exploration was a complimentary effort made in post monsoon season. It covers the enumeration of a small part of flora. This study revealed the occurence of 180 herbaceous species. Among them 19 species are reported as new for Eastern Nepal. Similarly 43 species are found with different altitudinal ranges in which 32 species recorded at higher range, 8 species were recorded at lower range and for 3 species no altitudinal range was reported in Hara *et al.* (1978, 1979, 1982), Press *et al.* (2000) and Joshi (2005) (Table 2).

Table 2. List of the species with different (regional or altitudinal) distribution than recorded in Hara *et al.* (1978, 1979, 1982), Press *et al.* (2000) and Joshi (2005)

S.N.	species	Species distribution	Collection from Imja Valley
1	*Aconogonum sibiricum* (Laxm.) H. Hara	Western-Central: 4000-4700m	*4000m
2	*Acronema johrianum* Babu	Central: 3700-4300m	*3980m
3	*Adenocaulon nepalense* M. Bittmann	Central-Eastern: 2550-3750m	# 3950m
4	*Ainsliea aptera* DC.	Western & Eastern: 1600-3500m	# 3670m
5	*Anaphalis margaritacea* (L.) Benth.	Central-Eastern: 1800-3100m	# 3980m
6	*Anaphalis subumbellata* C.B. Clarke	Central: 4300m	*# 3770m
7	*Anaphalis triplinervis* var. *intermedia* (DC.) Airy Shaw	Western-Eastern: 2900-4100m	# 4130m
8	*Arenaria depauperata* (Edgew.) H. Hara.	Western-Central: 3500-4400m	*# 4610m
9	*Artemisia dubia* Wall. ex Besser	Western-Eastern: 1200-3400m	# 4130m
10	*Bistorta milletii* Lev.	Western-Central: 3000-3400m	*# 4020m
11	*Bromus himalaicus* Stapf. apud. Hook. f	Central-Eastern: 2500-3700m	# 3990m
12	*Clematis montana* Buch.-Ham. ex DC	Western-Eastern: 1600-4000m	# 4130m
13	*Clinopodium umbrosum* (M. Bieb.) K. Koch	Western-Eastern: 1800-3400m	# 3750m
14	*Comastoma pedunculatum* (Royle ex D. Don) Holub	Western-Eastern	# 3990m
15	*Delphinium stapeliosum* Bruhl ex Huth,	Western-Eastern: 1200-3000m	# 3720m
16	*Drosera peltata* var. *lunata* (Buch.-Ham. ex DC.) C.B. Clarke	Western-Eastern. 2500-3600m	# 3670m
17	*Elymus schrenkianus* (Fisch. & C.A. Mey.) Tzvelev	Western-Central: 2700-4300m	*4020m
18	*Elymus sikkimensis* (Melderis) Melderis	Central	*# 4220m
19	*Epilobium brevifolium* subsp. *trichoneurum* (Hausskn.) P.H. Raven	Eastern: 2100-3600m	# 4020m
20	*Epilobium latifolium* subsp. *Speciosum* (Decne.) P.H. Raven	Western-Central: 3600-4200m	*4020m
21	*Epilobium royleanum* Hausskn.	Western and Eastern: 4100m	# 3740m
22	*Galium aparine* L	Western-Central: 900-3600m	*# 4320m
23	*Gallium asperifolium* Wall.	Western-Eastern: 1500-3000m	# 3430m
24	*Hemiphragma heterophyllum* Wall.	Western-Eastern: 1800-3500m	# 3770m
25	*Heracleum nepalense* D. Don	Western-Eastern: 1800-3700m	# 3990m
26	*Kobressia fragilis* C.B. Clarke	Western-Eastern: 3500-3800m	# 4220m
27	*Koenigia deliculata* (Meisn.) H. Hara	Western-Eastern: 2700-4000m	# 4050m
28	*Ligularia amplexicaulis* DC.	Western-Eastern: 2900-3300m	# 3980m
29	*Lomatogonium chumbicum* (Burkill.) H. Sm	Eastern: 3900m	# 4020m
30	*Oryzopsis munori* Stapf	Western: 3800-4600m	*# 3760m
31	*Pedicularis gracilis* Wall. ex Benth	Western-Eastern: 2200-3800m	# 4050m
32	*Pedicularis oxyrhyncha* T. Yamaz.	Central-Eastern: 3900-4400m	# 3670m
33	*Physospermopsis obtusiuscula* (DC.) C. Norman	Central: 300-4600m	*4200m
34	*Pleurospermum apiolens* C. B. Clarke	Western-Eastern: 3600-4500m	# 4610m
35	*Pleurospermum brunois* (DC.) C.B. Clarke	Western-Central: 4400-5000m	*# 3930m
36	*Poa annua* L.	Central-Eastern: 2300-3500m	# 4030m
37	*Poa nemoralis* L.	Central: 2600-4100m	*# 4220m
38	*Polygonatum oppositifolium* (Wall.) Royle	Central-Eastern: 1800m	# 3750m
39	*Primula capitata* Hook. f.	Eastern: 3800m	# 3750/4050m
40	*Rheum acuminatum* Hook. f. & Thomson ex Hook.	Central-Eastern: 3300-4200m	# 4270m
41	*Rhodiola crenulata* (Hook. f. & Thomson) H. Ohba	Central-Eastern: 4800-5300m	# 4070m
42	*Rhodiola chrysanthemifolia* (Leveille) Fu	Western-Central: 2500-3500m	*# 3660m
43	*Saxifraga aristulata* Hook. f. & Thomson	Western-Eastern: 4200-5600m	# 4020m
44	*Saxifraga filicaulis* Wall. ex ser.	Western-Central: 2700-3800m	*# 4220m
45	*Saxifraga montana* H. Sm.	Central: 4100-5000m	*4610m
46	*Selinum candollei* DC.	Western-Central: 3000-3800m	*34030m
47	*Senecio chrysanthemoides* DC.	Western-Eastern: 1400-4000m	# 4050m
48	*Silene khasiana* Rohrb.		*# 2850-3430m
49	*Silene stracheyi* Edgew.	Western-Central	*# 2750-3450m
50	*Thalictrum reniforme* Wall.	Central-Eastern: 2800-3300m	# 3430m

Note: *= not reported from Eastern Nepal, # = with different altitudinal distribution

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Exploration of High Value Medicinal Plants from Sagarmatha National Park, Nepal

Sangita Shrestha, Deepa Shree Rawal, Jaishree Sijapati, Neesha Rana, Prabina Rana and Anjana Giri

Abstract

Medicinal plants are second most valuable natural resources of Nepal. Most of such valuable medicinal plants grow in wild conditions as natural components of vegetation of particular region. However, due to various factors such as anthropogenic activities and climate change, several such high value plants have become threatened. So far, Nepal hasn't been able to rationally utilize and benefit from its highly valuable genetic resources such as medicinal plants. Hence, an integrated approach comprising of biodiversity characterization, documentation, bio-prospecting, their sustainable utilization and conservation has become an urgent need. Consequently, focusing all these needs, a collaborative research project has been initiated by EV-K2-CNR Italy and NAST at Sagarmatha region of Nepal. Altogether 40 medicinal plants were collected from Sagarmatha National Park and its buffer zone (2700m asl – 4470 m asl) area. Among them 14 were identified as high value medicinal plants.

Background

Many highly demanded and globally important medicinal plants such as *Swertia chirayita* (Roxb. ex Fleming) H. Karst., *Neopicrorhiza scrophulariiflora* (Pennell) Hong, *Podophyllum hexandrum* Royle, *Taxus wallichiana* Zucc., *Nardostachys grandiflora* DC are harbored in various geo-climatic region of Nepal (Polunin & Stainton 1985; Press *et al.* 2000). Total number of medicinal and aromatic plants reported in Nepal is 1950 (Ghimire 2008). Himalayan region of Nepal is one of the major storehouses of various high value medicinal plant species.

Tremendous demand of Nepalese high value medicinal plants exists for national and international pharmaceutical industries. The world market for herbal medicines, including herbal products and raw materials has been estimated to have an annual growth rate between 5 and 15%. Total global herbal drug market is estimated as US$ 62 billion and is expected to grow to US$ 6 trillion by the year 2050 (Joshi *et al.* 2004). The annual demand of various medicinal plants in India is estimated to be 24 million metric tons which is increasing at the rate of 20% per annum (Karki 2001). In the similar manner, the traditional Chinese medicine market has been valued at US$ 4.3 billion, which is projected to reach over 12.1 billion by the year 2010 (Nagpal & Karki 2004). Furthermore, huge demand exists for the Himalayan plants for Tibetan medicine (Pandey 2006). Therefore, global trend for demands of medicinal plants for pharmaceuticals, phyto-chemicals, neutraceuticals, cosmetics and other products is rapidly increasing leading to increased pressure on these valuable resources. However, in the meantime this increased demand has also opened up new avenues for Nepal to enhance its trade and commerce via value addition in crude products as well as development of final products for national/international market, so that maximum benefits could be obtained for poverty alleviation. Furthermore, this increased demand has created alarming need to conserve and sustainably utilize these high value resources for future.

Most of such valuable medicinal plants grow in wild conditions as natural components of vegetation. The necessary plant material (roots, leaves, bark, etc.) is collected and sold by the local people to the traders and industries, and exporters purchase them from traders. Since there is no scientific system of collecting and regenerating these plants, several high value plants have become endangered.

The main objective of this project was to explore high value medicinal plant species of the study area for documentation, characterization, conservation and sustainable utilization for future.

Study Area

The study area lies in Solukhumbu district, north eastern region of Nepal. It encompasses the Sagarmatha National Park (1148 sq km) and its buffer zone. Study sites include Lukla, Monju, Namche, Khunde, Syangboche, Thame, Thyangboche, Periche and Gokyo (Fig. 1).

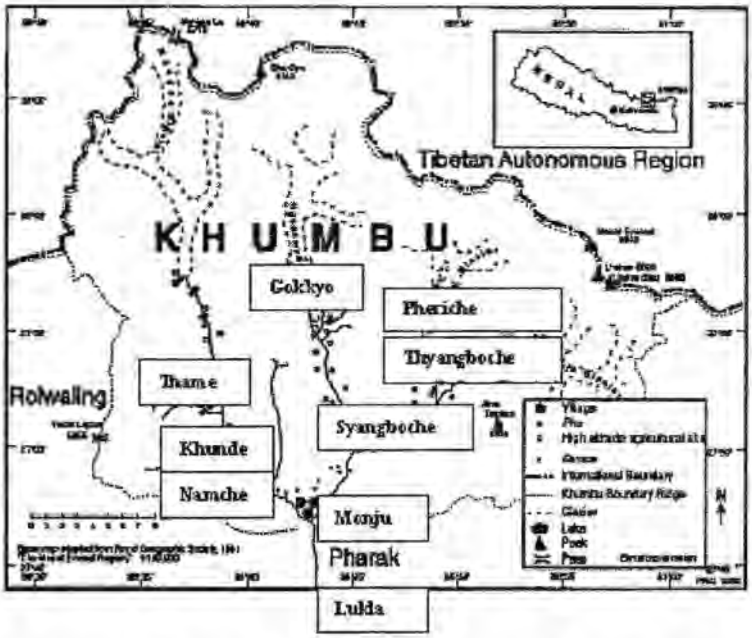

Fig. 1. Map of Khumbu (Sagarmatha National Park and Buffer Zone) region, Nepal.

Methods

Field survey was carried out during May 3-13, 2007. The medicinal plant samples were collected from their natural habitat. The collected herbarium specimens were identified with the help of standard taxonomic literature (Polunin & Stainton 1985, Press *et al.* 2000) and compared with previously identified herbaria in KATH (National Herbarium & Plant Research Laboratory, Godawari).

Medicinal properties and biochemical constituents of various high value medicinal plants of the region were gathered from secondary literature.

Results and Discussion

High value medicinal plants of Khumbu region

Altogether, 40 medicinal plants were collected, identified and documented from the study area (Table 1) Among them, 14 have been short listed as high value medicinal plants on the basis of IUCN category, lists of CITES list, Department of Plant Resources (DPR), IDRC/ICIMOD and other relevant literature (Table 2).

Table 1. List of Medicinal plants collected from Khumbu Sagarmatha National Park and Buffer Zone) region.

S. N	Species	Family	Nepali Name	Altitude (meters)	Longitude/Latitude
1*	*Abies spectabilis* (D. Don) Mirb.	Pinaceae	Talis Patra	3522	N – 27° 48.229' E – 86° 42. 908'
2	*Arisaema intermedium* Blume,	Araceae	Sarpa makai	2700	N – 27°45.048' E – 86°43.668'
3*	*Berberis mucrifolia* Ahrendt	Berberidaceae	Lekh chutro	2705	N – 27° 45.141' E – 86° 43. 615'
4*	*Berberis erythroclada* Ahrendt	Berberidacea		3522	N – 27° 48.229' E – 86° 42.908'
5*	*Berberis angulosa* Wall . ex.Hook and Thompson	Berberidaceae	Chutre Kanda	3522	N – 27° 48.229' E – 86° 42. 908'
6*	*Berberis aristata. var.micrantha* Hook. F. & Thomson	Berberidaceae	Chutro/ Kirmundo	3522	N – 27° 48.229' E – 86° 42. 908'
7	*Betula utilis* D.Don	Betulaceae	Bhoj Patra	3845	N – 27° 48.920' E – 86° 42.845'
8	*Clematis montana* Buch.-Ham.ex DC	Ranunculaceae	Junge lahara	3548	N – 27° 48.425' E – 86° 42.342'
9	*Cotoneaster microphyllus* Wall.ex Lindley	Rosaceae		2705	N – 27° 45.141' E – 86° 43.605'

10	*Digitalis purpurea*	Scrophulariaceae		2717	N – 27° 45.595' E – 86° 43. 044'
11*	*Ephedra gerardiana* Wall. ex. Stapf	Ephedraceae		4470	N – 27° 54.21' E – 86° 42.132'
12	*Euphorbia wallichi* Hook.f	Euphorbiaceae		2690	N – 27° 45.125' E – 86° 43.388'
13	*Fragaria nubicola* Lindl.ex Lacainta	Rosaceae	Bhui aiselu	2611	N – 27° 45.342' E – 86°43.209'
14	*Girardinia diversifolia* (Link) Friis	Urticaceae	Allo sissnu/ Thulo sissnu	2650	N – 27° 45.205' E – 86° 43.121'
15	*Gaultheria griffithiana* Wight	Ericaceae		2584	N – 27° 45.145' E – 86° 43.008'
16	*Iris kemaonensis* D. Don ex Royle	Iridaceae		3547	N – 27° 48.42' E – 86° 42.330'
17	*Juniperus indica* Bertol	Cupressaceae	Dhupi / Pamo	3522	N – 27° 48.229' E – 86° 42.908'
18	*Juniperus recurva* Buch. Ham. ex D. Don	Cupressaceae	Dhupi	3522	N – 27° 48.229' E – 86° 42.908'
19*	*Meconopsis grandis* Prain	Papaveraceae		3596	N – 27° 48.405' E – 86° 42.214'
20*	*Meconopsis paniculata* Prain	Papaveraceae	Mulapate	3596	N – 27° 48.405' E – 86° 42.214'
21*	*Neopicrorhiza scrophulariiflora* (Pennell) Hong	Scrophulariaceae	Kutki	3634	N – 27° 48.594' E – 86° 42.163'
22*	*Paris polyphylla* Smith	Liliaceae	Satuwa	2584	N – 27° 45.14' E – 86° 43.008'
23	*Pinus wallichiana* A.B.Jackson	Pinacene	Gobre Salla	3248	N – 26° 47.998' E – 86° 43.270'
24	*Plantago erosa* Wall.	Plantaginaceae	Isabgol	2503	N – 27° 45.002' E – 86° 43.843'
25*	*Podophyllum hexandrum* Royle	Berberidaceae	Laghu patra	3984	N – 27° 49.124' E – 86° 42.711'
26*	*Potentilla peduncularis* D. Don	Rosaceae		3845	N – 27° 48.920' E – 86° 42.845'
27	*Quercus semicarpifolia* Smith	Fagaceae	Ghaisi karsu	2584	N – 27° 45.145' E – 86° 43.008'
28	*Rhododendron anthopogan* D.Don	Ericaceae	Guras/Sunpate	3522	N – 27° 48.229' E – 86° 42.908'
29	*Rhododendron arboreum* Smith	Ericaceae	Guras/ Laleguras	3581	N – 27° 48.491' E – 86° 42.302'
30	*Rhododendron camparulatum Var. wallichi* (Hook F)	Ericaceae	Seto Guras	3867	N – 27° 48.954' E – 86° 42.708'
31	*Rhododendron lepidotum* Wall. ex. G. Don	Ericaceae	Bhale Sunpate	3522	N – 27° 48.229' E – 86° 42.908'
32	*Rhododendron triflorum* Hook.f.	Ericaceae		2789	N – 27° 45.984' E – 86° 42.745'
33	*Rubus ellipticus* Smith	Rosaceae	Aiselu	2717	N – 27° 43.595' E – 86° 42.044'
34	*Salix disperma* Roxb. ex D. Don	Salicaceae		3522	N – 27° 48.229' E – 86° 42.908'
35	*Selinum wallchianum* (DC)Raizada and Saxena	Umbelliferae	Bhutkesh	3728	N – 27° 48.734' E – 86° 42.936'
36*	*Taxus wallichiana* Zucc.	Taxaceae		2783	N – 27° 41.87' E – 86° 43.40'
37	*Tsuga dumosa* (D. Don) Eichler	Pinaceae	Thingo salla	3538	N – 27° 48.421' E – 86° 42. 747
38	*Utrica dioca* L.	Urticaceae	Lek Sisno	2649	N – 27° 45.197' E – 86° 43.130'
39	*Viola canescens* Wall.	Violaceae		2705	N-27°45.141' E – 86°43.605'
40*	*Zanthoxylum nepalense* DC	Rutaceae	Timur	2717	N – 27° 45.595' E – 86° 42.044'

* -Listed high value medicinal plants of the region.

Table 2. List of high value medicinal plant species based on IUCN category, lists of CITES, IDRC, DPR/ ICIMOD and other relevant publications.

S. N.	Species	CITES listed	IUCN category	IDRC listed	DPR/ ICIMOD and others
1.	**Podophyllum hexandrum*	Appendix II	Vulnerable (V)	Priority plant for South Asia (Internet visit, 2009; http://mappa.icomod.org)	Threatened due to over collection (rbg- web2.rbge.org.uk) www. plantlife.org.uk
2	**Taxus wallichiana*	Appendix II	-	-	National list (DPR, 2002) www. plantlife.org.uk
3.	**Neopicrorhiza scrophulariiflora*	-	Vulnerable (V)	Priority plant for South Asia (Internet visit, 2009; http://mappa.icomod.org)	National list (DPR, 2000) www. plantlife.org.uk
4.	*Abies spectabilis*	-	-	-	National listed plant banned for export(Internet visit, 2009; rbg-web2.rbge.org.uk)
5.	*Paris polyphylla*	-	Vulnerable (V)	-	Threatened due to over collection(Internet visit, 2009; rbg-web2.rbge.org. uk) www. plantlife.org.uk
6.	*Ephedra gerardiana*	-	-	-	Threatened due to over collection (Internet visit, 2009; rbg-web2.rbge.org. uk)/www.plantlife.org.uk
7.	*Potentilla peduncularis*	-	-	-	Based on other relevant literature
8.	**Berberis mucrifolia*	-	-	-	Based on other relevant literature
9.	**Berberis erythroclada*	-	-	-	Based on other relevant literature
10.	**Berberis angulosa*	-	-	-	Based on other relevant literature
11.	**Berberis aristata*	-	-	Priority plant for South Asia (Internet visit, 2009; http://mappa.icomod.org)	Based on other relevant literature
12.	**Meconopsis paniculata*	-	-	-	Based on other relevant literature
13.	**Meconopsis grandis*	-	-	-	Based on other relevant literature
14	**Zanthoxylum nepalense*	-	-	-	Based on other relevant literature

(*) – Species selected for future molecular genetic diversity, molecular phylogeny, bio-prospecting, chemo-prospecting and other relevant studies.

Most of the collected plants were found to have different medicinal uses in different alternative medicines such as Tibetan medicine, herbal drugs, modern medicine and homoeopathy (Alam *et al.* 2008, Dhama & Dhama 1996), While many others were found to have been mentioned in ethno medicinal literatures (Ghimire 1999, Rajbhandari 2001, Joshi & Joshi 2001, Pandey 2006, Bhattarai *et al.* 2006, Rajbhandari *et al.* 2007, Bhattarai *et al.* 2008, Ghimire 2008).

Medicinal properties and biochemical constituents of listed species

Description of medicinal values and biochemical constituents of 14 listed high value medicinal plant species of the study area are described here.

Abies spectabilis D. Don

Medicinal properties: The dried leaves are astringent, stomachic, carminative, expectorant and aphrodisiac. Juice of the leaves is taken for asthma and bronchitis. Essential oil from the needles is used for colds rheumatism and nasal congestion (DPR 2007).

Important biochemical constituents: leaves produce essential oil which contains mainly α and β-pinene, camphene, lemonene, bornyl acetate (DPR 2007).

Berberis spp.

(*Berberis aristata var micrantha* Hook.F.& Thomson, *Berberis erythroclada* Ahrendt, *Berberis mucrifolia* Ahrendt, *Berberis angulosa* Wall.ex.Hook.f.& Thomson)

Medicinal properties: Numerous species of Berberis are reported to have various medicinal properties. *Berberis aristata* has been reported to have wound healing properties (Biswas & Mukherjee 2003). *Berberis aristata* produces a bitter tonic, which is anti periodic and diaphoretic. The chief constituents are those of *Berberis* bark, the principle being the alkaloid Berberine (Internet visit 2007; www. botanical.com).

Berberis erythroclada Ahrendt

Berbamine, an ingredient of *Berberis* is widely utilized in Chinese folk medicine as a source of leukogenics, anti-arrhythmic and anti-hypertensive. In recent years, the immunosuppressive effect of Berbamine has been demonstrated (Luo *et al.* 1998). Root decoction is used in fever. The extract of wood and root bark is used as purgative for children. It is also used as blood purifier, tonic, febrifuge, jaundice and skin diseases. Root bark is also used externally in eye diseases and as medicine for gall bladder (DPR 2007). 'Rasaut' extract of bark, roots and lower part of stem is used in eye diseases, piles, sores and glandular swelling.

Important biochemical constituents: Berberine, berbamine. Root's bark contains berberis aristratin (DPR 2007).

Ephedra gerardiana Wall

Medicinal properties: The plant is astringent and has a strong pine odor. Its active constituent ephedrine is prescribed in ailments like rhinitis, asthama, hey fever, emphysema, epilepsy, nocturnal enuresis, myasthenia gravis and urticaria accompanying angioneurotic oedema. Ephedrine salt is used as nasal spray in congestion and swelling. Sub-cutaneous injection is used in hypotension caused by anesthesia. Pseudoephedrine is effective in nasal congestion. Both ephedrine and pseudoephedrine are used in expectorants and bronchodilator (Joshi & Joshi 2001).Plant powder is taken to control asthma. Fresh or dried fruits are chewed to overcome altitude sickness in Karnali zone, Nepal. In central Nepal, decoction of stem is drunk as an antiasthmatic drug. Hot decoction of plant is drunk in case of acute bronchial congestion. In Manang valley, plant paste mixed with water is used to take bath to treat skin diseases (Rajbhandary 2001)

Important biochemical constituents: Ephedrine and pseudoephedrine. Aerial part contains ephedrine and ephedroxyne (Husain *et al.* 1992).

Meconopsis grandis Prain

Medicinal properties: roots, leaves and flowers are used as medicine. The plant is used to treat bone fracture, kidney problems, to remove accumulated body fluid and as pain killer.

Important biochemical constituents: Cyanidin 3-malonylsambubioside 7-glucoside as the anthocyanin. They also contain large amount of kaempferol 3-gentiobioside and very small amounts of kaempferol 3-xylosylgentiobioside. The ratio of flavonol to anthocyanin was found to be 5.6:1, suggesting that the flavonol plays a role as a co-pigment in the blueing of *Meconopsis* flowers, rhoeadine alkoliod in traces (Kosaku *et al.* 1996).

Meconopsis peniculata Prain

Medicinal properties: According to Tibetan medicine, the plant has bitter taste, cool and blunt qualities for heat disorders (http://globaltibetmed.org/blog/).

Important biochemical constituents: alkaline phosphatases, glucose 6-phosphate dehydrogenase and malate dehydrogenase (Irshad *et al.* 1993), Papaverrubin A, B, C, D, E, rhoeadine alkoliod in traces (Arnold *et al.* 1986).

Neopicrorhiza scrophulariiflora (Pennell)Hong

Medicinal properties: It furnishes the drug, picrorhizin, obtained from dried rhizomes and roots; which is used as an adulterant or as a substitute for Indian gentian (*Gentiana kurroa*). The rhizomes of this Himalayan perennial herb is used in several traditional medicine systems to treat a wide variety of ailments (Traffic International 1999; Press *et al.* 2000). Rhizome is used in cathartic, stomachic and purgative. Rhizome is also used in dropsy, antiperiodic fever, anemia and jaundice. It promotes secretion of bile, improves appetite and stimulates gastric secretion. A paste of rhizome is used in cough and cold (Manandhar 2002). It promotes an appetite and is useful in cough, biliousness, billow fever, urinary discharge, hiccough, blood troubles, burning sensations, leucoderma and jaundice, and purifies the nurse's milk (Ayurveda).

Important biochemical constituents: Kutkin (glucosidal), kurrin (a non bitter product), kutkiol (alcohol), kutkisterol (Watanabe *et al.* 2005). Rhizomes and roots contain Picroside I, Picroside II, Kutkoside, Minecoside, Phenol glycoside (Picein & androsin) and 4-hydroxyacetophenone (Anynomous 2002). Kutkiol (an alcohol), Pocrorhizin, Pocrorhizitin, Picroside, Picroliv, Kuthoside, Androsine, Aucubin, Catalpol, Valinic acid and kutkisterol (Kunwar 2006).

Paris polyphylla Smith

Medicinal properties: A paste is applied to cuts and wounds. Pieces of the roots are fed to cattle in diarrhea and dysentery. Rhizome is anthelmintic and also used as a tonic. It acts as depressant on carotid pressure, myocardium and respiratory movements. It produces vasoconstriction in kidney, but vasodilatation in the spleen and limbs and stimulates isolated intestines (Joshi & Joshi 2001).

Important biochemical constituents: Rhizome contains Pariphyllin A, Pariphyllin B, parsterone, pollyphylin D, and Trillin (Buckingham 1994).

Podophyllum hexandrum Royle

Medicinal properties: It is recognized for its anticancer properties (Alam *et al.* 2008) and has been employed in the treatment of cancer (Prakash *et al.* 2005).Rhizomes are stimulant and purgative (Manadhar 2002) . Essential oil from the needles is used in colds rheumatism and nasal congestion. Root paste is applied on ulcer, cuts and wounds. It is also used in the treatment of skin diseases and the growth of tumor (Baral & Kurmi 2006).Utility of *P. hexandrum* has also been reported against constipation, cold, biliary fever, septic wound, burning sensation, erysipelas insect bite, mental disorders, rheumatism, plague and to provide symptomatic relief in some of the allergic and inflammatory conditions of skin.

Important biochemical constituent: Podophyllotoxin,4'-demethyldesoxypodophyllotoxin, α and β–pellatin, deoxypodophyllotoxin, podophyllotoxone, isopicropodophyllone, 4'-demethyldesoxy podophy (Husain *et al.* 1992).

Potentilla peduncularis D. Don

Medicinal properties: Extracts of the aerial and/or underground parts have been applied in traditional medicine for the treatment of inflammations, wounds, certain forms of cancer, infections due to bacteria, fungi and viruses, diarrhea, diabetes mellitus and other ailments.

Important biochemical constituents: Most of the pharmacological effects can be explained by the high amount of tannins and to a lesser extent by triterpenes, present in all parts of plant. Polyphenols; Flavonoids are also present in this plant (Michal & Klaus 2009).

Taxus wallichiana Zucc.

Medicinal properties: Taxol extracted from bark and leaves of this plant is used as anti-tumor agent and also to cure cancer particularly of breast and uterus. The juice is also used in cough, asthma and bronchitis (DPR 2007). Leaves tincture is also used for the treatment of headache, giddiness, feeble and falling pulse, coldness of the extremities, diarrhea and severe biliousness. The leaves are credited with emmenagogue and antispasmodic properties. Used for the treatment of hysteria, epilepsy and nervousness. Taxol (extract of leaves & bark) used as anti cancer drug (IUCN Nepal 2000).

Important biochemical constituents: Taxine alkaloids and Taxol (Joshi & Joshi 2001).

Zanthoxylum nepalense DC.

Medicinal properties: A paste of immature fruit is kept between the teeth about ten minutes to relieve the toothache. It is also considered as carminative and stomachic (Manandhar 2002, Baral & Kurmi 2006).

Important biochemical constituents: Tannic acid, gallic acid, starch, mineral salts mucilage from the rhizome (Anonymous 1994).

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Diversity, Ethnomycological Knowledge and Nutritional Value of Some Wild Mushrooms in Sagarmatha National Park, Nepal

Prabina Rana and Anjana Giri

Abstract

The diversity of mushrooms collected from Lukla to Pangboche were higher at an altitudinal range of 3500-4000 m above sea level (asl), followed at 2500-3000 m asl and 3000-3500 m asl. Twenty nine species of mushrooms were identified having ethnomycological use: 26 were identified as edible, 2 as medicinal and 1 for decorative purpose. Proximate composition; moisture, ash, carbohydrate, crude fat, crude protein, crude fibre and minerals such as calcium, phosphorous and iron of 11 most prized mushrooms were investigated. The results showed that some of the wild edible mushrooms have nutritional values comparable to the commonly cultivated mushroom species viz. *Agaricus bisporus* (Lange) Imbach, *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, *Lentinula edodes* (Berk.) Pegler, *Volvariella volvacea* (Bull.: Fr.) Singer.

Introduction

Nepal possesses distinct phytogeographical zones related to altitude and other factors. The contrasting topographies, varieties of climate and vegetational zones offer heterogeneous and dense habitats for mushroom flora (Bhandary 1985). Furthermore, the dominant or mixed or pure forest types (*Shorea - Pinus – Quercus – Abies – Rhododendron – Betula - Junipers*) from tropical to alpine zones with their ecological environment provide different micro-ecological conditions suitable for the origin, development and growth of diverse and specific mycotaxa. The western part of the country is much drier than the eastern and central part. Hence, the concentration of different groups of fungi varies from region to region. Moreover, the fungal diversity is favored in central and eastern as compared to western region.

Wild edible mushrooms are one of the important natural resources on which the local people of all nationalities rely heavily, and these mushrooms certainly play a role in improving the food nutrition (Yang 2002). Edible mushrooms contain high level of dietary fiber, substantial amount of protein, vitamins and minerals but are low in fat. They also have various properties for health benefits such as antioxidative, antitumour and hypercholesterolic effects (Wong & Cheung 2001).

In Nepal, mushroom collection and consumption have been continuing since time immemorial by different ethnic groups. Information on documentation of indigenous mushrooms is limited and poor. The wild edible mushrooms serve as an important natural seasonal crop which play an important role in the development of economic condition of the rural people (Adhikari 2000). The nutritive value of wild edible mushrooms have been analyzed by Singh and Nisha (1973), Adhikari (1996) and Pandey and Budathoki (2007a, b). The main objective of our study was to record the diversity of wild mushrooms, document ethnomycological knowledge of the Sherpa people and to analyse the nutritive value of prized edible mushrooms.

Study Area

The study area lies in Solu-Khumbu district of the northeastern region of Nepal. It encompasses the Sagarmatha National Park (1148 sq km) and its Buffer Zone area (275 sq km) (Fig. 1).The Park includes the upper catchment areas of the Dudhkoshi and Bhotekoshi rivers. Due to its altitudinal diversity, various types of vegetation are found in different climatic zones (temperate, subalpine, alpine, nival & permanent snow zones). The dominant vegetation at the lower elevation of the park below 3000 m asl is composed mostly of blue pine and Hemlock forest. The lower subalpine region above 3000 m asl comprises *Pinus wallichiana, Abies spectabilis* and *Juniperus recurva* forests. The upper subalpine, above 3600 m asl consists of Birch - Rhododendron forest (*Betula utilis, Rhododendron campanulatum* and *R.*

campylocarpum) and the lower alpine region above the timber–line at 3800-4000 m asl houses scrubs (*Juniperus* spp, *Rhododendron anthopogaon* & *R. lepidotum*).

Fig. 1. Map of the study area Sagarmatha National Park

Materials and Methods

Collection and identification of mushrooms

The mushroom specimens were collected from Lukla (2800 m asl.) to Pangboche (4000 m asl) during the months of August and September from 2004 to 2006. The mushroom specimens were collected carefully by digging them with the help of a sharp knife. Data on habit and habitat, ecological parameters such as altitude, forest type, etc. were recorded in the field. Each collection was placed in butter paper bags and tag numbers were assigned to them. The morphological characters such as spore colour, pileus and tube colour, colour change induced after bruising or cutting and exposing to chemicals such as Potassium hydroxide (KOH) and Ferrous sulphate ($FeSO_4$) were noted. The specimens were either sun dried or dried by placing them on tin foil over a local oven. Dried specimens were placed in butter paper bags. Naphthalene balls were used as insect repellents. The dried specimens were identified in the laboratory with the help of literature and studying their macro and microscopic characters. The collected specimens have been deposited in the laboratory of Nepal Academy of Science and Technology (NAST).

Ethno mycological information survey

During the field survey, a total of 150 respondents within the age bracket of 10 to 70 years were interviewed by applying artifact and inventory interviewing methods (Nepal *et al.* 1999) and filling questionnaire.

Nutritional analysis

Eleven mushrooms taxonomically and locally identified as prized wild edible mushrooms were collected, shed dried and further subjected to nutritional analysis. Proximate analyses (moisture, carbohydrate, crude protein, crude fat, crude fiber, moisture and ash) were performed at the Department of Food Technology and Quality Control (DFTQC) in accordance with the official methods of analysis of the Association of Official Analytical Chemist (AOAC 1995). All the calculations were carried out on dry weight basis of mushrooms. The minerals such as phosphorous (P), calcium (Ca) and iron (Fe) were read on Atomic Absorption Spectrophotometer (AAS).

Results and Discussion

Ecological distribution of mushrooms

A total of 150 mushroom species belonging to 37 families and 65 genera were collected from Lukla to Pangboche (Rana & Giri 2006, Giri & Rana 2007). During the survey, different microhabitats of mushrooms were also recorded (Fig.2). *Pulverboletus ravenelii* (Berkeley & Curtis) Murrill, a wild mushroom of class basidiomycetes collected from Ghat under coniferous forest (dominanted by *Pinus wallichiana*) at an altitude of 2604 m asl has been recorded as new to Nepal (Rana *et al.* 2006). Two species of *Boletus*, *Boletus auripes* Peck, *Chroogomphus tomentosus* (Murr.) O.K. Miller, *Russula metachroa* Hongo, *Tylopilus eximus* (Peck) Singh, and *Hypholoma capnoides* (Fr.) Kumm., *Paxillus involutus* (Batsch: Fr.) Fr. were found new to the list of edible mushrooms of Nepal(Giri & Rana 2007).

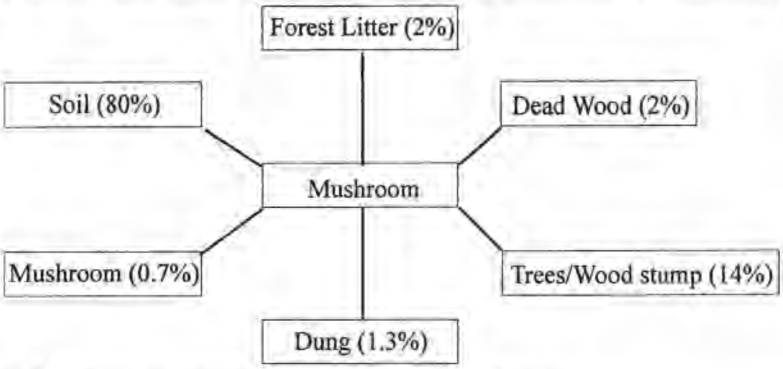

Fig. 2. Different microhabitats of mushrooms in the study area.

In Nepal, the distribution of mushrooms in a particular locality depends on topography, soil, rainfall and temperature, and vegetation pattern. The appearance and occurrence of mushrooms and their dominance are controlled by different factors such as altitude, phytogeography, etc. The mushroom specimens were found high on south and south west facing slope. The diversity of mushroom specimens was found high at an altitudinal range between 3500-4000 m asl followed by 2500-3000 m asl and 3000-3500 m asl (Fig 3).

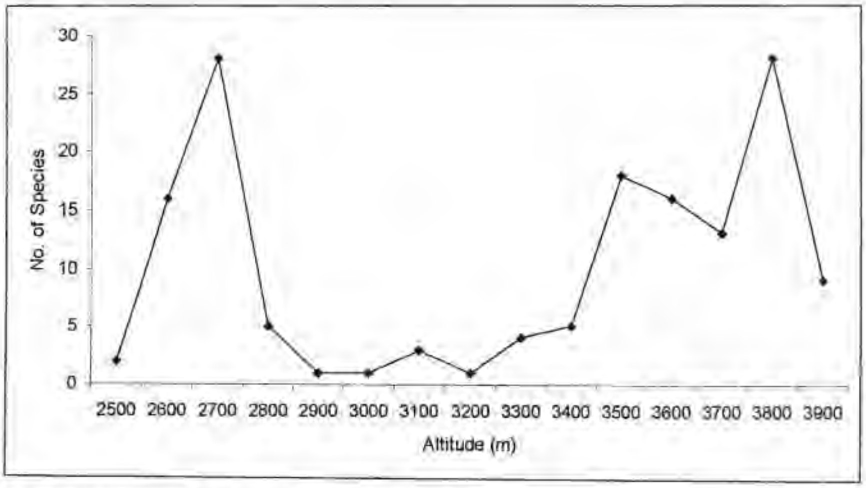

Fig. 3. Altitudinal distribution of collected mushroom species in the SNPBZ

Eighty-four species, belonging to 30 families were recorded within 3500-4000 m asl *(Abies, Rhododendron, Betula, Juniperus* forest). Similarly, 14 species of 11 families and 52 species belonging to 15 families were recorded between 3000-3500 m asl (*Abies, Rhododendron, Pinus & Betula* forest) and 2500 to 3000 m asl (*Rhododendron, Abies & Betula* forest) respectively. The presence of highest diversity of mushroom at 3500-4000 m asl may be due to cloud formation, which is a decisive factor for the vegetation of Nepal (Beug & Miehe 1999). The cloud zones are a source of moisture which creates microhabitats and favour high species richness (Rahbek 1995). The cloud forest is rich in diversity (Falkenberg & Voltonilini 1994).

Local nomenclatural practices for mushrooms

Twenty nine ethnomycologically important mushrooms were identified with the help of Sherpa community residing in the study area (Table 1). Among them 26 species of mushrooms were used for edible, 2 species for medicinal and one for decorative purposes.

Table 1. Scientific names, family, local names, locality and uses of wild mushrooms in SNPBZ.

SN	Species	Family	Local Name	Locality (forest)	Altitude (m)	Uses
1	*Amanita hemibapha* (Berk. & Br.) Sacc.	Amanitaceae	Rato anda chyau	Muse (Lukla)	2783	Edible
2	*Amanita vaginata* (Bull.:Fr.) Vitt.	Amanitaceae	Seto anda chyau	Muse (Lukla)	2783	Edible
3.	*Amanita* sp.	Amanitaceae	Sano anda chyau	Muse (Lukla)	2783	Edible
4.	*Armillariella* mellea (Vahl.:Fr.) Kummer	Tricholoma-taceae	Chiple chyau	Muse (Lukla), Phurte, Jamikhiau (Khumjung)	2783-3800	Edible
5.	*Auricularia polytricha* (Mont.) Sacc	Auriculariaceae	Durkho chyau	Chire (Kunde), Deboche, Omakha (Pangboche)	3900-4000	Edible
6.	*Boletus* sp.	Boletaceae	Fhe shyamo/ Muse chyau	Chire (Kunde),Jamikhiau (Khumjung) Deboche, and Omakha (Pangboche)	4000	Edible
7.	*Boletus edulis* Bull.: Fr. Steinpliz	Boletaceae	Pani chyau	Muse (Lukla)	2783	Edible
8.	*Boletus* sp.	Boletaceae	Rato martip	Muse (Lukla), Chire (Kunde), Jamikiau (Khumjung) Deboche, and Omakha (Pangboche)	2783-4000	Edible
9.	*Boletus auripes* Peck	Boletaceae	Seto martip	Muse (Lukla) Phurte (Namche)	2783-3615	Edible
10.	*Boletus pulverulentus* Opat.	Boletaceae	Kalo martip	Muse (Lukla)	2783	Edible
11.	*Cantharella cibarius* (Fr.: Fr.) Fr.	Cantharellaceae	Kujir (pothi)	Chire (Kunde), Jami khiau (Khumjung) Deboche	3665-3990	Edible
12.	*Chroogomphus tomentosus* (Murr.) O.K.Miller	Gomphidiceae	Kujir (Keta)	Chire (Kunde), Jamikhiau (Khumjung) Deboche, Omakha (Pangboche)	3665-4000	Edible
13.	*Clavulina cinerea* (Bull.: Fr.) Schroet	Clavulinaceae	Che shyamo	Phurte (Namche)	3615	Edible
14.	*Rhodocollybia butyraceae* (Bull.: Fr.) Lennox	Marasmiaceae	Karshya (Pothi)	Phurte (Namche)	3615	Edible
15.	*Gomphus clavatus* (Pers. : Fr.) S. F. Gray	Gomphaceae	Ee- shyamo	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung)	3615-3900	Edible
16.	*Gomphus floccosus* (Schw.) Singer var *floccosus*	Gomphaceae	Khumbhe chyau	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung) Deboche	3615-3900	Edible
17.	*Hydnum repandum* L. : Fr.	Hydnaceae	La shyamo / kasturi chyau	Jamikhiau (Khumjung)	3800	Edible
18.	*Hygrophorous* sp.	Hygrophoraceae	Omi shyamo / dudh chyau	Phurte (Namche)	3615	Edible
19.	*Laccaria laccata* (Scop.: Fr.) Cooke	Tricholomataceae	Chinduk shyamo	Jamikhiau	3800	Edible
20.	*Leccinum* sp.	Boletaceae	Petok shyamo	Chire (Kunde), Jamikhiau (Khumjung)	3800-3900	Edible
21.	*Ramaria flava* (Sch.: Fr.) Quel.	Ramariaceae	Che shyamo	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung) Deboche	3615-3900	Edible
22.	*Ramaria botrytis* (Pers.:Fr.) *Ricken*	Ramariaceae	Che shyamo	Phurte (Namche), Deboche	3615-3665	Edible
23.	*Russula metachroa* Hongo	Russulaceae	Che shyamo	Phurte (Namche), Jamikiau (Khumjung)	3615-8000	Edible
24.	*Hypholoma capnoides* (Fr.) Kumm.	Strophariaceae	Taktale	Phurte (Namche)	3615	Edible

25.	*Paxillus involutus* (Batsch: Fr.)Fr.	Paxillaceae	Dyangba	Jamkhian (Khumjung)	3800	Edible
26.	*Tylopilus eximus* (Peck) Sing.	Boletaceae	Kyakti / Jiri chyamlo	Chire (Khunde), Jamkhian (Khumjung), Deboche	3665-3900	Edible
27.	*Lycoperdon perlatum* Pers.: Pers.	Lycoperdaceae	Phusphuse	Deboche	3655	Medicinal
28.	*Lycoperdon pyriforme* Schaeff.: Pers.	Lycoperdaceae	Phusphuse	Phungi tenga	3590	Medicinal
29.	*Ganoderma* sp.	Ganodermataceae	Chale chyau	Muse (Lukla)	2783	Decoration

The local inhabitants have accumulated rich traditional knowledge and experience in utilization of the wild edible mushroom resources. They are well familiar about the morphological features, habitats and qualities of various edible mushrooms. Mushrooms are called "Shyamo" in Sherpa which means cap. In Nepali it is known as "Chyau". The local name of the wild edible mushrooms has been mostly derived on the basis of their morphological features, nature of growth, colour, etc. *Gomphus clavatus* is known as "Ee-shyamo" (Eng: mother-in-law) in Sherpa dialect. The name of this mushroom is derived from its growth characteristic. As the mother-in-law plays a dominant role in the family this mushroom grows dominantly (about 5-7 kg) in one place. Since *Gomphus floccosus* looks like a burning "Diyo" (Eng: oil lamp) it is called "Diyo chyau" or "Khumbhe chyau". *Clavulina cinerea, Ramaria flava* and *Ramaria botrytis* are known as "Che shyamo" due to its grass like appearance. *Hydnum repandum* is locally named as "La shyamo" (Eng: musk deer) or "Kasturi chyau" due to its resemblance to the skin of musk deer. *Amanita* spp which have an egg like volva are known as "Anda (Eng: egg) chyau" Further depending on its size or colour of the cap a prefix may be added such as "sano" (small), "rato" (red), "seto" (white), etc. *Amanita hemibapha* is known as "Rato anda chyau. *Lycoperdon perlatum* is known as "*Phusphuse*" because when pressed its powdery spore eject out slowly making a *phus-phus* sound. *Ganoderma* sp is known as "Chhale (Eng: skin) chyau" because its outer layer resembles thick skin. It was also observed that the local people have adopted both Sherpa as well as Nepali names for the mushrooms.

General findings

The study reveals that 90% of the total population of the area consume wild edible mushrooms. It was also observed that mushroom were collected mainly by lower economic group of people aged between 15 - 45 years by utilizing local knowledge handed down from generations. Among them, men played a crucial role in collecting mushrooms.

Wild edible mushrooms are regarded as a delicacy food in that region. It was found that even economically sound people hired men to collect mushroom occasionally. Meat and fresh green vegetables are scarce, very expensive and rarely eaten item especially in higher region above Namche. They are brought from Jiri and are usually available only in the weekly market (Haat Bazaar) of Lukla and Namche. They are dependent on the seasonal mushrooms as a substitute of meat and vegetables. However, in local hotels the use of wild mushrooms has not been observed.

Below Namche the local people hardly preserve mushrooms for culinary uses. This may be due to availability of the variety of vegetables in this region as compared to the upper high altitude region. Field survey reveals that a superstition "storing mushrooms results in the death of their livestock" still prevails among some Sherpas of lower region (Lukla, Benkar, etc.). However, above Namche drying mushrooms in "*Nanglo*" (Bamboo woven) and preserving them as pickle was frequently observed. Most of the respondents believe that due to prolonged snow fall the snow remains longer on the ground resulting in better growth of mushrooms.

The regionwise popularity of these mushrooms is as follows; *Amanita hemibapha, Boletus edulis* and *Boletus pulverulentus* were popular among informants residing between Lukla and Jorsalle. Similarly, *Gomphus clavatus*, *Gomphus floccosus*, *Hygrophous* sp. and *Ramaria flava* were popular among local residents of Namche and surrounding area. *Leccinum* sp, *Boletus* sp, *Paxillus involutus* and *Chroogomphus tomentosus* are popular among people living in Khunde and Khumjung area. *Boletus* spp and *Cantharella cibarius* are popular in Thangboche and Pangboche.

In addition to food item, mushroom is also used in health treatment by the rural people. According to some local informants spores of *Lycoperdon perlatum* Pers.:Pers and *L. pyriforme* Schaeff.:Pers are used as powder to heal wounds and to cure baby rashes. Hobbs (1987) reported that several species of *Lycoperdon* can be used to stop bleeding in a fresh wound. Similar results were reported by Adhikari (1988), Bhandary (1991) and Ghimire *et al.* (2001) from eastern and western regions of Nepal. Some locals use mushrooms like *Ganoderma* sp for decorative purpose while some polypores are used to make lids of bottle and stop cork. This was also reported by Kharel (1998) and Adhikari (2000). In this study, it was observed that mushrooms were not used for religious or ceremonial purposes.

The poisonous mushrooms are referred to as "bhoot chyau in Nepali dialect and "Sindi shymo" in Sherpa dialect. As per interviews with local people, hospital staff and traditional healers no mushroom poisoning cases were reported till date in the study area. The local people aged between 10 to 70 years can easily distinguish the poisonous mushrooms from edible ones on the basis of traditional knowledge handed down from generations. Some local people even believe the mushrooms become poisonous only after being bitten by a poisonous snake. Therefore, in their view the mushrooms are not that poisonous because there are no poisonous snakes in the region.

Chemical composition of prized wild edible mushrooms

Out of 26 wild edible mushrooms proximate and chemical analyses were done in eleven wild edible mushroom species. Mushrooms were selected on the basis of region-wise availability and popularity among local residents of the study area. The selected edible mushrooms were *Ramaria flava* (Schaeff. : Fr.) Quel., *Paxillus involutus* (Batsch : Fr.), *Gomphus clavatus* (Pers.: Fr.), *Leccinum* sp, *Ramaria botrytis* (Pers.: Fr.) Ricken, *Hygrophorous* sp, *Gomphus floccosus* (Schw.) Singer, *Tylopilus eximus* (Perk) Sing., *Chroogomphus tomentosus* (Murr.) O.K.Miller, *Amanita hemibapha* (Berk. & Br.) Sacc., *Boletus* sp. During analysis nine parameters such as crude protein, crude fat, crude fibre, moisture, ash, carbohydrate, calcium, phosphorous and iron in mushroom samples were carried out (Tables 2, 3 & 4).

The chemical composition of edible mushrooms determines their nutritional value. It differs according to species but also depends on the substratum, atmospheric conditions, age and part of the fructification (Manzi *et al.* 2001). The average crude protein content of edible mushrooms range between 19-40% (Kurtzman 1978). *Chroogomphus tomentosus* (11.84%) had the lowest value while *Ramaria flava* (28.32%) had the highest protein content. The crude protein content of these mushrooms was 28.32% (*Ramaria flava*), 16.46% (*Paxillus involutus*), 22.68% (*Gomphus clavatus*), 21.33% (*Leccinum* sp), 16.98 % (*Ramaria botrytis*), 22.97% (*Hygrophorous* sp), 20.97% (*Gomphus floccosus*), 25.89% (*Tylopilus eximus*), 11.84% (*Chroogomphus tomentosus*), 25.87% (*Amanita hemibapha*) and 27.75% (*Boletus* sp). Mushrooms like *Ramaria flava* (28.32%), *Gomphus clavatus* (22.68%), *Hygroporous* sp (22.97%), *Amanita hemibapha* (25.87), *Gomphus flocosus* (20.97%) and *Tylopilus eximus* (25.89%) have protein content comparable to commonly cultivated mushrooms in Nepal such as *Agaricus bisporous* (26.3%) and *Volvariella volvacea* (29.5%). These mushrooms have higher protein content than *Lentula edodes* (17.5%) and *Pleurotus ostreatus* (10.5 %).

In Khumbu region the Sherpa people do not sacrifice or kill animals. Meat is very expensive and rarely eaten item. It is only available in the weekly market (hat bazaar) and is brought all the way from Jiri by carrying it in a "*doko*" (bamboo woven basket). Therefore, the people of higher region rely on seasonal mushrooms as a major source of protein substitute to meat. Mushrooms have good nutritional value particularly as a source of protein that can enrich human diet especially in developing countries where animal protein may not be available and are expensive (Pandey & Budathoki 2007a).

The average range of carbohydrate content in edible mushrooms vary from 27.6 to 71.1% of dry wt (Purkayastha & Chandra 1985). In the present study, the values of carbohydrates ranged from a low of 31.02% in *Amanita hemibapha* on a dry weight basis to a high of 62.63% for *Chroogomphus tomentosus*. The crude fat content of mushrooms is 2-8% of the dry weight but it can vary from less than 1% to as high as 15-20 % (Crisan & Sands 1978). The lowest crude fat constituent was found in *Ramaria botrytis* (0.39%) and the highest value was observed in *Amanita hemibapha* (6.48%). The moisture content in dry mushroom ranges from 10-12% (Crisan & Sands 1978). The moisture in eleven samples of mushroom varied from the lowest value of 8.30% in *Gomphus floccosus* to the highest of 11.95% in *Hygrophorous* sp. Crisan and Sands (1978) stated that fibre content in mushrooms is very high and varies from 3-33%. In the present study *Hygrophorous* sp. had the lowest (7.73%) fibre content while *Tylopilus eximus* had the highest (19.84 %.) value. The calcium content in eleven samples of mushroom varied from 1.82 mg/100g (*Boletus* sp) to 33.09 mg/100 g (*Gomphus floccosus*). The quantity of phosphorous was lowest in *Ramaria flava* (62.51 mg/100g) while *Paxillus involutus* (944 mg/100 g) had the highest calcium content. *Leccinum* sp (0.576 mg/100g) had the lowest iron content while the *Amanita hemibapha* (307.26 mg/100 g) had the highest calcium content. The chemical composition (crude protein, carbohydrate, crude fat, crude fibre, iron, calcium and phosphorous) of mushroom samples were comparable to the range given by Crisan and Sand (1978). Most of the mushrooms are comparable with the cultivated common edible mushrooms such as *Pleurotus ostreatus, Volvariella volvacea, Agaricus bisporus* and *Lentinula edodes*.

Table 2. Proximate analysis of mushrooms collected from the SNPBZ.

S.N	Mushroom species	Crude Protein (%)	Crude Fat (%)	Moisture (%)	Ash (%)	Carbo-hydrate (%)	Crude Fibre (%)
1	*Ramaria flava*	**28.32****	1.35	10.88	16.53	42.96	8.85
2	*Paxillus involutus*	16.46	3.37	10.27	**28.88****	41.02	12.55
3	*Gomphus clavatus*	22.68	0.97	10.86	18.44	47.05	8.69
4	*Leccinum* sp.	21.33	0.89	10.73	18.06	48.99	10.17
5	*Ramaria botrytis*	16.96	**0.39***	10.15	23.5	53.32	7.97
6	*Hygrophorous* sp.	22.97	1.53	**11.95****	19.18	40.05	**7.73***
7	*Gomphus floccosus*	20.97	1.89	**8.30***	16.36	40.35	14.02
8	*Tylopilus eximus*	25.89	1.78	11.46	24.36	36.51	**19.84****
9	*Chroogomphus tomentosus*	**11.84***	2.42	9.69	**13.42***	**62.63****	8.78
10	*Amanita hemibapha*	25.87	**6.48****	10.23	26.4	**31.02***	13.37
11	*Boletus* sp.	27.75	1.99	9.40	24.42	36.44	19.05

*Lowest value **Highest value

Table 3. Mineral analysis of mushrooms collected from the SNPBZ.

SN	Mushroom Species	Calcium (Ca) (mg/100 g)	Phosphorous (P) (mg/100 g)	Iron (Fe) (mg/100 g)
1	*Ramaria flava*	6.66	**62.51***	4.07
2	*Paxillus involutus*	5.40	**944****	6.16
3	*Gomphus clavatus*	26.22	389.49	25.37
4	*Leccinum* sp.	6.10	480	**0.576***
5	*Ramaria botrytis*	6.30	441	6.21
6	*Hygrophorous* sp.	6.17	649.37	13.23
7	*Gomphus floccosus*	**33.09****	518.9	54.56
8	*Tylopilus eximus*	2.92	359	0.93
9	*Chroogomphus tomentosus*	4.01	264	2.81
10	*Amanita hemibapha*	20.09	721.75	**307.26****
11	*Boletus* sp.	**1.82***	163.77	10.66

*Lowest value ** Highest value

Table 4. Crude protein, carbohydrate, fat, fibre, calcium (C), phosphorous (P) and Iron (Fe) content of some cultivated edible mushrooms.

Mushrooms	Protein (%)	Carbohydrate (%)	Fat (%)	Fibre (%)	Ca (mg/100g)	Fe (mg/100g)	P (mg/100g)
Agaricus bisporous	26.3	49.5	1.8	8-10.4	23	0.2-19.0	790-1425
Pleurotus ostrateus	10.5	74.3	1.6	7.5-8.7	33	15.2	1348
Volvariella volvacea	29.5	40.0	5.7	4.4-13.4	35	6.0	978-1337
Lentinula edodes	17.5	59.5	8.0	7.3-8.0	98	8.5	476

Source: Crisan and Sands (1978) and Li and Chang (1982)

Conclusions

- This study documents the diversity of wild mushrooms in the Sagarmatha National Park and its Buffer Zone.
- It documents ethnomycological knowledge of the Sherpa people and to safeguard knowledge handed down from generations.
- In order to ensure continued production of these wild edible mushrooms effective conservation of natural habitat, regeneration of forest and proper harvesting techniques are recommended.

- The overall nutrition and protein content of analysed wild edible mushroom are good. Therefore, efforts should be directed towards domestication of the wild edible mushrooms. Government and private institutions should promote and develop cultivation techniques of the wild edible mushrooms which have good nutrient value.

Acknowledgements

We are grateful to Prof. Dr. H.N. Bhattarai, Vice Chancellor and Prof. Dr. D. Subba Secretary, of NAST for their kind support. We express our profound gratitude to Ev-K2-CNR, Italy for the financial support. Our special appreciation goes to the Department of National Park and Wildlife Conservation and the staff and people of Sagarmatha National Park (SNP). We would like to thank Mr. Sanjiv K. Shrestha and Mr. Pragun S. Sainju for assistance in the field. We are indebted to Mr. M. Christensen, the Royal Veterinary and Agricultural University, Denmark for helping us in the identification work.

Domestication of Some Wild Edible and Medicinal Mushrooms of Sagarmatha National Park (SNP), Nepal

Chandra P. Pokhrel, Prabina Rana and Anjana Giri

Abstract

The study was aimed to isolate and culture the medicinal and edible mushrooms. The study was conducted in the Solukhumbu district. All three Village Development Committees of Sagarmatha National Park namely Chaurikharka (buffer zone area), Namche bazaar and Khumjung were the target VDCs for experiment. During the field visit, PDA (Potato Dextrose Agar) and Agar media were used to culture the isolated mushrooms. A total of 19 species of mushrooms were collected and cultured in the synthetic media. Out of 19 species, six were successfully grown in the culture media.

Introduction

Mushrooms are the primary recyclers and are essential to recycling organic waste and efficient return of nutrients back into the ecosystem. Not only are they recognized for their importance within the environment but also for their effects on human health. Wild edible mushrooms are a natural resource with a high nutritional and economic value. The consumption of edible and medicinal mushrooms by humans is an age old practice (Pokhrel *et al.* 2007). Numerous species of higher basidiomycetes mushrooms have a long history of the application as a food source and for medicinal purposes. These mushrooms attract intense industrial interest, being a source of many pharmaceutical substances with potent and unique valuable properties, as well as various health enhancing substances known under a myriad of names: dietary supplements (DSs), functional foods, nutraceuticals, nutriceuticals, mycochemicals, and designer foods (Zeisel 1999, Wasser & Weis 1999, Wasser *et al.* 2000). Almost 650 of the known 14,000 species on earth possess significant pharmacological properties, and almost 100 species have been tested for cultivation (Hobbs 1995, Kirk *et al.* 2001, Miles & Chang 1997, Reshetnikov *et al.* 2001). Of these, about 40 species are commercially cultivated, and 7 are cultivated on an industrial scale (Chang 1999).

In Nepal, research has only been concentrated in the pre-commercial experimentation of commercially important edible mushrooms. However, focus has not yet been centered on the potential wild edible and medicinal mushrooms. The local communities of Sagarmatha National Park and its buffer zone have been using more than 26 species of mushrooms for culinary purposes. The aim of this study was to explore the possibility of domestication of wild edible mushrooms which are highly prized by the local people for both culinary and medicinal purposes.

Study Site

The study area lies in Solukhumbu district (Chaurikhark VDC, Namche VDC, Khumjung VDC) of northeastern region of Nepal. It encompasses the Sagarmatha National Park (1418 sq km.) and its buffer zone area. The park is largely composed of the rugged terrain and gorges of the Himalayas ranging from 2845 m at Monju to the top of the world Sagarmatha (Mount Everest) at 8848 m above sea level (m asl). The study sites ranging altitudinally from 2600 to 4200 m asl include Lukla, Namche, Phurte, Khunde, Khumjung, Thangboche, Deboche and Periche.

Materials and Methods

Isolation of fungal strains

Nineteen edible/ medicinal mushrooms locally used by the Sherpa people were collected from the study site during the peak season of fruiting (August- September) in 2008. A young and healthy fruiting body was chosen to establish mycelial culture of the mushrooms. Pieces of tissue were cut aseptically with a sharp razor blade from the stipe, near the point where it joins the cap and inoculated in PDA and Agar petridishes and slants. The petridishes and slants were brought to the laboratory and incubated at

23 ^{0}C for 3 weeks. The isolated mycelial cultures of mushroom were maintained in PDA slants and subcultured every 3 months.

Sterilization and pouring of media

The PDA (Potato Dextrose Agar) and Agar media were sterilized in the autoclave at 15 lb/sq inch pressure and 121^{0}C temperature for 15 minutes. The sterilized media were poured asceptically into the petridishes (9 mm in diameter) which were previously sterilized.

Determination of temperature

The effect of temperature on the mycelial growth of isolated strains of *Ramaria botrytis*, *Ganoderma* sp and *Hericium* sp were determined by the radial growth of colony measurement method. To test the effect of temperature on mycelial growth the basal medium contained glucose 1gm/L, peptone 2gm/L, KH_2PO_4 2gm/L, $MgSO_4.7H_2O$ 0.5gm/L and Agar 20gm/L in distilled water. The prepared basal medium was autoclaved at 121^{0}C for 15 minutes and than 20 mL media was poured into the petridish. A 5 mm diameter mycelial disk from newly grown PDA medium was inoculated and incubated for nearly a week at 20 ^{0}C, 22 ^{0}C and 24 ^{0}C. Five replicates of each temperature were made. For each replication the mycelial growth was determined by calculating average of the different measures of diameter after 6 days of incubation.

Results and Discussion

Collection and isolation of mushroom strains

Domestication of edible and medicinal mushrooms is one of the parts of bioprospecting of mushrooms. Out of 26 mushrooms locally used, nineteen prized wild culinary and medicinal mushrooms were collected from various forest situated between Lukla to Periche (Table 1). Out of 26 species, 15 species of mushrooms were reported to be locally used as edible or other purpose by Giri *et al.* (2006) and 4 species of edible and medicinal mushrooms namely *Ramaria* sp, *Agaricus* sp, *Anellaria semiovata* (Sow.:Fr.) Person et Denni and *Hericium erinaceous* (Bull.:Fr.) Pers were added as new to the previous list from this study. Out of 19 mushroom species, mycelial cultures of six species namely *Laccaria laccata, Ramaria botrytis, Agaricus* sp., *Anellaria semiovata, Hericium erinaceous* and *Ganoderma luciderm* were successfully grown by tissue culture method. These strains were maintained in PDA and were subcultured every three months.

Table 1. Scientific names, locality and uses of wild mushrooms.

S.N.	Species	Locality (forest)	Altitude (m)	Local use
1	*Amanita hemibapha* (Berk. & Br.) Sacc.	Muse (Lukla)	2783	Edible
2	*Ramaria* sp	Muse (Lukla)		Edible
3	*Auricularia polytricha* (Mont.) Sacc	Chire (Kunde), Deboche, Omakha (Pangboche)	3900-4000	Edible
4	*Boletus pulverulentus* Opat.	Muse(Lukla)	2783	Edible
5	*Cantharella cibarius* (Fr.: Fr.) Fr.	Chire (Kunde), Jami khiau (Khumjung) Deboche	3665-3900	Edible
6	*Chroogomphus tomentosus* (Murr.) O.K.Miller	Chire (Kunde), Jamikhiau (Khumjung) Deboche, Omakha (Pangboche)	3665-4000	Edible
7	*Gomphus clavatus* (Pers. : Fr.) S. F. Gray	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung)	3665-4000	Edible
8	*Gomphus floccosus* (Schw.) Singer var *floccosus*	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung) Deboche,	3615-3900	Edible
9	*Hydnum repandum* L. : Fr.	Jamikhiau (Khumjung)	3800	Edible

10	*Hygrophorous* sp.	Phurte (Namche)	3615	Edible
11	*Laccaria laccata* (Scop.:Fr.) Cooke	Jamikhiau	3800	Edible
12	*Leccimum* sp.	Chire (Kunde), Jamikhiau (Khumjung)	3800-3900	Edible
13	*Ramaria flava* (Sch.: Fr.) Quel.	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung) Deboche.	3615-3900	Edible
14	*Ramaria botrytis* (Pers.:Fr.) Ricken	Phurte (Namche) Chire (Kunde), Jamikhiau (Khumjung) Deboche	3615-3665	Edible
15	*Paxillus involutus* (batsch: Fr.)Fr.	Jamikhiau (Khumjung)	3800	Edible
16	*Agaricus* sp	Periche	4200-4300	Edible
17	*Anellaria semiovata* (Sow.:Fr.) Person et Dennis	Periche	4200	Edible
18	*Hericium erinaceous* (Bull.:Fr.) Pers	Jamikhiau (Khumjung), Thangboche	3800-3900	Edible/ Medicinal
19	*Ganoderma luciderm* (Leyss.Fr.) Karst.	Muse (Lukla)	2783	Decorative/Medicinal

Effect of temperature on mycelial (vegetative) growth

The effect of temperature on mycelial growth of *Ramaria botrytis*, *Ganoderma* sp and *Hericium* sp are shown in Fig 1. The maximum mycelial growth of *Ramaria botrytis* was obtained at 22 ^{0}C (2.3 mm). Similarly, mycelial growth of *Ganoderma* sp was best at 20 ^{0}C (1.6 mm) and growth of *Hericium* sp was best at 22 ^{0}C (8.8 mm). Outstanding growth was observed in *Hericium* sp in comparison to the other two species, this may be due to the influence of medium used for the experiment.

It has been suggested that at the optimum temperature, growth for each fungus, enzyme and metabolic activities increases and energy is released and good mycelial growth occur (Garraway & Evens, 1994). These mushrooms might be difficult to cultivate in hot climatic regions, as well as during the summer season under natural condition. Therefore, a well equipped growing house is needed for the year round production of these mushrooms.

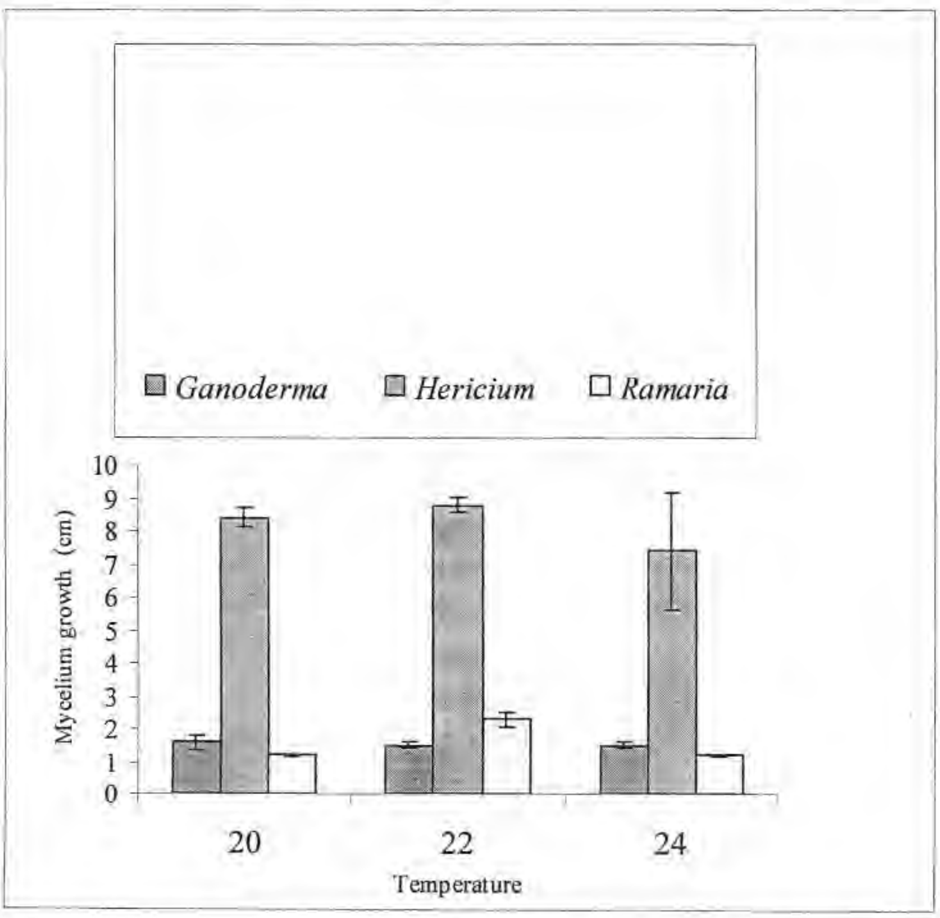

Fig 1. Effect of temperature on mycelial growth. Error bars represent standard deviation of the means (n=5).

Conclusion

The consumption of mushrooms is one of the nutritional sources of the people in the study area. However, deforestation and over harvesting are the major threats to the wild mushrooms. Therefore, artificial cultivation of such mushroom is safe and economically viable. During the field visit, PDA (Potato Dextrose Agar) and Agar media were used to culture the isolated mushrooms. A total of 19 identified species of mushrooms were collected and cultured in the synthetic media. Out of 19 species, six were successfully cultured in the media. The effect of temperature on the growth of three different species was studied. Further, their chemical and nutritional requirement is being experimented in the laboratory.

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Ethnobotanical Knowledge on Plant Uses in Khumbu Region, Sagarmatha National Park, Nepal

Dinesh R. Bhuju, Prabina Rana, Anjana Giri and Sangeeta Shrestha

Abstract

A survey was undertaken to list the plant species used by local people, the Sherpa of the Khumbu area of east Nepal. A total of 130 plant species, representing 55 families, were reported during the interview with 72 local residents in major settlements comprising traditional healers, farmers, house wives and elderly people. Ninety eight plant species were found to be used for medicinal purposes such as antipyretic, carminative, expectorant. Notably, the plant was used for more than one purpose, i.e. they had multipurpose in use such as fodder, firewood, ceremonial, vegetable etc. The Sherpa people had a long tradition of wise use of resources and used to collect medicinal and other plants for trading as well. This made their indigenous knowledge rich. However, tourism has changed their life style and economic activities as well. It is feared that the knowledge could erode with modernization Therefore timely understanding of the knowledge in the use and preparation method of high valued plant species is emphasized.

Introduction

Nepal is a multiethnic and multilingual country with more than 60 ethnic groups speaking about 75 languages in Nepal. Many people specially dwelling in the remote rural areas still do not have access to the modern medicinal facilities and depend on the plant resources to meet their daily needs including medicinal herbs. Since ancient time people inhabiting in those areas have been depending on the traditional medical supports based on locally available medicinal plants and herbs and they amass deep knowledge on the use of those items. However such knowledge lacks written records and is confined to limited elderly rural illiterate people who pass such knowledge orally to next generation.

The first scientific ethnobotanical study was on some edible and medicinal plants of Nepal in 1955 followed by several other studies (Gewali 2008). Among the ethnic groups, Sacherer (1979) conducted an ethnobotanical study of edible and medicinal plants in Sherpa community of Rolwaling, while Bhattarai (1989) studied the traditional phytotherapy among the Sherpa people of Helambu in central Nepal. Manandhar (2002) has listed 88 plant species with Sherpa names. Stevens (1996) noted limited forest terminology in Khumbu dialects of Sherpa. An ethno-botanical baseline study was carried out to document local knowledge on the use of plants and herbs in the Khumbu region of Sagarmatha National Park (SNP).

Study site

Khumbu valley lying in SNP Nepal is significant as an ecological, cultural and geographical treasure of the world. In 1979 United Nations Educational, Scientific and Cultural Organization (UNESCO) declared the SNP encompassing the Khumbu valley a World Heritage Natural Site in recognition of the significance of the world's highest mountain, its sub alpine types of flora and fauna, together with the unique cultural heritage of the Sherpa people who are the local residents. Also known as Mount Everest region, the Khumbu valley was very little known till 1950. Today, it is one of the most travelled destinations in the world.

The SNP (declaration 1976; area: 1,150 sq km; coordinates: 27°45'-28°07'N, 86°28'-87°07'E) occupies the central ecological landscape of the Himalaya in Nepal. Encompassing the valleys of three major rivers, Bhotekoshi, Dudhkosi and Imja, it forms a distinct geographical unit enclosed on all sides by high mountain ranges with altitudes extending from 2,800m (Jorsalle river confluence) to 8848m (Mount Everest). Nearly 95% of the park area lies in the high altitudes (>4,000m asl) and 78.25% of the area is barren land (RN 1999). Thus, the forest including shrub-land (2.72%) and grazing land (18.28%) have been the utmost important natural resources for subsistence of the local people, mainly the Sherpa communities who have been living in the area since 300 years.

Material and Methods

A questionnaire was developed to carry out the study; however, the interview was open-ended type. Interviews were done on personal basis as well as in groups where such situation existed. During the interview, special attention was given to cover various strata (age, economy and education) of the society. We approached local healers for detailed documentation and also for techniques of extraction and/or preparing the plant material for use, while the common people to understand their practice of the knowledge. The plant specimens were collected for confirmation with interviewees, which were used for identification later.

The, study was carried out in major settlements covering all three VDCs (village development committee) of SNP and its Buffer Zone, namely: Chaurikhark, Khumjung and Namche. The interview sites were: Khunde, Kyangjuma, Lukla, Mongla, Monjoo, Namche, Phakdin, Phortse, Phurte and Thame. The field study was conducted during the month of April 2005. The reported plant species were verified with available references. Nomenclature has mainly been based on DPR (2001). The use of plants, their habit, distribution and conservation status was verified with Rajbhandari (2001), Joshi and Joshi (2001) and Manandhar (2002).

Interviews schedule

Altogether 72 persons were interviewed in 10 settlements. Majority of the interviewed persons were local residents. By gender composition, 36 were male and 36 female. By age class, 25 were between 41 and 60 years old, 18 were between 21 and 40 years old, 14 were below 20 years old and 15 were above 60 years old. The interviewed persons included traditional healers, house wives, farmers, lodge managers, students, porters, nursery men and monks.

Result and Discussion

Plant enumeration

Altogether 130 plant species were listed from the interviews with local people in ten different sites in Khumbu region. The plants comprise medicinal and aromatic plants (MAPs) and non-timber forest products (NTFPs) as well. The plant specimens are enumerated in alphabetical order by scientific names followed by Nepali name, and Sherpa name where available. The family, habit, distribution (m asl) of the plants and their use (medicinal and others) and parts used are mentioned. Also the conservation status is mentioned when applicable.

1. *Abies spectabilis* (D. Don) Mirbel
Nepali: Thingre Salla, Sherpa: Thasing
Family: Pinaceae
Habit: Tree
Distribution: 2,400-4,200 m
Medicinal value: Asthma, bronchitis carminative, expectorant, aphrodisiac, fever
Part used: Leaves juice
Other uses: Timber for house construction, firewood, fodder, incense
Conservation status: GON protected

2. *Abrus precatorius*
Nepali: Rati gedi
Family: Leguminosae
Habit: Perennial climber
Distribution: 300-1,100 m
Medicinal value: Boils; headache, throat pain, tonic, diuretic, jaundice, gonorrhea
Parts used: Paste of leaves, roots
Other uses: Beads in necklace

3. *Acacia intsia Willd*
Nepali: Arkhu
Family: Leguminosae
Habit: Spiny shrub
Distribution: 1,100 m
Other uses: Fodder, bark juice for fish poison

4. *Aconitum ferox* Wall ex Seringe
Nepali: Nib bikh
Family: Ranunculaceae
Habit: Herb
Distribution: 2,100— 3,800 m
Medicinal value: Antipyretic, analgesic, tubers narcotic, sedative,
Parts used: Flowers, tubers
Other uses: Poisoning arrows
Conservation status: Commercially threatened
(IUCN Cat.)

5. *Aconitum gammiei Stapf*
Nepali: Bish; Sherpa: Pongmar
Family: Ranunculaceae
Habit: Weak herb
Distribution: 3,300 - 43,00 m
Medicinal use: Stomachaches, antipyretic, antitoxic
Parts used: Juice from squeezed roots
Conservation status: Rare (IUCN Cat.)

6. *Aconitum hookeri* Stapf
Sherpa: Thukchenduk
Family: Ranunculaceae
Habit: Herb
Distribution: 4,300-4,800 m
Medicine: Remove lice in the body
Parts used: Leaves and flowers are crushed

7. *Aconitum laciniatum* (Bruhl) Stapf
Nepali: Kalo bikh
Family: Ranunculaceae
Habit: Herb
Distribution: 2,800 —4,600 m
Medicinal value: Roots poisonous
Part used: Root paste
Other uses: Poison animals
Conservation status: Thratened

8. *Aconitum spicatum* (Bruehl) Stapf
Nepali: Bikh, Sherpa: Chendu
Family: Ranunculaceae
Habit: Herb
Distribution: 1,800-4,200 m
Medicinal value: Tubers deadly poisonous, antipyretic, analgesic
Part used: Root paste
Other uses: Poison the hunting arrows
Conservation status: Commercially threatened

9. *Ageratum conyzoides Linnaeus*
Nepali: Ganaune jhar
Family: Compositae
Habit: Annual herb
Distribution: Up to 2,000 m
Medicinal value: Wounds
Parts used: Paste of roots

10. *Albizia lebbeck (Linnaeus)* Benth
Nepali: Sins
Family: Leguminosae
Habit: Decidious tree
Distribution: 1,000 m

Medicinal value: Gums bleeding, Ayurvedic treatment, tonic, astrigent, diarrhea
Parts used: Roots, bark, seeds, flowers
Other uses: Soap (bark; crush), useful in dan-druff

11. Algae
Nepali: Leu
Habit: Algal thallus
Other use: Edible, mixed in meat

12. Allium hypsistum Steam
Nepali: Jimbu
Family: Amaryllidaceae
Habit: Herb
Distribution: 2,000-4,500 m
Medicinal value: cough and cold
Part used: Leaves boiled with water, whole plant
Other use: Flavoring lentil soup, curry
Conservation status: Vulnerable

13. *Allium sativum* Li
Nepali: Lasun, Sherpa: Gogpa
Family: Amaryllidaceae
Habit: Bulbous herb
Distribution: 3,000 m
Medicinal value: High altitude sickness, carminative, aphrodisiac, expectorant
Part used: Bulb boiled as soup
Other uses: Garlic powder spice

14. *Allium wallichii* Kunth
Nepali: Ban lasun; Sherpa: Gokpa
Family: Amaryllidaceae
Habit: Bulbulous herb
Distribution: 2,400-4,500 m
Medicinal value: Cholera, diarrhea, altitude sickness, cough, cold
Part used: Bulbs, leaves
Other uses: Leaves as vegetable, pickle, flavoring

15. *Alnus nepalensis* D. Don
Nepali: Uttis, Sherpa: Ramsyang
Family: Betulaceae
Habit: Tree
Distribution: 1.000-2,200 m
Medicinal use: Burns
Parts used: Bark
Other uses: Erosion control, dyeing, tanning, fodder

16. *Anaphalis adnata* (Wall) DC
Nepali: Buki phul; Sherpa: Sechng
Family: Compositae
Habit: Hairy herb
Distribution: 800-3,200 m
Medicinal use: Fresh cuts and wounds
Parts used: Leaves juice
Other uses: Make fire by rubbing on the stone, parts used flower

17. *Anaphalis contorta* (D. Don) Hork.f.
Nepali: Buki phul
Family: Compositae
Habit: Herb
Distribution: 1,500-4,000 m
Medicinal value: Cough, cold

Part use: Leaves paste
Other use: Offered to gods, cockroach repellent

18. *Anaphalis triplinervis* (Sims) C.B. Clarke
Nepali: Boki phul; Sherpa: Trachung
Family: Compositae
Habit: Erect herb
Distribution: 1,800-3.300 m
Medicinal use: Wounds
Parts used: Flower paste
Other uses: Fodder

19. *Androsace globifera* Duby
Family: Primulaceae
Habit: Herb
Distribution: 3,200-4,700 m
Other use: Good incense

20. *Angelica cyciocarpa* (C. Norman) Cannon
Family: Toricelliaceae
Habit: Herb
Distribution: 1,600-2,500 m
Other use: Fodder

21. Artemisia dubia Wallich ex Besser
Nepali: Titepati; Sherpa: Khemo
Family: Compositae
Habit: Herb
Distribution: 1,200-3,400 m
Medicine: Nose bleeding, cut bruise, fever, cough, cold
Part used: Whole plant crushed and make juice
Other use: Incense, cleaning the floor, dry fodder, manure, bed for animals, to cover marcha, when mixed with Abies leaves and fern it degrades very fast

22. *Arthaxon sp.*
Nepali: Chitre bans
Family: Gramineae
Habit: Herb
Distribution: 1,000-2,000 m
Other uses: Ceiling poles

23. *Aster falconeri* (C.B. Clarke) Hutchin.
Nepali: Tare phul
Family: Compositae
Habit: Erect herb
Distribution: 3,300-4,300 m
Medicine: Cuts, wounds
Part used: Juice of roots

24. *Astragalus leucocephalus* Graham ex Bentham
Sherpa: Gorkhim
Family: Leguminosae
Habit: Shrub
Distribution: 1,500-3,700 m
Other uses: Fruit pisonous

25. *Berbens angulosa Wall.* ex Hook. f. & Thomson
Family: Berberidaceae
Habit: Shrub
Distribution: 3,400 - 4,500 m
Other use: Fodder, fire-wood

26. *Berberis aristata* DC
Nepali: Chutro, Sherpa: Namli, Chermang
Family: Berberidaceae
Habit: Spiny shrub
Distribution: 1,800-3,000 m
Medicinal value: Fever, diarrhea
Part used: Stem, root
Preparation: Extraction
Other uses: Fodder, firewood

27. *Bergenia ciliata* (Haworth) Stemb
Nepali: Pakhenbed; Sherpa: Chyucha
Family: Saxifragaceae
Habit: Herb
Distribution: 1,300-3,000 m
Medicinal value: Urinary troubles, body-ache, cooling, tumors, urinary discharges, cough
Part used: Rhizome paste
Other uses: Flowers as pickle
Conservation status: Commercially threatened (IUCN Cat.)

28. *Betula utilis* D. Don
Nepali: Bhojpatra; Sherpa: Takpa
Family: Betulaceae
Habit: Tree
Distribution: 2,000-4,000 m
Medicinal value: Throat pain, carminative, hysteria, cuts
Part used: Roots are boiled
Other uses: Juice from trunk as refreshing drink, handle for tools (kodalo), large wooden spoon (dadoo, panyo, dabilo), wooden pots (dongmu, bowl), firewood, put under soil for its durability, incense

29. *Bistorta milletii* Leveille
Nepali: Mhyakure; Sherpa: Rambu
Family: Polygonaceae
Habit: Herb
Distribution: 2,900-4,000 m
Other uses: Flour made from seeds, useful in bad years

30. *Buddleja asiatica* Loureiro
Nepali: Bhimseripati
Family: Buddlejaceae
Habit: Evergreen shrub
Distribution: 300 - 2,000 m
Medicinal value: Skin disease
Parts used: Juice from plant
Other uses: Leaves and flower for religious purposes

31. *Calotropis gigantea* (Li) Dryander
Nepali: Ank
Family: Asclepiadaceae
Habit: Branched shrub
Distribution: 1,000 m
Medicinal value: Body pain, pimples, skin disease, dysentery, expectorant
Parts used: Juice prepared from leaves, roots. Latex, flowers
Other uses: Making charcoal, thread

32. *Chesneya nubigena* (D. Don) Ali
Nepali: Sherpa: Gorkhim
Family: Leguminosae
Habit: Herb
Distribution: 3,000 - 4,000 m
Other uses: Seeds edible

33. Chielanthus albomarginata C.B. Clarke
Nepali: Damini sinka; Sherpa: Kalsing, Thokcho
Family: Pteridaceae
Habit: Fern
Distribution: Up to 3200 m
Medicinal value: Stomach disorder
Parts used: Rhizome juice
Other use: Young leaves for vegetable, leaves for potato storage and manure

34. *Cinnamomum tamala* (Buds-Ham)
Nees & Eberm.
Nepali: Tejpat
Family: Lauraceae
Habit: Evergreen tree
DistribUtion: 500 - 2,000 m
Medicinal value: Diarrhea, colic carminative, nausea, flatulence
Part used: Leaf and bark
Other use: Essential oil, flavoring meat

35. *Cirsium verutum* (D. Don) Springel
Nepali: Dhode kanda
Family: Compositae
Habit: Herb
Distribution: 700 - 3100 m
Medicine: Fever, nose bleed
Part used: Juice of root
Other uses: Tender roots chewed

36. *Citrus aurantium* Li
Nepal: Kagati
Family: Rutaceae
Habit: Small tree
Distribution: 1,000 - 1,400 m
Medicinal use: Source of vitamin C
Part used: Fruits
Other uses: Pickle

37. *Clematis buchananiana* D C
Nepal: Ghante phool
Family: Ranunculaceae
Habit: Trailing shrub
Distribution: 1,000 - 3,300 m
Medicinal use: Inflammation, toothache, cough, cold
Part used: Juice of root, leaves
Other uses: Fermenting cake Marcha, animal feed

38. *Cordyceps sinensis* (Berk.) Sacc
Nepal: Yarsagumba
Family: Hypocreaceae
Habit: Parasitic fungus, later becoming saprophytic on insect larva after its death
Distribution: 3,000 - 4,000 m
Medicinal use: Aphrodiasic, tonic, expectorant, asthma, impotency
Part used: Whole organism
Conservation status: Under Forest Act 1993, permission needed for export

39. *Cortia depressa* (D. Don) C. Norman
Nepali: Kholo
Family: Typhaceae
Habit: Herb
Distribution: 3,600 - 4,900 m
Other use: Fodder

40. *Cotoneaster microphyllus* Wall ex Lindley
Nepali: Ghangharu; Sherpa: Kyakpa, Pelma
Family: Rosaceae
Habit: Branched evergreen shrub
Distribution: 1300 - 3500 m
Other use: Fodder, preventing soil erosion, ripe fruits eaten.

41. *Curcuma angustifolia* Rox
Nepali: Haledo
Family: Zingiberaceae
Habit: Herb with green, bitter leaves
Distribution: 1,500 m
Medicinal value: Dislocated bones, nutritious, demulcent
Part used: Root
Preparation: Paste after boiling

42. *Cynodon dactylon* (Li) Persoon
Nepali: Dubo, Sherpa: Khemba
Family: Gramineae
Habit: Prostrate perennial grass
Distribution: 3,000 m
Medicinal value: Indigestion, dropsy, haemorrhages, dysentery
Part used: Whole plant, juice
Other uses: Fodder, religious rituals

43. *Dactylorhiza hatagirea* (D. Don) Soo
Nepali: Panchaunle; Sherpa: Omolakpa
Family: Orchidaceae
Habit: Ground orchid
Distribution: 2,800— 3,800 m
Medicinal value: Expectorant, nutritious, astringent, demulcent, urinary trouble
Parts used: Powder from root
Other use: Vegetable
Conservation status: Critically endangered; Collection banned by Forest Act 1993

44. *Daphne bholua* Buch-Ham ex D. Don
Nepali: Lokta; Sherpa: Syu
Family: Thymelaeaceae
Habit Evergreen shrub
Distribution: 1,000— 3,200 m
Medicinal value: Fever, intestinal problem
Part used: Juice from root, bark, seeds
Other use: Handmade papers, ropes

45. Dendranthema nubigenum (Wall ex DC) Kitamura
Nepali: Sun phul; Sherpa: Khembakaru
Family: Compositae
Habit: Shrub
Distribution: 2,600 - 4,000 m
Medicinal value: Lice, insect repellent
Parts used: Leaves powder
Other uses: Incense

46. Dendrocalamus hamiltonii Nees & Arnott ex Munro
Nepali: Choya
Family: Graminae
Habit: Bamboo
Distribution: 500 - 2,000 m
Other uses: Traditional baskets, young shoots cooked as vegetable

47. *Dendrocalamus* sp.
Nepali: Bans
Family: Gramineae
Habit: Bamboo
Distribution: 690 - 2,000 m
Other uses: Young shoots as vegetable or pickle, stem for scaffolding

48. *Drepanostachyum falcatum* (Nees) Keng f.
Nepali; Nigalo
Family: Gramineae
Habit: Bamboo
Distribution: 1,000 - 2,000 m
Otheruse: Construction, roofing, weaving basket, pipe used for tomba (Pipsi), comb, broom, lighting, leaves for fodder

49. *Dryopteris chrysocoma* C. Christ
Nepali: Chyamle
Family: Dryopteridaceae
Habit: Herbaceous fern
Distribution: 1,500 - 3,200 m
Medicinal value: Cuts and wounds
Parts used: Juice of rhizome

50. *Dryopteris cochleata* (D. Don) C. Christensen
Nepali: Niuro; Sherpa: Thokcho
Family: Dryopteridaceae
Habit: Terrestrial fern
Distribution: 1,600 m
Medicinal value: Diarrhea, dysentery
Parts used: Rhizome juce
Other use: Tender shoots and fronds are cooked as vegetable, pickle and manure

51. *Elsholtzia eriostachya* (Benth) Benth
Nepali: Bhotepati; Sherpa: Ki-lenja
Family: Labiatae
Habit: Hairy herb
Distribution: 3,000 - 4,800 m
Medicinal value: Cough, cold
Part used: Seeds

52. *Emilia sonchifolia* (Li) DC
Nepali: Mulapate
Family: Compositae
Habit: Erect hairy herb
Distribution: 1,700 m
Medicinal value: Cuts and wounds
Part used: Leaves are ground
Other use: Powder for marcha (yeast cake)

53. Ephedra gerardiana Wallich ex Stapf
Nepali: Kagcharo; Sherpa: Sangkaba
Family: Ephedraceae
Habit: Gymnospermous shrub
Distribution: 2,000 - 5,200 m
Medicine: Skin disease, asthma, respiratory problem, cardiac
Parts used: Whole plant made for juice
Other: Incense, potato storage, fuel

54. *Eupatorium adenophorum* Sprengel
Nepali: Banmara

Family: Compositae
Habit: Erect shrub
Distribution: 500 - 2.400 m
Medicinal value: Wounds and cuts
Part used: Leaves juice
Other uses: Green manure

55. *Euphorbia hirta* Li
Nepali: Dudhe jhar
Family: Euphorbiaceae
Habit: Prostrate herb
Distribution: 1,800 m
Medicinal value: Milk sap cause allergy, dislocated bone, body pain, anthelmintic, laxative, tonic, dysentery, diarrhea, asthma, fever, headache
Part used: Whole plant
Other uses: Vegetable and fodder

56. *Eurya acuminata* DC
Nepali: Jhingano; Sherpa: Pajan
Family: Theaceae
Habit: Evergreen shrub
Distribution: 1,000 - 2,800 m
Other use: Fuel, fodder

57. *Fagopurum esculentum* Moench
Nepali: Phapar; Sherpa: Tau
Family: Polygonaceae
Habit: Annual herb (crop)
Distribution: 1,000 - 2,500 m
Medicinal value: Pain
Part used: Seed, flower
Other uses: Flour from fruits, grass for washing and potato storage

58. *Fragaria daltoniana* Gay
Nepali: Bhui kaphal
Family: Rosaceae
Habit: Shrub
Distribution: 1.000 - 2,800 m
Medicinal value: Fever
Part used: Juice from root

59. *Fragaria nubicola* Lindley ex *Lacaita*
Nepali: Bhui ainselu, Bhui kafal, Sherpa: Nhynalarig dhambu
Family: Rosaceae
Habit: Herb
Distribution: 1,600 - 4.000 m
Medicinal value: Profuse menstruation
Part used: Whole plant juice
Other uses: Ripe fruits edible

60. *Fragaria* sp.
Sherpa: Chhusyambru
Family: Rosaceae
Habit: Herb
Distribution: >1,500 m
Medicinal value: Cough, cold, fever
Part used: Stem, Fruit

61. *Gaultheria fragrantissima* Wallich
Nepali: Dhasingre; Sherpa: Chenchen
Family: Ericaceae

Habit: Shrub
Distribution: 1,200 - 2,600 m
Medicinal value: Cough, aromatic, stimulant, carminative
Part used: Juice from fruit, seeds, twigs, leaves
Other use: Ripe fruits eaten

62. *Gerbera nivea* (DC) Schultz Bipontinus
Nepali: Jhulo
Family: Compositae
Habit: Herb
Distribution: 2.800 - 4,500 m
Other use: Fire starting tool

63. *Imperata cylindrica* (Li) Palisot de Beauvois
Nepali: Siru; Sherpa: Chaharu
Family: Gramineae
Habit: Perennial grass
Distribution: 2,400 m
Medicinal value: Anthelmintic
Parts used: Root boiled
Other use: Thatching roof, fodder

64. *Inula cappa* (Buch-Hem ex D. Don) DC
Nepali: Bakhre kane; Sherpa: Lhapi karpo
Family: Compositae
Habit: Erect shrub
Distribution: 2,500 m
Medicine: Ulcer, indigestion, headache
Preparation: Juice
Other uses: Useful for making Marcha (ferment in cake)

65. *Iris clarkei* Baker ex Hook f
Nepali: Bojho jhar; Sherpa: Thukchentu
Family: Iridaceae
Habit: Herb
Distribution: 3,000 - 3500 m
Medicine: Cuts and wounds
Parts used: Root paste
Other use: After eating, cows can't eat any food for about two days

66. *Juniperus indica* Bert
Nepali: Dhupi; Sherpa: Syukapa
Family: Cupressaceae
Habit: Evergreen coniferous shrub
Distribution: 2,100—4,300 m
Medicinal value: Fever, headache, vaginal discharges, stomachic, tonic, piles, laxative
Part used: Fruits, berries, wood
Other uses: Leaves as incense, groom (leaf and stem), holy book cover, wood to make water bowl, timber, firewood, flag post

67. *Juniperus recurva* Buch - Ham ex D. Don
Nepali: Dhupi; Sherpa: Sukpa
Family: Cupressaceae
Habit: Evergreen coniferous shrub
Distribution: 3,300 - 4,600 m
Other use: Incense, firewood, very important for lama puja, flag-post put outside the house when a son is born

68. *Kobressia* sp.
Sherpa: Phurchha
Family: Cyperaceae
Habit: Herb

Distribution: Above 3,000 m
Other uses: Fodder for yak

69. *Lagotis kunawurensis* (Royle ex Benth) Rupr.
Family: Scrophulariaceae
Habit: Herb
Distribution: 3,900 - 5,600 m
Medicinal value: By Amchis

70. *Lindera neesiana* (Wallich ex Nees) Kurz
Nepali: Sil timur
Family: Lauraceae
Habit: Tree
Distribution: 700 — 2,600 m
Medicinal value: Diarrhea, worm
Parts used: Fruits
Preparation: As it is
Other uses: Used in wine preparation

71. *Lycopodium clavatum* Li
Nepali: Nagbeli; Sherpa: Chhe Mehendo
Family:Lycopodiaceae
Habit: Trailing herb V
Distribution: 1.600 - 3,600 m
Medicinal values: Diuretic, antispasmodic, wounds, cracks
Parts used: Spores
Other uses: Vegetable sulphur, local dessication

72. *Lygodium japonicum* (Thunb) Swartz
Nepali: Ankhle jhar
Family: Lygodiaceae
Habit: Herbaceous fern
Distribution: 1,000 - 3,900 m
Medicine: Wounds, scabies, joint aches
Preparation: Juice and paste
Other uses: Tender part as vegetable

73. *Lyonia ovalifolia* (Wall) Drude
Nepali: Angeri; Sherpa: Rongle
Family: Ericaceae
Habit: Deciduous small tree
Distribution: 1.300 - 3,300 m
Medicinal value: Scabies, itching
Part used: Leaf juice
Other uses: Coal, young leaves poisonous to cattle

74. *Maharanga emodi* (Wall) A.DC
Nepali: Maharangi; Sherpa: Koma
Family: Boraginaceae
Habit: Hispid herb
Distribution: 2200 - 4500 m
Medicine: Hair tonic, cooling, laxative, anthelmintic
Preparation: Juice of rhizome
Conservation status: Data deficient

75. *Mahonia napaulensis* DC
Nepali: Jamne mandro; Sherpa: Chersing
Family: Berberidaceae
Habit: Evergreen shrub
Distribution: 1,400 - 2,900 m
Medicne: Fruit is diuretic, inflammation of eye, antidysenteric

Parts used: Bark juice, berries
Other uses: Fruits edible, pickles, dye

76. *Meconopsis grandis* Prain
Nepali: Kesar; Sherpa: Shiinakpa
Family: Papaveraceae
Habit: Hairy herb
Distribution: 3,000 - 5,000 m
Other use: Tender stem edible, seeds roasted for pickles

77. *Mentha spicata* Li
Nepali: Pudina, Bawari
Family: Labiatae
Habit: Herb
Distribution: 2,500 m
Medical value: Dysentery, aromatic, stimulant, stomachic, carminative
Part used: Leaves, flower tops
Other uses: Leaves as pickle

78. *Michelia champaca* Li
Nepali: Champ
Family: Magnoliaceae
Habit: Tree
Distribution: 600 - 1,300 m
Medicine: Wounds, diuretic, stimulant, astringent, expectorant, purgative
Preparation: Leaves, bark, fruits, seeds
Other: Wood, furniture, oil for perfume
Conservation status: Critically endangered; IUCN endangered cat.; under Forest Act 1993

79. *Myricaria rosea* W.W. Smith
Nepali: Akhrelu; Sherpa: Chhui syambu
Family: Tamaricaceae
Habit: Trailing herb
Distribution: 3,300 - 4,500 m
Medicinal value: Treat cold, relieve backaches, tuberculosis, cough, food poisoning
Part used: Whole plant boiled or raw
Other uses: Fruit edible

80. *Neopicrorhiza scrophulariifolia* (Pennell) Hong
Nepali: Kutki; Sherpa: Hodling
Family: Scrophulariaceae
Habit: Herb
Distribution: 3,500 - 4,800 m
Medicinal value: Fever, cough, cold, throat infection, anemia, jaundice, dropsy, appetizer
Part used: Rhizome paste or juice, root eaten or crushed and boiled
Other use: Incense, color
Conservation status: Endangered, protected by GON

81. *Parmelia* spp.
Nepali: Jhau; Sherpa: Myangmar
Family: Parmeliaceae
Habit: Lichen growing on rocks
Distribution: >2500 m
Medicinal use: Cuts
Part used: Thallus rubbed
Other use: Manure
Conservation status: Under Forest Act 1993

82. *Phyllanthus emblica* Li
Nepali: Amala
Family: Euphorbiaceae
Habit: Deciduous tree

Distribution: 1,600 m
Medicinal value: Diarrhea, dysentery
Part used: Bark juice

83. *Pieris formosa* (Wall) D. Don
Nepali: Bulu; Sherpa: Praba
Family: Ericaceae
Habit: Small tree
Distribution: 2,000 - 3,300 m
Medicinal value: Scabies
Part used: Leaves juice
Use: Poisonous to livestock; leaf, both leaves mixed with each other and put in cow dung to make manure

84. *Pinus roxburghii* Sargent
Nepali: Rani salla; Sherpa: Metang
Family: Pinaceae
Habit: Evergreen coniferous tree
Distribution: 800- 1,400 m
Medicinal value: Cuts, wounds, expectorant, purgative, asthma, scabies, gonorrhea
Part used: Resin paste, seeds
Other uses: Leaves for manure, also as incense, timber for construction and furniture

85. *Pinus wallichiana* A.B. Jackson
Nepali: Gobre salla; Sherpa: Metang
Family: Pinaceae
Habit: Evergreen coniferous tree
Distribution: 1,800 - 2,300 m
Medicinal value: Bone fracture, pain reliever
Part used: Resin
Other uses: Leaves for manure, also as incense, timber for construction and furniture, resin for commercial purpose

86. *Piptanthus nepalensis* (Hook) D. Don
Nepali: Suga phul
Family: Leguminosae
Habit: Shrub
Distribution: 2,000 - 3,800 m
Other use: Beams walking stick; bark and leaves fish poison; fodder nutritious for wild animals

87. *Plantago erosa Wall*
Nepali: Gahun phul; Sherpa: Gandhap
Family: Plantaginaceae
Habit: Perennial herb
Distribution: 2,000 - 4,600 m
Medicinal value: Bruises, rash, skin allergies, fever, diarrhea, dysentery
Part used: Leaves paste
Other uses: Vegetable

88. *Podophyllum hexandrum* Royle
Nepali: Laghupatra; Sherpa: Hamung
Family: Berberidaceae
Habit: Herb
Distribution: 3,000 - 4,500 m
Medicinal value: Stimulant and purgative, used by Amchis, hepatic stimulant
Part used: Rhizomes, fruits, roots

89. *Potentilla fruticosa* Li
Nepali: Bairungpati, Sherpa: Kisur
Family: Rosaceae
Habit: Erect shrub
Distribution: 2,400 - 4,600 m

Medicinal value: Indigestion
Part used: Root juice, leaves
Other uses: Incense, substitute of tea

90. *Potentilla indica* (Andrews) Wolf
Nepali: Bhuin ainselu; Sherpa: Aprak
Family: Rosaceae
Habit: Creeping herb
Distribution: 500 - 2,600 m
Medicinal value: Diarrhea, dysentery, cough
Part used: Root juice
Other uses: Edible fruits, fodder

91. *Potentilla kleiniana* Wight
Nepali: Kauwa Kaphal
Family: Rosaceae
Habit: Annual herb
Distribution: 800 - 2,500 m
Medicinal value: Cold, cough, fever
Part used: Whole plant for juice
Other use: Ripe fruits edible

92. *Potentilla monanthes* Wall ox Lehm
Family: Rosaceae
Habit: Herb
Distribution: 3,000 — 4,500 m
Other value: ripe fruits edible

93. *Potentilla pedunularis* D. Don
Nepali: Kali ainselu: Sherpa: Kitulu
Family: Rosaceae
Habit: Herb
Distribution: 3,000 - 4,700 m
Medicinal value: profuse menstruation, fever
Part used: Leaves paste

94. *Primula irregularis* Craib.
Nepali: Bhuichampa phul
Family: Primulaceae
Habit: Herb
Distribution: 2,800 - 3,400 m
Use: Browsed by domestic animals

95. *Prinsepia utilis* Royle
Nepali: Dhatelo; Sherpa: Yormag
Family: Rosaceae
Habit: Deciduous shrub
Distribution: 1,300 - 2,900 m
Medicinal value: Muscular pain, rheumatism
Parts used: Oil from seeds
Other uss: Fodder, fences

96. *Prunus cornuta* (Wall ox Royle) Steudel
Nepali: Arupate painyu
Family: Rosaceae
Habit: Tree
Distribution: 2,100 - 3,500 m
Use: Ripe fruit eaten, foliage for fodder

97. *Pyrus pashia* Buch - Hamilton ex D. Don
Nepali: Mayal; Sherpa: Seplung
Family: Rosaceae

Habit: Deciduous tree
Distribution: 700 - 2,600 m
Medicinal value: Treat conjunctivitis, diarrhea
Part used: ripe fruit
Preparation: juice
Other use: Leaves boil as tea, fodder; Wood for walking stick

98. *Quercus semecarpifolia* Smith
Nepali: Kharsu; Sherpa: Tadumpa, Bhelo
Family: Fagaceae
Habit: Tree
Distribution: 700 - 3,800 m
Medicinal value: Bodyache
Part used: Resin
Other use: Resin boiled as tea, fodder, firewood

99. *Rheum australe* D. Don
Nepali: Padamchal; Sherpa: Chyurcha
Family: Polygonaceae
Habit: Herb
Distribution: 3,200 - 4,200 m
Medicinal value: Tonic, purgative, astringent, dysentery, appetizer
Part used: Rhizome boiled and drink
Other use: Leaves boiled and drink as tea
Conservation status: Vulnerable

100. *Rhododendron anthopogon* D. Don
Nepali: Sunpati; Sherpa: Balu-changsing, Lhe Masur
Family: Ericaceae
Habit: Shrub
Distribution: 3,300 - 4,600 m
Medicinal value: Fever, cough, cold
Parts used: Leaves boiled
Other uses: Incense

101 . *Rhododendron arboreum* Smith
Nepati: Gurans; Sherpa: Kalma
Family: Ericaceae
Habit: Tree
Distribution: 1,400 - 3,300 m
Medicinal value: To swallow fish bone, wounds, diarrhea, cough, menstrual disorder
Part used: Flower chewed, bark
Other uses: Flower juice as drinks, firewood, wooden tools

102. *Rhododendron barbatum* Wall. ex G. Don
Nepali: Chimal; Sherpa: Kalma
Family: Ericaceae
Habit: Tree
Distribution: 2,400 - 3,600 m
Medicinal value: Rheumatic pain, syphilis
Parts used: Juice of leaves
Other uses: Excellent firewood, young leaves poisonous

103. *Rhododendron campanulatum* D. Don
Nepali: Chimal; Sherpa: Khamapu
Family: Ericaceae
Habit: Shrub
Distribution: 2,800 - 4,400 m
Medicinal value: Wounds
Part used: Flowers juice
Other uses: Firewood, manure

104. *Rhododendron grande* Hooker f
Nepali: Chimal; Sherpa: Tongmar
Family: Ericaceae
Habit: Tree
Distribution: 1,700 - 2,900 m
Other uses: Mainly firewood

105. *Rhus javanica* L
Nepali: Bhokyamlo
Family: Anacardiaceae
Habit: Deciduous tree
Distribution: 1,000 - 2,600 m
Medicinal value: Profuse menstruation, paralysis, diarrhea, appetizer
Part used: Fruit powder
Other uses: Fuel-wood

106. *Roscoea alpina* Royle
Nepali: Bharda
Family: Zingiberaceae
Habit: Herb
Distribution 2,400 - 3,100 m
Use: Tuber poisonous

107. *Rubus ellipticus* Smith
Nepali: Ainselu; Sherpa: Nanyungma
Family: Rosaceae
Habit: Straggling shrub
Distribution: 1,600 - 2,300 m
Medicinal value: Sore throat, tonsil, fever, astringent, tonic, cooling
Part used: Root
Preparation: Ground and mixed with water
Other values: Leaves browsed by cattle, fruit edible when ripe

108. *Rubus splendidissimus* Hara
Nepali: Chande ainselu; Sherpa: Phamilumu
Family: Rosaceae
Habit: Shrub
Distribution: 2,400 - 3,000
Other use: Ripe fruits edible

109. *Rumex nepalensis* Sprengel
Nepali: Halhale, Sherpa: Damshima
Family: Polygonaceae
Habit: Herb
Distribution: 3,300 m
Medicinal value: Body wash
Part used: Whole plant boiled with water
Other uses: Vegetable, Fodder

110. *Salix babylonica* Li
Nepali: Bains
Family: Salicaceae
Habit: Tree
Dostribution: 1,400 - 3,600 m
Medicinal value: Tonic, astringent, fever, anthelmintic
Part used: Leaves, bark
Other use: Firewood, leaves for manure, soil conservation

111. *Seilnum candollii* DC
Nepali: Bhutkesh; Sherpa: Rhuji
Family: limbelliferae
Habit: Herb

Distribution: 3,000 - 3,800 m
Medicinal value: Minor cuts
Part used: Flowers
Other uses: Locket

112. *Silene stracheyi* Edge
Family: Caryophyllaceae
Habit: Herb
Distribution: 2,000 - 3,500 m
Use: Powder root to wash clothes

113. *Sinarundinaria maling* (Gamble) C.S. Chao & Renvoize
Nepali: Malingo
Family: Gramineae
Habit: Bamboo
Distribution: 1,000 - 3,000 m
Other use: Mats, Pipsi (used to drink tómba), roofing, broom, comb, fences, baskets, flag post

114. *Sonchus wightianus* DC
Nepali: Mulapate
Family: Compositae
Habit: Errect perennial herb
Distribution: 600 - 2,500 m
Medicinal value: Fever, typhoid, indigestion
Parts used: Juice of plant, root
Other use: Young leaves and shoot used as vegetable

115. *Sorbus microphylla* Wenzig
Nepali: Charuwa; Sherpa: Lami chasing
Family: Rosaceae
Habit: Small tree
Distribution: 3,000 - 4,500 m
Other use: Handle, fodder

116. *Swertia chirayita* (Roxb. ex Fleming) Karsten
Nepali: Chiraito
Family: Gentianaceae
Habit: Annual herb
Distribution: 1,500 - 2,500 m
Medicinal value: Fever, cold, headache, skin disease, intestinal worms, bronchial asthma
Part used: Roots crushed and boiled with water
Conservation status: Vulnerable (IUCN cat.)

117. *Swertia multicaulis* D. Don
Nepali: Sarmaguru; Sherpa: Hogling
Family: Gentianaceae
Habit: Tufted herb
Distribution: 4,000 - 4,900 m
Medicinal value: Fever, cold, cough, infection of blood clot, stomach ache
Part used: Rhizome juice

118. *Tagetes erecta L*
Nepali: Sayapatri
Family: Compositae
Habit: Erect herb
Distribution: 2,200 m
Medicinal value: Throat pain
Part used: Flower juice
Other use: Fresh receptade eaten

119. *Tanacetum nubigena* (DC) Shih
Sherpa: Khemsang

Family: Compositae
Habit: Perennial herb
DistributiOn: 3,000 m
Use: Dry leaves for incense

120. *Taxus baccata* L subsp. wallichiana (Zucc) Pilger
Nepali: Loth salla
Family: Taxaceae
Habit: Evergreen coniferous tree
Distribution: 2,200 - 3,400 m
Medicinal values: Cough. bronchitis, cancer cure, asthma
Parts used: Juice of leaves, barks
Other uses: Pole, firewood
Conservation status: CITES Append II

121 . *Trichosanthes wallichiana* (Seringe) Wight
Nepali: Ban pharsi
Family: Cucurbitaceae
Habit: Climber
Distribution: 600 - 2,700 m
Medicinal value: Fever
Part used: Juice from root, fruit
Other uses: seeds put inside the grass for about a week then it tastes good

122. *Tsuga dumosa* (D. Don) Eichler
Nepali: Thingre salla; Sherpa: Lashing
Family: Pinaceae
Habit: Evergreen coniferous tree
Distribution: 2000 - 3600 m
Other use: Leaf for manure and incense, wood for construction

123. *Urtica dioica* Li
Nepali: Sisnoo; Sherpa: Jhaduk, Syak
Family: Urticaceae
Habit: Stringing shrub
Distribution: 500-4,500 m
Medicinal value: Cough, cold, wounds, bleeding from nose, diuretic, astringent
Part used: Leaves, roots, whole plant
Preparation: Boiled
Other use: Vegetable

124. *Utricularia aurea* Loureiro
Nepali: sim-jhar
Family: Lentibulariaceae
Habit: Aquatic herb
Distribution: 1,400 m
Medicinal value: Cuts and wounds
Parts used: Dried powered plants

125. *Viburnum erubescens* Wal ex DC
Nepali: Ban chulo
Family: Sarmbucaceae
Habit: Tree
Distribution: 1,300 - 3,300 m
Medicinal value: Cough
Parts used: Root juice
Other use: Fruits edible when ripe

126. *Viburnum gandiflorum* Wal ex dc
Nepali: Ganaune
Family: Sambucaceae

Habit: Shrub
Distribution: 3,000 - 3,700 m
Use Ripe fruits edible

127. *Viscum album* L.
Nepali: Harchur
Family: Viscaceae
Habit: Parasitic herb
Distribution: 600 - 2,300 m
Medicinal value: Dislocated bones, wounds, tumors, ear diseases, tonic, aphrodiasic
Part used: Bark, plant, berries

128. Vitis repanda Wight & Arnott
Nepali: Jhuleti; Sherpa: Syolmak
Family: Vitaceae
Habit: Large climber
Distribuon 1,400 - 2,500 m
Use: Ripe fruits edible

129. *Waldheimia glabra* (Decne) Regel
Family: Compositae
Habit: Herb
Distribution: 4,100-5,400 m
Use: Good scent

130. *Zanthoxylum armatum* DC
Nepali: Timur; Sherpa: Kiermang, Yerma
Family: Rutaceae
Habit: Spiny shrub
Distribution: 1,100 - 2,900 m
Medicinal value: Altitude sickness, throat pain, intestinal worms, allergic body rashes
Parts used: Root, leaves boiled and paste made, seeds,, bark
Other uses: Intoxicate fish, add in pickle, firewood

The reported species represented 55 plant families and 106 genera (Fig. 1). Family Ranunculaceae contained the highest number of plant species (16 spp.) followed by Compositae (15 spp.). The other families with relatively high number of species (>6 spp.) were Ericaceae, Gramineae, Leguminosae, and Ranunculaceae. Genera (Aconitum, Potentilla and Rhododendron) was represented by five species each, the highest, while Allium, Anaiphalis and Fragaria had three species each. By habit, the herbaceous species represented the highest number (59 spp.) followed by shrub species (28 spp.) and tree species (26 spp.). The others were grass (6 spp., majority bamboo), fern (5 spp.) and climber (3 spp.). Algae, lichen and orchid were represented one species each (Fig. 3).

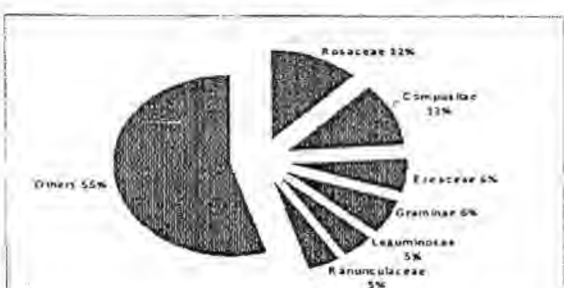

Fig. 1. Species Sharing by Plant Family in Sagarmatha National Park

The reported speőies occurred at various altitudinal ranges. Majority of the species occurred at arctic (> 4,000m asl) and alpine bioclimatic zone (3,000 - 4.000m asl) with 40 species in each zone. Some species are recorded from very high altitude, such as Lagotis kunawurensis, a herbaceous plant used by the Amchis occuring up to 5,600m asl. Thirty five species were occurring at cool temperate (2,000-3,000m asl) and the rest 15 at warm temperate (1,000 - 2,000m asl). However, it is assumed that the local people of Khumbu region mentioned about those plants occuring at tropical zone because of their popularity in their daily life (e.g. Phyllanthus emblica, Citrus aurantium).

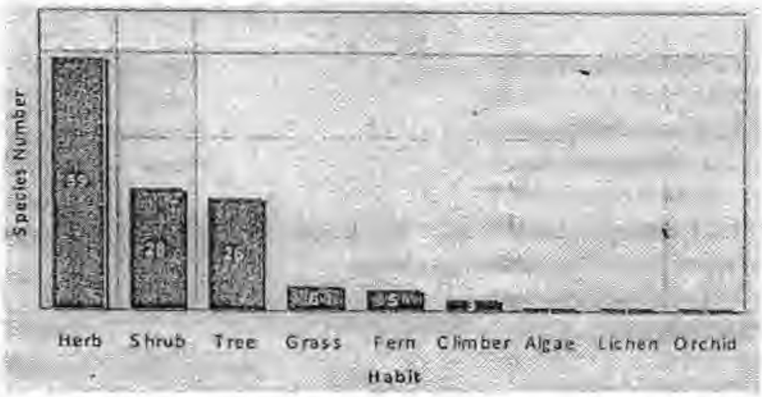

Fig. 2. Number of Species by Habit in Sagarmatha National Park Used by Local People for Various Purposes.

Himalayan medicinal and aromatic plants comprise a diverse array of species varying in their life forms and habitat specifications. Preliminary analysis of the distribution pattern along altitudinal gradient in the Nepal Himalaya shows that the lower sub-tropical level (1,000-1,500m asl) harbor proportionately maximum number of medicinal plants. The alpine and sub-alpine zones contain relatively lower medicinal plant species; however, they provide habitats supporting diversity of potential species which are highly valued by regional and international trade (Ghimire 2008).

Purposes

Local people used the plant species for various purposes, viz. medicine, food, fodder, fuel-wood, furniture, timber, incense, manure, etc. Out of 130 species reported, 98 species were found used for medicinal purposes (Fig. 3). Such plants included commonly found tree species of the area such as *Abies spectabilis* (to treat asthma, bronchitis, fever) and *Betula utilis* (to treat throat pain, hysteria, cuts), *Rhododendron* spp. (to treat fever, cough, wounds) and also high altitude herbaceous species such as *Aconitum* spp. (to treat fever, pain), *Allium* spp. (to treat altitude sickness, cough and cold), and Potentilla spp. (to treat cough, diarrhea, profuse menstruation, fever). Some species of shrub (e.g. Zanthoxylum armatum, Urtica diolca), climbers (e.g. Abrus precatorius, Trichosanthes wallichiana) were also found to be used for medicinal purposes.

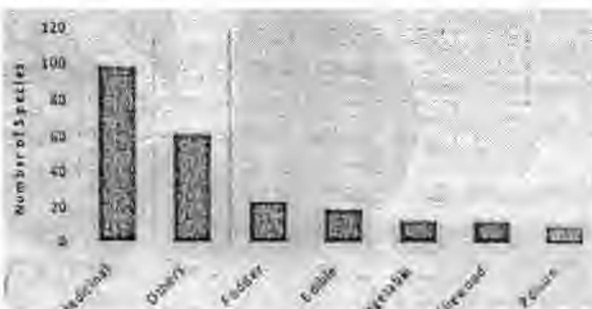

Fig. 3. Number of species used by local people for various purposes

Fodder is an integral part of agricultural livelihood. The Khumbu people reported some 24 species of plants being used to feed their livestock (e.g. *Artemisia dubia, Berberis angulosa, Kobressia* sp., *Prunus cornuta).* Some 13 species of plants were found to be used for firewood (e.g. *Betula* utilis, *Juniperus indica, Quercus semecarpifolla, Rhododendron* spp.), and other 13 species including some fern *(Chielanthus albomarginata)* and bamboo species *(Dendrocalamus hamiltonii)* were found used as vegetable. Beside used for furniture and timber (e.g. *Pinus roxburghii, P. wallichiana, Abies spectabilis)* plants were also a part of their culture, the leaves and twigs of *Juniperus* spp., *Rhododendron anthopogan, Tanacetum nubigena* were reported as profusely used as incense any kind of ceremonies and rituals. The Sherpa people would erect a flag post of Juniperus recurva when a boy child is blessed to a house.

It was notable that nearly half of the species (63 spp) mentioned were found to be used for two purposes, while about 20 per cent (25 spp) were used for three or more than three purposes, and the rest (42 spp) were used for single purpose (Fig. 3). It shows the optimal use of the plant species by the local people, and also indicates the importance of plant species for multi purposes.

The forest products including medicinal plants were formerly especially important to poorer families in the region. Some medicinal herbs were formerly collected and taken south even as far as India for sale (Stevens 1996). However, it is no more a valid practice. It was found that nowadays most of the people use modern medicines after the establishment of Khunde hospital at Khunde. The knowledge of medical uses is generally confined only to the Lamas and Sherpas at the Gumbas.

Threatened species

Of the total 130 plant species, 16 species were noted as threatened as classified by international standard or government rules/regulations The species categorized under CAMP were Aconiturn ferox, *A. spicatum, Allium hypsistum, Dactylorhiza hatagirea, Maharanga emodi, Michelia champaca, Neopicrorhiza scrophthariifolia, Rheum austral,* and *Swertia chirayita.* There were eight species put under IUCN category of rare (*Aconitum gammiei)* threatened *(Aconitum laciniatum, Aconitum spicatum, Bergenia ciliata)* vulnerable (*Neopicrorhiza scrophularifolia Swertia chirayita)* inadequate knowldege (*Maharanga* emodi) and endangered *(Michelia champaca)* Similarly there was one species under CITES Appendix III, *Taxus baccata.* Six species were protected by the Government of Nepal by its Forest Regulation, they were: *Abies spectabilis, Dactylorhiza hatagirea, Micheia champaca, Parmelia* sp., *Swertia chirayita* and *Taxus baccata.*

Conclusion

Only about two per cent of Khumbu, approximately 2,200 ha is forested, but for Sherpas this small area is a critical component of their homeland and way of life. The forests are, therefore, very important and so scare for Sherpa community. The shrub land comprises high valued medicinal herbs. Out of 30 prioritized medicinal plants for bioprospecting and research by the Government of Nepal, nearly half are recorded from the present study in Khumbu. What strikingly lacking is the total study of such important plants, their distribution, habitat status, detailed study of morphological and growth pattern, and extraction of bioactive compounds for commercial use. Moreover, it is high time that such studies including molecular characterization of such high valued plants are conducted and safeguard its intellectual property right.

Acknowledgements

We grateful to the local people who voluntarily provided us with valuable information on the use of various plants which they have amassed so carefully for years. This study was carried out with support fund of Ev-K2-CNR, Italy.

Antimicrobial Activity of Some High Altitude Mushrooms of Sagarmatha National Park and Its Buffer Zone

Nabin Bhattarai, Rishi Baniya, Anjana Giri and Prabina Rana

Abstract

Antimicrobial properties of ethanol extracts of four wild mushrooms *(Gomphus clavatus, Tylopilus eximus, Ramaria flava* and *Boletus pulverulentus)* collected from Sagarmatha National Park, Khumbu region were investigated against pathogens. The mushroom extracts showed antibacterial activity against six pathogenic bacteria. *Bacillus subtilis* was susceptible towards all the mushroom extracts. *Tylopilus eximus* was active against *Bacillus subtilis, Escherichia coil, Salmonella paratyphi and Acinetobacter* sp. *Ramaria flava* was active against *Bacillus subtilis* and *Salmonella paratyphi. Boletus pulverulentus* was active against *Escherichia coli, Bacillus subtilis, Staphyloccus aureus* and *Shigella dysenteriae* F44 DH. *Gomphus clavatus* was active against *Escherichia coli, Bacillus subtilis* and *Salmonella paratyphi. Candida albicans* was not inhibited by the mushroom extracts.

Introduction

Mushrooms have long been used as a valuable food source and as traditional medicines around the world since ancient times (Akyuz and Kirbag 2009). It is believed that mushrooms need antibacterial and antifungal compounds to survive in their natural habitat. Both the fruiting body and the mycelium of mushrooms contain compounds with wide ranging antimicrobial activity. They are rich sources of natural antibiotics, where the cell wall glucans are well known for their immunomodulatory properties and many of the externalized secondary metabolites combat bacteria, fungi and viruses (Benedict and Brady 1972, Suzuki *et al.* 1990, Collins *et al.* 1997). Researchers showed antimicrobial activity of several mushrooms (Gezer *et al.* 2006; Mercan *et al.* 2006; and Turkoglu *et al.* 2007). Extracts from fruiting bodies and the mycelia of various mushrooms have been reported for antimicrobial activity against wide range of infectious bacteria (Hirasawa *et al.* 1999; Dulger *et al.* 2002). Shrestha *et al.* (2005) studied the antagonistic behaviour of ectomycorrhizal fungi, isolated from Baluwa forest of Kavre district of Nepal (Central Nepal), against pathogenic fungi and bacteria. G.C. *et al.* (2004) studied the microbial control of white grubs with entomopathogenic fungi and its value in integrated pest management (IPM) in Syangja and Prabat districts of Nepal.

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation led scientist to search for new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents (Karaman *et al.* 2003). Among the sources of bioactive metabolite less intensively studied organisms like higher fungi seem highly promising as new source of useful bioactive compounds. Giri and Rana (2008) reported 26 wild edible mushrooms consumed by the local people of Sagarmatha National Park Khumbu region. The aim of study was to investigate the antimicrobial potential of some locally consumed wild edible mushrooms of Khumbu region.

Study area

The study area lies in the Northeastern Region of Nepal. It encompasses the Sagarmatha National Park (1418 sq. Km.) and its Buffer Zone area. The park includes the upper catchment areas of the Dudh Kosi and the Bhote Kosi rivers. Due to its altitudinal diversity, various types of vegetation are found in different climatic zones. The park is largely composed of the rugged terrain and gorges of the Himalayas ranging from 2845 m at Monju to the top of the world Sagarmatha (Mount Everest) at 8848 m above sea level.

Material and Methods

Fruiting bodies

Fruiting bodies of four wild mushrooms (Table 1) coonsumed by the locals (Giri and Rana 2008) were collected from Sagarmatha National Park and its Buffer Zone in August and September in

2008. The mushroom specimens were identified with the help of standard literatures (Adhikari 2000, Imazaki and Hongo 1979, Svrech 1983). The fruiting bodies were cut into small pieces, shade dried and ground to fine powder.

Table 1. List of Mushrooms Collected from Sagarmatha National Park and its Buffer Zone

S.N.	Mushroom	Local name	Collection site	Altitude m asl
1	*Boletus pulverulentus* Opat	Kalo martip	Lukla	2783
2	*Gomphus clavatus* (Pers. ex Fr.) S. F. Gray	Ee-shyamo	Phurte	3619
3	*Ramaria flava* (Sch. : Fr.) Quel.	Cheshyamo	Phurte	3619
4	*Tylopilus eximus* (Peck) Sing.	Kyati	Khumjung/ Khunde	3825

Test organisms

Four mushroom extracts were assayed for antibacterial activity against 14 pathogenic bacteria and 1 yeast isolate obtained from National Institute of Science and Technology (NIST) and Tribhuvan University Teaching Hospital (TUTH). Bacteria used were *Bacillus subtilis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Acinotobacter* spp, *Enterococcus faecalis, Staphylococcus aureus, Kleibsella pneumoniae, Citrobacter freundii, Salmonella paratyphi, S. typhi, Shigella* spp., *Shigella dysenteriae* F44 DH and *Shigella dysenteriae*. Yeast used was *Candida albicans*.

Extract Preparation

Powder of dried samples of fruiting body (15gm each) of four mushrooms were separately extracted with 100 mL ethanol using soxhlet apparatus. The residual solvent was removed by evaporation at 40°C for 24 h in vacuum using a rotatory evaporator. The resulting organic extracts were further reconstituted with ethanol (1000 mg/mL) followed by storage in sterile capped bottle under refrigeration condition (4°C) prior to use for subsequent assays.

Antimicrobial activity

Antimicrobial activities of aqueous ethanolic extracts of mushrooms were determined by agar well diffusion method (Prescott *et al.* 2002). Pure isolate of each bacterium was cultured in Nutrient broth and *Candida albicans* was incubated in Yeast Extract Dextrose (YED) broth. The culture suspension were prepared and adjusted by comparing against 0.5 Mc Farland turbidity standard tubes and it was seeded on Mueller Hinton Agar (MHA) plates and Yeast Extract Dextrose (YED) agar plates respectively and left for few minutes at room temperature. Then wells were made on inoculated plate with 5 mm sterile cork borer and 50µL solutions were loaded in these wells. It was left for 30 minutes for diffusion. Then the plates were incubated at 37°C for 24-48 hours for bacteria and 28°C for 48 hours for yeast. A control well was made on each plate by applying ethanol for organic solvent extract (Shrestha and Piya 2002; Shrestha *et al.* 2005). The antimicrobial activity was determined by measuring the diameter (cm) of clear inhibition zone around each well.

Results and Discussion

The ethanol extract of all four mushrooms used for this investigation possessed varying degrees of antibacterial activity against six bacterial pathogens. Figure 1 and 2 show the zone of inhibition of mushroom extracts against bacterial strains. *Bacillus subtilis* was found susceptible towards all mushroom extracts. The extract obtained from the *Tylopilus eximus* was active against *Bacillus subtilis, Escheria coli, Salmonella paratyphi* and *Acinetobacter* spp. The extract of *Ramaria flava* inhibited the growth of *Bacillus subtilis* and *Salmonella paratyphi*. The extract obtained from *Boletus pulverulentus* inhibited the growth of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Shigella dysenteriae* F44 DH. The extract obtained from *Gomphus clavatus* inhibited the growth of *Escheria coli, Bacillus subtilis* and *Salmonella paratyphi*. The extract obtained from the bitter mushroom *Tylopilus eximus* showed higher inhibition zone towards the *Bacillus subtilis* in comparison to the other mushroom extracts. The mushroom extracts did not exhibit anticandidal activity towards *Candida albicans*. It was observed that the control (absolute ethanol) had no inhibitory activity against any of the microorganisms used.

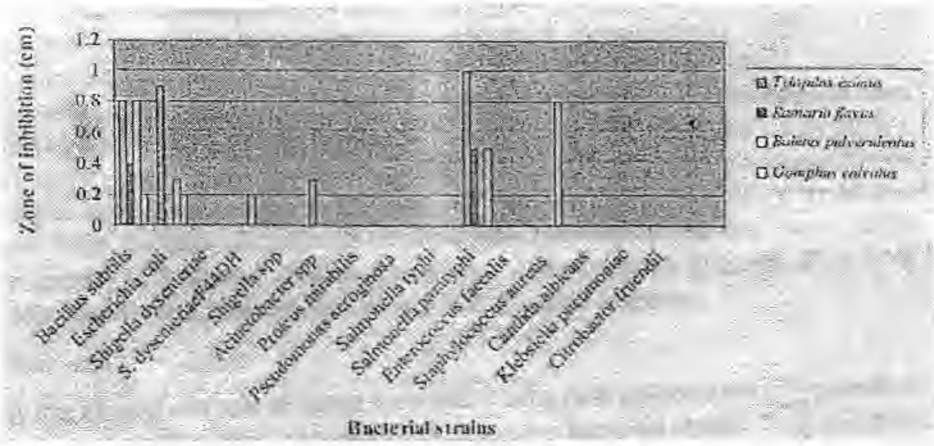

Fig. 1. Antimicrobial activity of ethanolic extracts of four edible mushrooms

Salmonella sp. causes typhoid and paratyphoid fever on mammals. *Staphylococcus aureus* causes wound infection i.e. post operative, food poisoning, boils, abscesses, endocarditis, and toxic shock syndrome diseases on human and mastitis on cattle. *Escherichia coli* cause gastroenteritis and urinary tract infections (UTI). *Shigella* spp. causes bacillary dysentery. *Acinetobacter* spp. causes neonatal septicemia. *Bacillus subtilis* causes post operative cellulitis, septicemia, respiratory disease, endocarditis, pneumonia, etc. (Sneath *et al.* 1986). Mushrooms are a vital source of medicinal compounds that may be used to cure different disorders and prevent pathogenic microorganisms. The result shows that the above mentioned bacterial strains were inhibited by mushroom's extracts and could be possibly used to control the disease caused by these bacterial organisms. The intake of these edible mushrooms by the locals could provide a natural covering of antibiotics to fight against pathogenic bacteria.

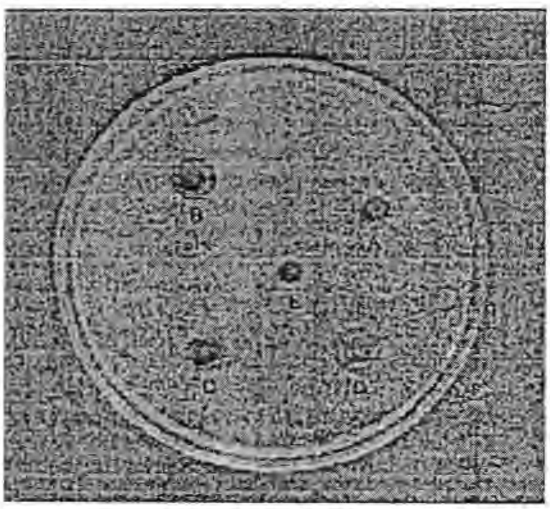

Fig. 2. Zone of inhibition shown by ethanol extracts of A. *Gomphus clavatus*, B. *Boletus pulverulentus*, C. *Ramaria flava*, D. *Tylopilus eximus*, E. Control (ethanol) against *Bacillus subtilis*

All mushroom extracts showed antibacterial activity towards the gram positive bacteria *(Bacillus subtilis* and *S. aureus)* and gram negative bacteria *(E. coli, Salmonella paratyphi, Acinetobacter* spp. and *Shigella dysenteriae* F44 DH). Opige *et al.* (2006) reported that the extracts obtained from methanol inhibited the gram negative bacteria *Pseudomonas aeruginosa* and the extracts obtained from the petroleum ether inhibited the gram positive bacteria *S. aureus* but *E. coli* bacteria were resistant towards these extracts. Gram positive bacterial strains have been inhibited by mushroom extracts which is analogues to the work of Imitiaj and Lee (2007). They found that the gram positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* were sensitive towards the Korean wild mushrooms. Fagade and Oylade (2009) found that among twelve fungi, the ethanolic extracts obtained from *Pleurotus florida* and *Panus fulvus* inhibited the growth of *S. aureus, Streptococcus* spp., *Streptococcus pyogens, Escherichia coli, Klebsiella pneumoniae, Flavobacterium* spp. and *Candida albicans.*

Conclusion

In this study the ethanol extracts obtained from wild mushrooms collected from Sagarmatha region showed antibacterial activity towards six pathogenic bacterial strains. They could be promising antibacterial agents. With an increasing number of bacteria developing resistance to commercial antibiotics, extracts and derivatives from mushrooms hold a greater promise as new source of antibacterial agents. Therefore, wild edible mushrooms consumed by the indigenous people could be a vital source for antimicrobial agents so they should be explored for their antimicrobial potential. Further in depth analysis of the bioactive compounds should be carried out. Studies on antifungal properties should be explored.

Acknowledgements

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