INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences

Research Article…!!!

Received; accepted

ISOLATION AND CHARACTERIZATION OF FUNGAL SPECIES FROM THE RHIZSPHERE OF *JUGLANS REGIA* L. OF KASHMIR VALLEY

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Keywords:
Rhizosphere, walnut tree, fungi, culturing, dominant species, genus, soils

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ABSTRACT

The fungal diversity scaling relationships relative to that of plants is important to understand ecosystem functioning. We marked 26 isolates of fungi from different sites. The genus *Aspergillus* had been found to be the most dominant fungus at generic level, followed by *Penicillium* and *Cladosporium*, *A.niger*, *Mucor himalayensis* and *Penicillium notatum* were the most dominant fungi at species level as they have been found at every site and in different seasons. Most of the fungi were present during all the seasons at different sites, *A. terricola*, *Trichophyton terrestre* and the black fungus *Capnobotryella renispora* were found during winter only. *Capnobotryella renispora* was the rare fungus isolated in the winter from one site only. The fungal diversity tended to be more during rainy season, followed by summer and winter. The most frequent species were *A. niger*, *Mucor himalayensis*, *Penicillium notatum* and most rare species were *Capnobotryella renispora*, *A. terricola* and *Trichoderma odoratum*.

INTRODUCTION

Soil is a rich habitat for the growth of microorganisms than other microbial habitats. Among these microorganisms, fungi are one of the dominant groups present in the soil. Fungi live, multiply and die or disintegrate in the soil and thus they provide rich organic matter, which could be recycled as plant nutrition. Thus developed humus complex is a natural fertilizer mixed with soil and plays a very important role in the composition of soil. The rhizosphere is the soil environment directly under the influence of living roots [1]. There is a close link between plant species and microbial community structure in the rhizosphere, and the hypothesis is that bacteria and fungi have also developed a unique diversity pattern over time, which may give insights into microbial evolution. The soil microorganisms colonizing the rhizosphere assist plants in the uptake of several vital nutrients from the soil, such as phosphorus, potassium and nitrogen[2]. Microbial communities are supported by nutrients released by root exudates, mucilage, and sloughed-off root cells [3]. In undisturbed soils, most of the nutrient cycling, roots, and biological activity are found in the top 20 to 30 cm, called the rooting zone. The rhizosphere is characterized as a zone of intense microbial activity, and represents a close relationship between the plant, soil and soil organisms [4]. Very little information is available regarding biodiversity and taxoecology of soil fungi of Baramulla district of Kashmir. Hence the present investigation was undertaken for three years (2007, 2008 and 2009).

MATERIALS AND METHODS

Study area

The facts that J & K state is predominant in alluvial soils have high organic matter content than soils of low altitude due to the low temperature and heavy rainfall which restricts the microbial population [5].All the five experimental plants of *Juglans regia L.* were of the same age but differed only in the growth patterns, preferred soil types and microclimatic conditions. The selection of experimental plants was based on growth characteristics (vigorous growth, medium and poor growth) and microclimatic variations.

To study the fungal community, soil samples were collected from a depth of approximately 10 cm around the rhizospheres of five experimental plants of *Juglans regia L.* of Baramulla district (Kashmir) during winter, rainy seasons and summer. Samples were placed into cold-based sterile bags and transported to the laboratory. Samples from this soil were used for

plating culturable fungi on three media viz., Potato Dextrose Agar (PDA), Malt Agar Medium (MAM) and Czapek-Dox Agar Medium.

Description of the five experimental plants

1. Plant 'A' and 'B' belong to site 1. They vary in growth patterns as plant 'A' was vigorously growing and plant 'B' was relatively moderate in growth. The soil of the site was alluvial type.

2. Plant 'C' and 'D' belong to site 2. They also differ in growth patterns from each other as plant 'C' showed vigorous growth than plant 'D'. The soil of the site was sandy in texture.

3. Plant 'E' belong to the site 3 which differed from rest of the plants as it grows on slightly hilly area and the soil was laterite type.

Soil inoculation and incubation

The soil was air dried and sieved, and 1 gram of the soil was taken from each sample and was serially diluted up to 10^{-6}, after completion of serial dilution 0.2 ml soil solution from each test tube was inoculated on three types of petri plates containing Potato Dextrose Agar (PDA) medium, Malt Agar Medium and Czapek-Dox Agar Medium (CAM), (Chloramphenicol was added to the media as 250mg/100ml to check the growth of bacteria). Inoculated petri plates were kept in incubator (adjusted at a temperature of 25^0C) for a period of 3-5 days for culturing. Subculturing was done in order to get the pure colonies. Pure cultures were periodically transferred onto fresh medium to allow continuous growth and viability of fungi.

Isolation and Identification of fungi

Fungi were isolated as mono-cultures on three media: potato dextrose agar (PDA, Hi Media), malt-extract agar (Hi Media) and Czapek's agar (Hi Media). Preliminary identification of the isolated fungi was done under the Research microscope (Olympus; CH 20i) on the morphological basis and growth patterns [6,7,8,9,10,11].

RESULTS AND DISCUSSION

Soil is a rich habitat for the growth of microorganisms than other microbial habitats. Among these microorganisms, fungi are one of the dominant groups present in the soil. Fungi live, multiply and die or disintegrate in the soil and thus they provide rich organic matter, which

could be recycled as plant nutrition. Thus developed humus complex is a natural fertilizer mixed with soil and plays a very important role in the composition of soil.

There were 26 isolates of fungi isolated from different sites. The genus *Aspergillus* had been found to be the most dominant fungus at generic level, followed by *Penicillium* and *Cladosporium*. *A. niger*. *Mucor himalayensis and Penicillium notatum* were the most dominant fungi at species level as they have been found at every site and in different seasons. Though most of the fungi were present during all the seasons at different sites, *A. terricola*, *Trichophyton terrestre* and the black fungus *Capnobotryella renispora* were found during winter only. *Capnobotryella renispora* was the rare fungus isolated in the winter from one site only.

There are 26 fungal species that had been isolated from the rhizosphere of walnut tree in different seasons. The fungal diversity tended to be more during rainy season, followed by summer and winter in that order. Among most frequent species were *A. niger, Mucor himalayensis, Penicillium notatum.* Among most rare species were *Capnobotryella renispora, A. terricola* and *Trichoderma odoratum*.

Earlier Chaudhary and Sachar (1934)[12], Saksena (1955)[13], Miller et al., (1957)[14], Saksena and Sarbhoy (1964)[15] studied seasonal variation in forest soil fungi and pointed out seasonal changes in soil mycoflora and fungal population, which drastically differ from season to season in a particular soil. Tresner et al., (1954)[16], Mishra (1966)[17], Rama Rao (1969)[18], Persiani et al., (1998)[19] and many others also observed seasonal variations in forest soil mycoflora. Dwivedi (1966)[20] and Dkhar and Mishra (1987)[21] discussed seasonal variations of fungal population in some soil types and concluded that the changes in soil, organic contents, water holding capacity, temperature and pH of respective season were the probable factors associated with fungal population. Similar results are obtained in the present investigation where some of the species appeared only sporadically after distinct seasonal interruption while other species were predominant in all seasons. These species were constant in their occurrence throughout the year and repeatedly isolated in all the seasons. Many species therefore maintain seasonal rhythm in their occurrence and are treated under all seasonal species or constant species. Though several species were found to maintain their occurrence in all seasons, some species were also restricted in their occurrence in specific season because of suitable condition.

Among all the isolated species, *Aspergillus* was the most dominant genus and repeatedly isolated from the rhizosphere of all the experimental plants in all the seasons. It occurs on all sorts of organic debris. These observations in the present investigation are similar to Gangawane and Deshpande (1972)[22], Reddy et al., (1987)[23], Saksena et al., (1967)[24], Manoharachary et al., (1989)[25], etc. who have reported that *Aspergillus* occur more frequently than *Penicillium* genus in the soils.

An exhaustive work has been done on the taxonomy of forest soil fungi in India. Saksena (1955)[26], Bakshi and Singh (1956)[27] , Shrivastava and Bhargava (1966)[28], Deshpande and Deshpande (1966)[29], Kamal and Bhargava (1972,1973)[30,31], Manoharachary (1977)[32], Madhusudan Rao and Manoharachary (1981)[33], Manoharachary et al., (1989)[34], Venugopal Rao et al., (1984)[35], Mohanty and Panda (1994)[36] etc. have studied the soil fungi of different forests.

Table: Seasonal variation of fungi in the rhizosphere of five experimental plants of *Juglans regia L*. in three different seasons.

S NO.	FUNGAL STRAINS	WINTER A B C D E	RAINY A B C D E	SUMMER A B C D E
1	*Aspergillus. Niger*	+ + + + +	+ + + + +	+ + + + +
2	*A. fumigates*	- - + - +	+ + + + +	+ + + + +
3	*A. terreus*	+ + - - +	+++++	+ + - - +
4	*A. terricola*	+ + - - -	- - - - -	- - - - -
5	*A. nidulans*	+ + - - -	+ + - - -	+ + - + -
6	*A. versicolor*	- - + - -	+ + - - -	+ - - - -
7	*Capnobotryella renispora*	- + - - -	- - - - -	- - - - -
8	*Cladiophialophora caronii*	+ - - - +	+ - - - +	+ - - - +
9	*Cladosporium spherospermum*	+ - + - +	+ - + - -	- + - - +
10	*C. cladosporoides*	+ + - - -	+ + + - -	+ + + - -
11	*Curvularia lunata*	- - + - -	+ + + - -	+ + + - -

12	*Curvularia spp.*	+ + + - -	+ + - - -	+ + - - -
13	*Exophila spp.*	+ + - - -	+ + - - -	+ + + - -
14	*Fusarium oxysporum*	- + - + -	- - + + -	- - + + -
15	*F. solani*	- - + + -	-- + + +	- - + + -
16	*Hyaline spp.*	- - + + -	- - + + -	- - + + -
17	*Mucor himalayansis*	+ + + + +	+ + + + +	+ ++ + +
18	*Oospora virabilis*	- - - + +	+ - - + +	+ - - + -
19	*Penicillium aurantiogriseum*	+ + - - -	+ + - + -	+ + - + -
20	*P. camembertii*	+ + + + -	+ + + + +	+ + + + +
21	*P. notatum*	+ + + + +	+ + + + +	+ + + + +
22	*Phoma spp.*	+ + - - -	+ + - - -	+ + - - -
23	*Trichophyton terrestre*	+ + + - -	- - - - -	- - - - -
24	*Trichoderma harzianum*	- - + - -	- - + + +	- - + + -
25	*T. koningii*	+ - + - -	+ - + + +	+ - + + -
26	*T. odoratum*	+ - - - -	+ + - - -	+ + - - -

+ =Present; - = Absent

CONCLUSION

This is the first report of fungal diversity in the rhizosphere of the walnut tree which seems to be almost similar as in the case of rhizosphere of other plants. The most frequently species were of the genus *Aspergillus* which were observed in all the three experimental seasons. The species *Trichophyton terrestre* which is rarely present in Indian soils have been isolated during the winter season only. The most important finding of the study is the isolation of black fungus *Capnobotryella renispora.* This fungus was earlier reported from granite column of Greek island the Delos. It is very slow growing and loses viability if not subcultured in 10-15 days.

REFERENCES:

1. Kent, A. D. and E. W. Triplett (2002). Microbial community and their interactions in soil and rhizosphere ecosystems. *Annu. Rev. Microbiol.* 56: 211-236.

2. Trolove, S. N., M. J. Hedley, G. J. D.Kirk, N. S. Bolan and P. Loganathan. (2003). Progress in selected areas of rhizosphere on P. acquisition Aust. J. *Soil Res.* 41: 471-499.

3. Hutch, B. W., Augustine, J., & Embrace, W. (2002). Plant rhizodeposition- an important source for carbon turn over in soil. *J plant Nutr. Soil Sci.* 165: 397-407.

4. Clapperton, M. J. (1997). Weeds, the rhizosphere and the underground story. In: weeds as Teachers, S.K. Hilander (Ed.), AERO, Helena, Montana, USA.

5. Lahiri, T. and Chakravarti,S.K.(1989). Characterictics of some soil of Sikkim at various altitudes. *Journal of Indian Society of Soil Science* 37: 451- 454.

6. Raper, K. B., Fennel, D. I. (1977). *The Genus Aspergillus*. New York: Robert Erieger Publishing Company Huntimgton. 685 p.

7. Domsch, K. H., Gans, W., Anderson, T. H. (1980). Compendium of Soil Fungi. London, New York, Torroute, Sydney, San Francisco: Academic Press. 869 p.

8. Samson, R. A., Hoekstra E. S., Ftrisvald O. (2000). Introduction to Food-and Airborne Fungi. Ultrecht: Centraalbureau Voor Schimmelcultures.383 p.

9. Pitt, J. I. (1979). *The Genus Penicillium*. London, New York, Toronto Sydney, San Francisco: Academic Press. 635 p.

10. Carmichael, J. W., Kendrick W. B., Conners I. I., Sigler L. (1980). Genera of Hyphomycetes. Edmonton, Alberta, Canada: The University of Alberta Press. 386 p.

11. Kiffer E., Morelet M (2000). The Deuteromycetes. Mitosporic Fungi. Classification and Generic Keys. U.S.A: *Science Publishers Inc.* 273 p.

12. Chaudhary, H. and G. S. Sachar (1934). A study of fungus flora of Punjab soils. *Annales of Mycology* 32: 90-110.

13. Saksena, S. B. (1955). Ecological factors governing the distribution of soil microfungi in some forest soils of Sagar. *Journal of Indian Botanical Society* 34: 262-298.

14. Miller, J. H., J. E., Giddins and A. A. Foster (1957). A survey of fungi of forest and cultivated soils of Georgia. *Mycologia.* 49: 779-808.

15. Saksena, R.K. and A.K. Sarbhoy (1964). Ecology of the soil fungi of Uttar Pradesh-I. *Proceedings of National Institute of Science India* 29B: 207-224.

16. Tresner, H. D., M. P. Bakus and J. T. Curtis (1954). Soil microfungi in relation to hardwood forest continuum in southern Wiskinson. *Mycologia* 46: 314-333.

17. Mishra, R. R. (1966). Some members of Mucoraces isolated from soils of Gorakhpu/Shorea forests. *Proceedings of Indian Science Congress Association* 53(3): 235.

18. Rama Rao, P. (1969). Studies on soil fungi-III, seasonal variation and distribution of microfungi in some soils of Andhra Pradesh. *Transactions of British Mycological Society* 52(2): 277-298.

19. Persiani, A. G., M. A. Maggi and F. D. C. Pineda (1998). Diversity and variability in soil fungi from a distributed tropical rain forest. *Mycologia* 90 (2): 206-214.

20. Dwivedi, R. S. (1966). Ecology of soil fungi of some grasslands of Varanasi-II, Distribution of soil mycoflora. *Bulletin of International Society of Tropical Ecology* 7: 84-99.

21. Dkhar, M. S. and R. R. Mishra (1987). Microbial population, fungal biomass and CO2 evolution in maize (*Zea mays*) field soils. *Plant and soil* 99: 277-283.

22. Gangawane, L.V. and K.B. Deshpande (1972). Microbial ecology in some forest soils of Marathwada-I, Rhizosphere microfungi of some common trees of Ajanta hills. *Marathwada University Journal of Science B* 11: 1-4.

23. Reddy V.N., P. Prakash and C. Manoharachary (1987). Fungistasis in forest soil fungi using the dominant soil fungi. *Biome* 2(1): 126-128.

24. Saksena, R. K., Krishna N and A. K. Sarbhoy (1967). Ecology of the soil fungi of Uttar Pradesh-II, soils of the Himalayan forests and their microfungi. *Proceedings of National Institute of Science India* 33B (3-4): 144-153.

25. Manoharachary, C., V.R.T. Reddy and U.Prasad (1989). Population studies and phenology of fungi in scrub jungle forest soils of Andhra Pradesh, India. *Geophytology* 19(2): 131-139.

26. Saksena, S.B. (1955). Ecological factors governing the distribution of soil microfungi in some forest soils of Sagar. *Journal of Indian Botanical Society* 34: 262-298.

27. Bakshi, B.K. and S. Singh (1956). Wilt disease of shisham (*Dalgergia sisoo Roxb*): Studies on soil fungi (excluding *Aspergillus* and *Penicillium*) isolated from shisham forests *Indian Phytopathology* 9 (2): 114-123.

28. Srivastava, S. and K.S. Bhargava (1966). Fungi isolated from Eugenia forest soil of Gorakhpur. *Proceedings of Indian Society Congress Association* 53: 441.

29. Deshpande, K.B. and K.S. Deshpande (1966). Studies on soil fungi of Marathwada (MS), India. *Proceedings of Indian Science Congress Association Chandigrah,* 53: 223.

30. Kamal and K.S. Bhargava (1972). Studies on soil fungi from Teak forest of Gorakhpur, II. A contribution of Indian *Aspergilli. Mycopathologia Mycological Applicata* 119: 240-259.

31. Kamal and K.S. Bhargava (1973). Studies on fungi from Teak forests of Gorakhpur. Edaphic factors and distribution of soil microfungi in teak stands of different ages. *Proceedings of National Academy of science* 43(B): 9-1

32. Manoharachary, C. (1977). Microbial ecology of scrub jungle and dry wasteland soils from Hyderabad district. Andhra Pradesh (India). *Proceedings of Indian National Science Academy* 43: 6-18.

33. Madhusudan Rao, M. and C. Manoharachary (1981). Studies on the myco-ecology of two pond muds and forest soils of Mannaur Forest, Andhra Pradesh. *Indian Journal of Botany* 4(2): 137-148.

34. Manoharachary, C., V.R.T. Reddy and U.Prasad (1989). Population studies and phenology of fungi in scrub jungle forest soils of Andhra Pradesh, India. *Geophytology* 19(2): 131-139.

35. Venugopal Rao, K., N.K. Rao and C. Manoharachary (1984). Thermophilic fungi from forest soils of Andhra Pradesh. *Indian Botanical Report* 3(2): 158.

36. Mohanty, R.B. and T. Panda (1994). Ecological studies of the soil microfungi in a tropical forest of South Orissa in relation to deforestation and cultivation. *Journal of Indian Botanical Society* 73: 213.