ANTAGONISTIC STUDY OF ECTOMYCORRHIZAL FUNGI ISOLATED FROM BALUWA FOREST (CENTRAL NEPAL) AGAINST WITH PATHOIGENIC FUNGI AND BACTERIA

G. Shrestha Vaidya*, **K. Shrestha**** and **H. Wallander*****
*Royal Nepal Academy of Science and Technology, Kathmandu, Nepal
**Natural History Museum, Swayambhu, Kathmandu, Nepal
***Microbial Ecology Lab, Lund University, Sweden

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 bacteria *Agrobacterium tunifaciens*, *Escherichia coli*, *Pseudomonas solanacerum*, *Pseudomonas aeruginosa* and *Shigella dysentriaie 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*, *Kleibsiella* sp. *Staphylococcus aureus*, *Shiegella dysenteria* and , *Escherichia coli* but no activity was found against A*spergillus 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.

Key words: Antagonistic; Ectomycorrhizae; Metabolites; Pathogenic fungi and bacteria; *Pisolithus* sp. and *Scleroderma* sp.

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 forest lies in Kavre district and was totally eroded before and covering with red soil. Its altitude ranges from 980 – 1020m. But Fodder department of Nepal Agriculture center, 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 micro-organisms. 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 tunifciens, Bacillus* sp., *Escherichi coli*, *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 incresed nutrient uptake and exposing a greater absorbing surface. It makes plant drought and frost resistant and protects root ends from attack by *paqrasite* fungi.

METHODOLOGY

Collection and Identification

Sexual spores were collected from Baluwa forest of Kavre district (Fig. 2). They were identified according to Adhikari

Author for Correspondence: G. Shrestha Vaidya, Royal Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal. GPO Box: 3323. E-mail: geeta_shrestha1@yahoo.com

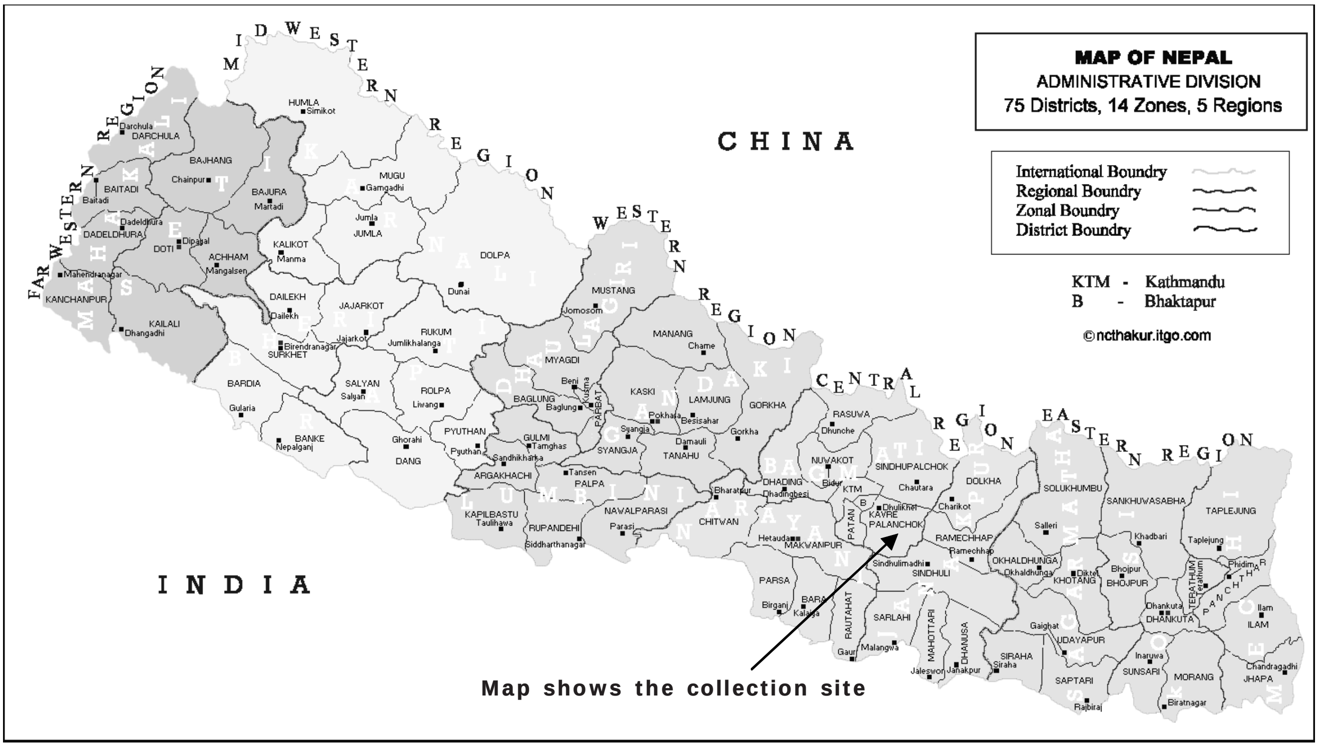


Fig.1: Baluwa forest (Kavre district) showing the sampling site.

(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) , Maharajgung, Nepal.Isolation of ectomycorrhizal fungi from collected sexual spores were done according to Shrestha (1999) and selection criteria was done according to Shrestha (1999).

ANTAGONISTIC ACTION STUDY

A. Direct cross inoculation

Ectomycorrhizal fungi were isolated from *Pisolithus* sp. and *Scleroderma* sp.and were incubated at 27^{0}C (Shrestha 1999). 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 in 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 27^0 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 37.c for 18-24 hrs. After incubation then 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^{0}C for 1-2 weeks. After incubation then observed for growth of inhibition.

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

tomycorrhizal fungi	*Pythium* sp.	*Rhizoctonia* sp.	. *Fusarium* udum	*Fusarium oxysporum*	*Geotrichum* sp.
Pisolithus sp.	+ + +	+ + +	+ + +	+ +	+ +
Scl*eroderma* sp.	+ + +	+ + +	+ +	+ + +	+

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

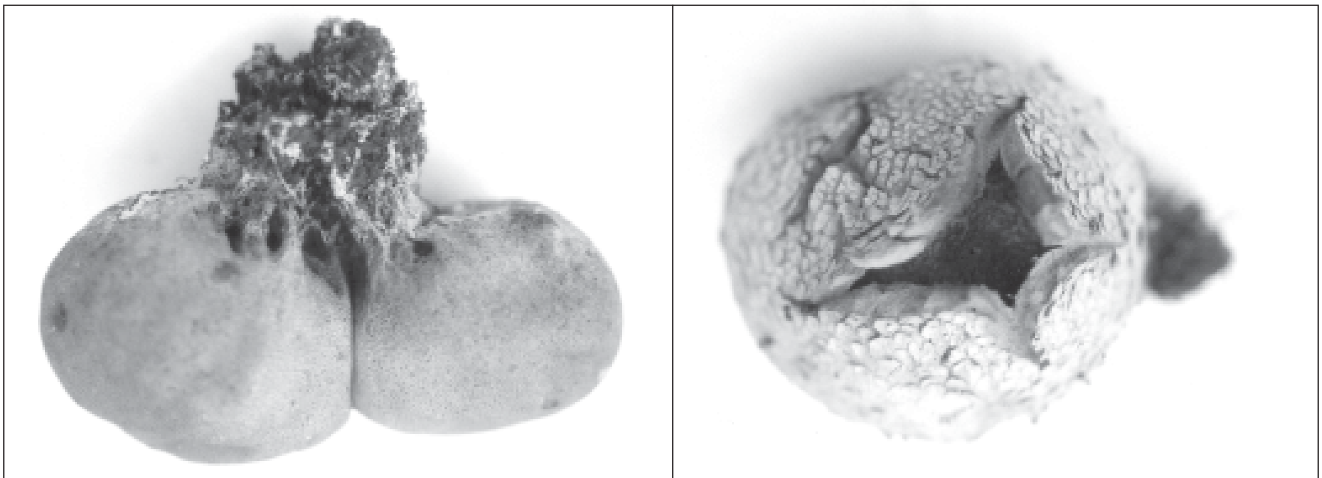
Table 2: Antifungal activity of metabolites

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

RESULTS AND DISCUSSION

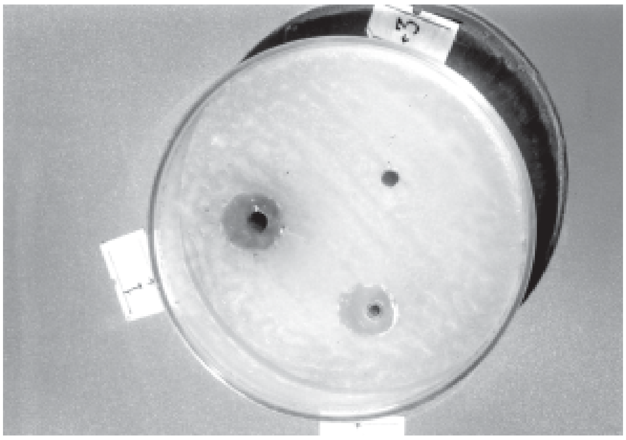
The cross inoculation method has shown that *Pisolithus* sp. was highly active against *Pythium* sp., *Rhizoctonia* sp., *Fusarium udum* and moderately active against Fusarium *oxysporum* and *Geotrichum* sp. (Table 1). *Scleroderma* sp. was highly active against *Pythium* sp., *Rhizoctonia* sp., *Fusarium oxysporum* moderately active against *Fusarium udum* and low active against *Geotrichum* sp.(Table1).

The metabolites of *Pisolithus* sp. in concentration 10 mg./ml show higher zone of inhibition *Rhizoctonia* sp., *Fusarium udum,* Pythium *sp.and slightly decreaces in Fusarium oxysporum* and *Geotrichum* sp. and *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 decreases in Fusarium oxysporum* and *Geotrichum* sp but *A.niger, A.ustus,* and *A.granulosis* did not show zone of inhibition (Table 2).



Pisolithus sp. *Scleroderma* sp.

Fig. 2



Inhibition shown by extract of 1. *Pisolithus* sp. 2. *Scleroderma* sp. 3. Control against *E.coli*

Fig. 3

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).

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.coli*, *Agrobacterium tunifaciens, Pseudomonas aeruginosa* lowest i *Staphylococcus aureus* and *Shigella dysenteriae in agar well difusion method.*(Fig. 5).

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

Due to inhibitory effect of these ectomycorrhizal fungi

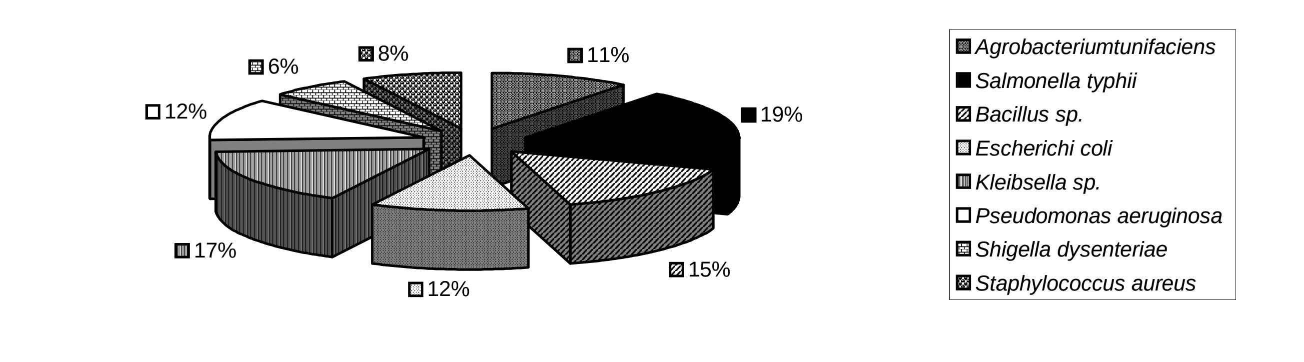


Fig. 4: Pie chart shows the percentage of inhibition shown by *Scleroderma* sp. against with pathogenic bacteria.

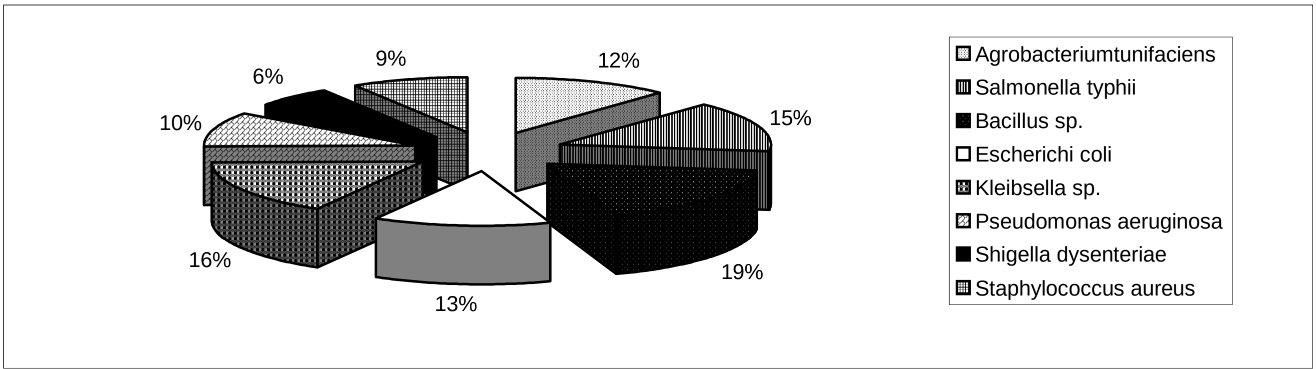


Fig. 5: Pie chart shows the percentage of inhibition shown by *Pisolithus* sp. against with pathogenic bacteria.

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.

REFERENCES

Adhikari M.K. 1999. Mushrooms of Nepal.

Bakshi B.K and D.Kumar 1968. For tree mycorrhiza. Indian Forester 94 pp.80 – 84.

Brundrett M.N. Bougher, B.Dell, T.Grove and N.Malajzuk. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR monograph 32.374+xP. Pirie Printers, Canberra, Austrailia.

Buscot F.G. Weber and F. Oberwinkler 1992. Interaction between *Cylindrocarpon destructions* and ectomycorrhizas of *Piceas abies* with *Laccaria lacata* and *Paxillus involutus*. Trees 6:83-90.

Duchesne L.C., R.L Peterson and B.E .Ellis 1988. Interaction between the ectomycorrhizal fungus *Paxillus involutus* and *Pinus resinosa* induces resistance to *Fusarium oxysporum*. Can. J. Bot. 66:558-562.

Marx D.H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infection. Phytopathology, 59:153-163, 411-417, 558-565.

Perrin.R. and J.Garbaye. 1983. Influence of ectomycorrhizae on infectivity of *Pythium* infested soils and substraites. Plant soil 71:345-351.

Shrestha G. 1999. The role of ectomycorrhizae in the forestry Development in Nepal. In proc. International workshop BIO-REFOR, Nepal 28 Nov-2 Dec 1999, pp 178-182.

Shrestha G. 1999. Ectomycorrhizal effect of mushrooms in Pine forest of Nepal. In Proc. Of III National Conference on Science and Technology 8-11 March 1999, pp 1272-1275. RONAST, Kathmandu.

◆◆◆