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STUDIES ON THE RHIZOSPHERE MYCOFLORA OF MUSTARD

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ABSTRACT
Rhizospheric soil sample of mustard (*Brassica* sp.) was collected from the cultivated land area near Gurukul Kangri University, Haridwar. It was reported that *Trichodema* and *Aspergillus* were dominant in mustard rhizosphere. During the growth rate measurement (colony diameter method and dry mycelial weight method) of the isolates, it was observed that *Trichoderma* grew at a faster rate whereas *Aspergillus* grew at much lower rate. During the study of effect of temperature on growth of the isolates, it was observed that all the isolates were able to grow at temperature ranges (15º, 25º and 35ºc). Hence they are mesophiles. While studying the effect of pH on growth of the isolates, it was observed that *Trichoderma* is acidophile whereas *Aspergillus* is alkalophile. *Trichoderma* and *Aspergillus* has potential to solubilize phosphate. Antifungal sensitivity was determined by Poisoned Food Technique and it was found that nystatin is most effective among all antifungal drugs.

KEYWORDS: Fungi, Rhizosphere and Mustard.

INTRODUCTION

(Lorenz Hiltner, 1904) coined the term "Rhizosphere" to denote the area of inter microbiological activity that extends several millimetre from the root system of the growing plant. The term "Rhizosphere Soil" refers to the thin layer adhering to a root after the loose soil clump have been removed by shaking. The growth of many microorganisms in the rhizospheric region depends on the root exudates released by the plants.

Fungi in rhizosphere soil are present as mycelial bits, rhizomorph or as different spores. Their number varies from a few thousand to a few million per gram of soil. Certain genera, like *Aspergillus, Penicillium, Fusarium, Trichoderma, Rhizopus* and *Mucor* are the most frequent in the rhizosphere **(Alexander, 1997)**. These mycoflora are known as Plant Growth Promoter Fungi (PGPF). PGPF belongs to genera *Penicillium, Fusarium, Aspergillus, Trichoderma* and *Phloma*. Some filamentous fungi present in the rhizosphere widely used as producer of organic acids particularly *Aspergillus* and some species of *Penicillium* **(Saraf, 2010)**.

Mycoflora perform important function within the soil in relation to transport, storage and recycling of nutrients, disease suppression and water dynamics all of which help plants become healthier and regenerates soil fertility. In **1991, Hawksworth** a mycologist estimated the world′s fungal diversity at 1.5 million species. Any deterioration in fungal population and diversity can therefore have a considerable impact on ecosystem health.

MATERIALS AND METHODS

The rhizospheric soil sample was taken from mustard. The present study was carried out in the winter season from January to March 2012.

(i) Isolation and enumeration of rhizospheric mycoflora

Soil dilution plate method of **Waksman and Fred (1922)** was used to isolate fungi from rhizosphere of mustard. One gram of soil suspension was suspended in 9 ml of sterilised distilled water which gave a dilution of 1:10 from which the dilution of 1:100, 1:1000, 1:10000 were prepared. One ml of each dilution was transferred aseptically into sterilized Petri plates and poured with 10-15 ml of melted Potato dextrose agar medium supplemented with ciprofloxacin. The plates were rotated by hand in a broad and slow swirling motion to disperse the soil suspension. Three Petri plates were provided for each dilution. Plates were incubated at 25 ± 1ºc for 5 days.

(ii) Morphological characterization of fungal isolates

Fungi growing on plates were identified by observing its macroscopic (colour, texture, appearance and diameter of colonies) and microscopic (microstructures) characteristics by fungal staining **(K.R. Aneja, 1993)**.

(iii) Measurement of growth rate of fungal isolates by colony diameter method

Melted Potato dextrose agar media supplemented with ciprofloxacin was poured in sterile Petri plates. Agar discs of pure culture of each fungal isolate was inoculated aseptically at the center of solidified Petri plates. Three replicates of each observation were prepared. The plates were incubated at 25 ± 1ºc in an inverted position for 5 days.

(iv) Measurement of growth rate of fungal isolates by dry mycelial weight method

Potato dextrose broth supplemented with ciprofloxacin was prepared. Agar discs of pure culture of each fungal isolate was inoculated aseptically into flasks containing broth then were incubated into shaker at 25 ± 1ºc for 10 days. Three replicates of each observation was prepared. After 10 days of incubation mycelial mat of each isolate was filtered through a pre-weighed Whattman No.1 filter paper which were dried at 80ºc for 24 hours and were re-weighed.

(v) Effects of temperature on growth of fungal isolates

Melted Potato dextrose agar media supplemented with ciprofloxacin was poured in sterile Petri plates. Agar discs of pure culture of each fungal isolate was inoculated at the center of each solidified plate and then the plates were incubated at different temperature ranges (0, 5, 15, 25 and 35°c) for 5 days.

(vi) Effects of pH on growth of fungal isolates

Melted Potato dextrose agar media supplemented with ciprofloxacin was adjusted with different pH ranges (2, 4, 6, 8 and 10) and was poured in sterile Petri plates. Agar discs of pure culture of each fungal isolate was inoculated at the center of each solidified plate of particular pH and then the plates were incubated at 25 ± 1ºc for 5 days.

(vii) Analysis of phosphate solubilization by fungal isolates

Melted Pikovskaya's medium was poured in sterile Petri plates. Agar discs of pure culture of each fungal isolates was inoculated at the center of each solidified plate and then the plates were incubated at 25 ± 1ºc for 5 days.

(viii) Antifungal sensitivity of fungal isolates

Antifungal sensitivity was performed by Poisoned Food Technique **(Nene and Thapliyal 1993)**. 0.05% solution of each commercially available antifungal drug (nystatin, griseofulvin, ketocip and fluconazole) was prepared. Melted Potato dextrose medium supplemented with ciprofloxacin was mixed separately with 100µl of each antifungal solution and was poured in sterile Petri plates. A disc of each fungal isolate was aseptically inoculated at the center of solidified plates of each antifungal drug. The plates were incubated at 25 ± 1ºc for about 5 days. PDA plates supplemented with ciprofloxacin inoculated with each isolate were used as control.

RESULTS

(i) Isolation and enumeration of rhizospheric mycoflora

Total fungal colonies were observed and enumerated after 5 days of incubation from mustard rhizosphere soil sample. Number of colonies decreases as dilution increases (Table:1).

Table 1: Enumeration of rhizosphere mycoflora from mustard.

S.No.	Sample	Dilution	Dilution factor	Mean ± S.E.	Colony Forming Unit
1.	Mustard rhizosphere	10^{-1}	10^{1}	33 ± 0.471	33×10^{1}
2.		10^{-2}	10^{2}	25 ± 1.699	25×10^{2}
3.		10^{-3}	10^{3}	9 ± 1.413	9×10^{3}
4.		10^{-4}	10^{4}	3 ± 0.471	3×10^{4}

- **Each result is the mean of 3 replicates ± the standard error.**

(ii) Morphological Characterization

All isolates were identified and characterized on the basis of Fungal stain (Lactophenol Cotton Blue). In this study it was found that *Trichoderma* and *Aspergillus* and was dominant in mustard rhizosphere. (Table:2).

Table 2: Morphological characteristics of fungal isolates from mustard rhizosphere.

S.No.	Fungi	Morphological characteristics			
		Colour	Shape	Mycelium	Fruiting body shape
1.	***Trichoderma***	White to green colonies	Phialides shape	Non-septate	Conidia in balls
2.	***Aspergillus***	Greenish blue, black or green colonies	Conidiophore arising from a foot cell	Septate	Basipetal conidia on phialides and 1 or 2 series on vesicles

(iii) Measurement of growth rate of fungal isolates by colony diameter method

Growth of fungal isolates was measured as per day growth in their colony. After 5 days of incubation, it was observed that *Trichoderma* grew at faster rate than *Aspergillus* (Table:3) and (Fig: 1).

Table 3: Measurement of growth rate of fungal isolates by colony diameter method.

S.No.	Fungi	Colony Diameter (mm) Days of incubation					Growth rate (mm/day) (Mean ± S.E.)
		1	2	3	4	5	
1.	*Trichoderma*	18	27	35	43	50	34.6 ± 6.54
2.	*Aspergillus*	11	12	15	19	22	15.8 ± 2.41

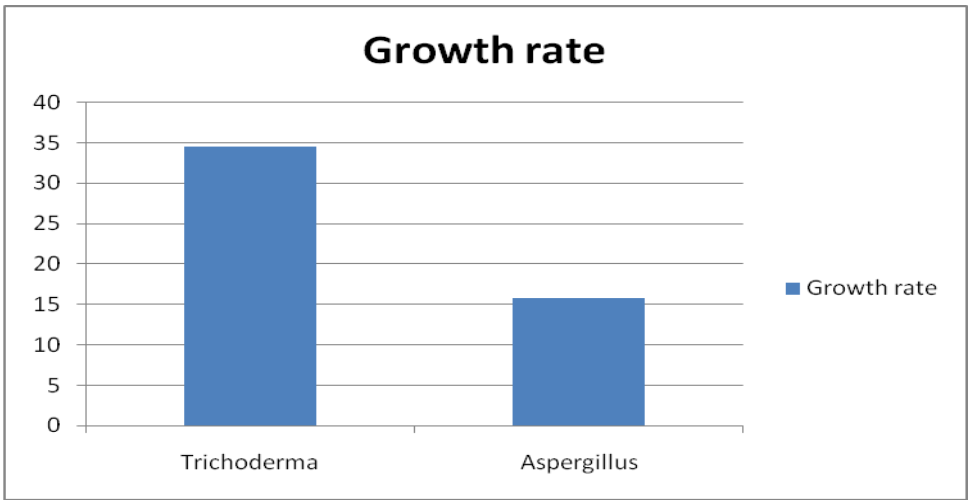

Fig. 1: Graph showing growth rate (mm/day) of fungal isolates by colony diameter method.

(iv) Measurement of growth rate of fungal isolates by dry mycelial weight method

Dry mycelial weight of fungal isolates was measured after 10 days of incubation. Maximum growth rate was observed in *Trichoderma* (Table:4) and (Fig:2).

Table 4: Measurement of growth rate of fungal isolates by dry mycelial weight method.

S.No.	Fungi	Dry mycelial weight(g) (Mean ± S.E.)	Growth rate (g/day)
1.	*Trichoderma*	0.569 ± 0.038	0.0569
2.	*Aspergillus*	0.388 ± 0.011	0.0388

- **Each result is the mean of 3 replicates ± the standard error.**

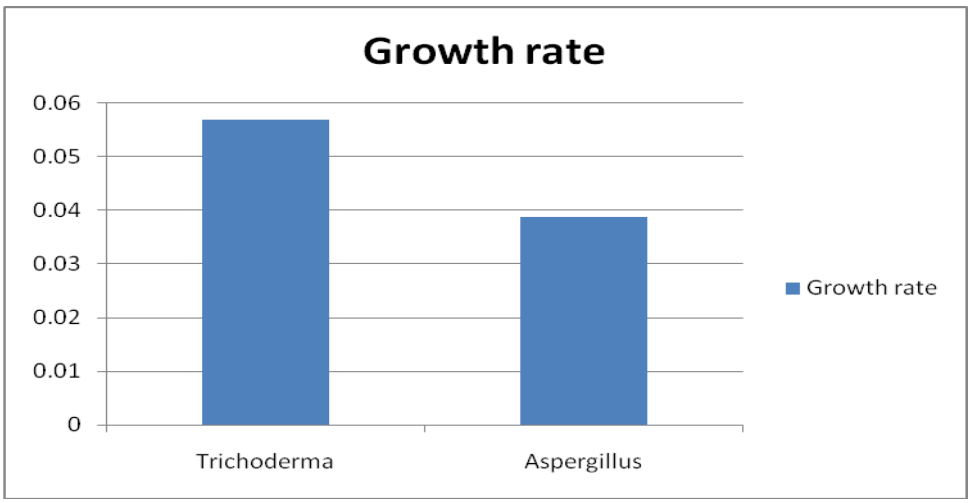

Fig 2: Graph showing growth rate (g/day) of fungal isolates by dry mycelial weight method.

(v) Effect of temperature on growth of fungal isolates
It was observed that all the isolates were able to grow at extreme temperatures (35°C). Hence, they are Mesophiles. Maximum growth of *Trichoderma* and *Aspergillus* was observed at 25°C (Fig.:3).

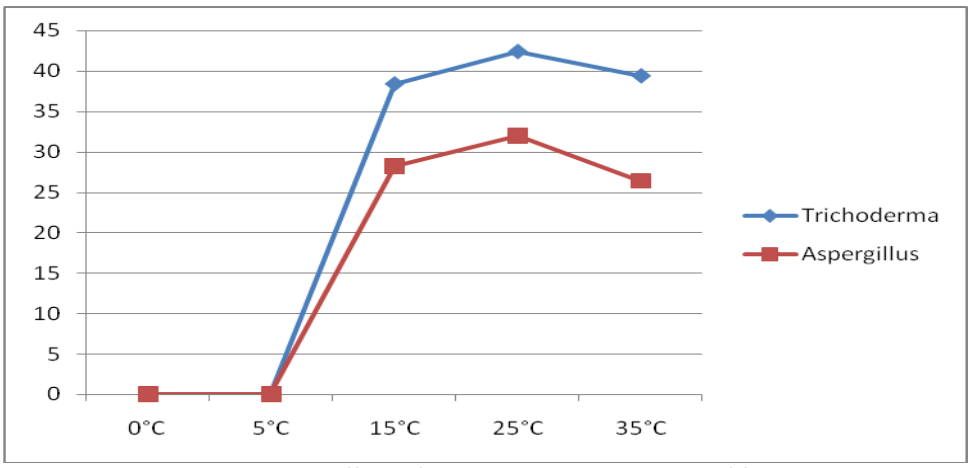

Fig. 3: Graph showing effect of temperature on growth of fungal isolates.

(vi) Effect of pH on growth of fungal isolates
Maximum growth of *Trichoderma* and *Aspergillus* was observed at 4and 10 pH, hence they are Acidophiles and Alkalophiles respectively(Fig.: 4).

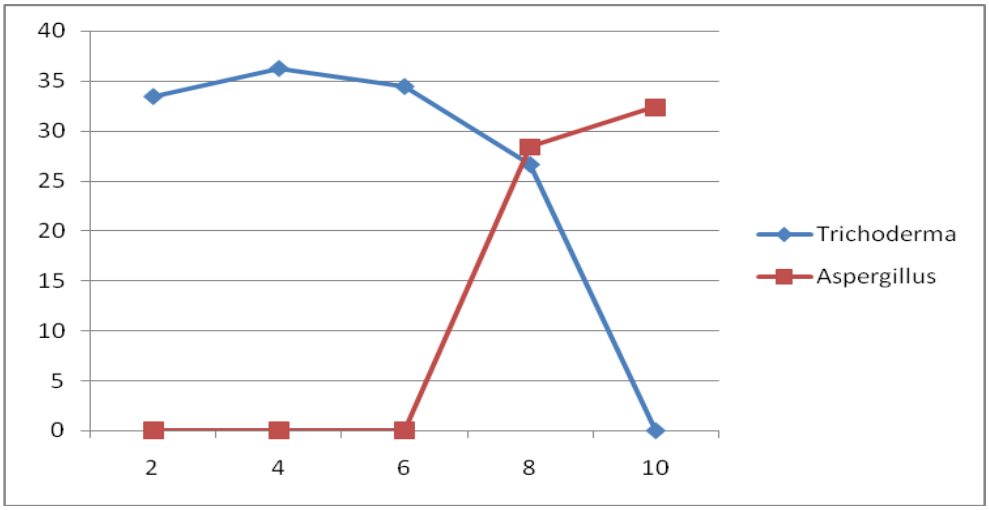

Fig. 4: Graph showing effect of pH on growth of fungal isolates.

(vii) Analysis of phosphate solubilization by fungal isolates
Clear zones were observed only around the *Trichoderma* and *Aspergillus*. Hence they are able to solubilize phosphate (Table:5).

Table 5: Analysis of phosphate solubilization by fungal isolates.

S.No.	Fungi	Phosphate solubilisation
1.	*Trichoderma*	+
2.	*Aspergillus*	+

(-) = Nil.

(viii) Antifungal sensitivity of fungal isolates
During the study of antifungal sensitivity of isolates, it was observed that maximum inhibition of isolates was obtained with nystatin (Table:6 and Fig.: 5).

Table 6: Antifungal sensitivity of fungal isolates.

S.No.	Fungi	% Mycelial growth inhibition			
		Nystatin	Fluconazole	Griseofulvin	Ketocip
1.	*Trichoderma*	100	50.8	48.5	57.8
2.	*Aspergillus*	100	46.8	100	100

- **Each reading is an average growth of 5 days.**

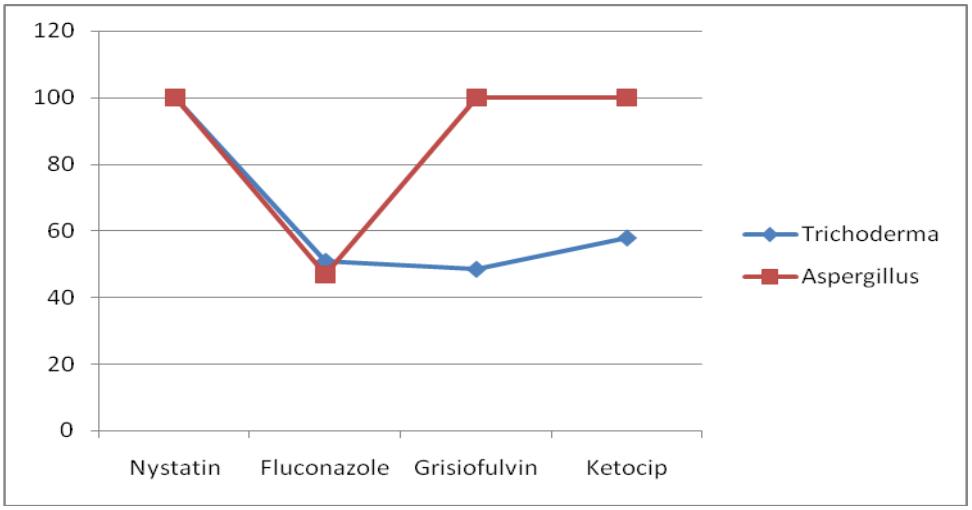

Fig. 5: Graph showing antifungal sensitivity of fungal isolates.

DISCUSSION

Soil can be defined as a natural medium for plant growth composed of minerals, organic materials and living organisms. The present study describes mycoflora of rhizosphere of mustard (*Brassica* sp.).

Shivpuri and Mali (2009) isolated five *Trichoderma* spp. from rhizospheric soil of mustard. Similarly in present study *Trichoderma* and *Aspergillus* were abundant in Mustard rhizosphere. Temperature is a vital factor to manipulate the fungal growth **(Woo *et al.*, 2006)**. Therefore, effect of temperature on the growth of fungal isolates was evaluated in order to determine the most suitable temperature for their growth. In the present study, maximum mycelial growth of *Trichoderma* and *Aspergillus* was observed at 25°c. Similarly **Pandey *et al.*, (2001)** observed that *Trichoderma* species prefer a mesophilic temperature range (15 to 35°c). The optimum temperature for growth differs among the *Trichoderma* isolates; although most *Trichoderma* strains are mesophilic **(Kredics *et al.*, 2003)**. *Aspergillus* species showed good growth at higher temperature (41°c) and was categorized in xerophilic fungi (**Cabrera *et al.*, 2005)**. **Kapri and Tewari (2010)** isolated 14 strains of *Trichoderma* spp. which were able to solubilize tricalcium phosphate with varying potential. Similarly in present study it was observed that *Trichoderma* and *Aspergillus* were able to solubilize insoluble phosphate.

CONCLUSION

Rhizosphere contains lots of organic substrates which harbours a high count of microorganisms. The loss of organic materials from roots provides the energy for the development of active fungal population in the rhizosphere around the root. From the present study it was concluded that *Trichoderma* and *Aspergillus* are dominant in mustard (*Brassica* sp.) rhizosphere. It was observed that *Trichoderma* and *Aspergillus* are able to solubilize phosphate, hence they are beneficial for plants.

REFERENCES

1. Abouziena HFH, Elham ZAE, Youssef RA and Sahab AF (2010). Efficacy of intercropping mango, mandarin or Egyptian clover plants with date palm on soil properties, rhizosphere microflora and quality and quantity of date fruits; *Journal of American Science*, 6(12): 230-238.
2. Alexander M (1997). In: Introduction to soil microbiology, 2nd ed. John Wiky and Sons. Inc., New York and London.
3. Alexopoulos CJ, Mims CW and Blackwell M (1996). In: Introductory mycology 4th ed. Johnwiley and Sons, Inc, New York.
4. Aneja KR (2003). In: Experiments of microbiology plant pathology and biotechnology 4th ed. New Age International (P) Limited, Publishers, New Delhi.
5. Brady NC and Weil RR (2000). In: Elements of the nature and properties of soil 12th ed. Prentice Hall, Inc., New Jersey.
6. Cabrera HP, Taniwaki MH, Hashimoto JM and Menezes HC (2005). Growth of *Aspergillus ochraceus*, *A. carbonarius* and *A. niger* on culture media at different water activities and temperatures. Brazilian J. Micro., 36: 24-28.
7. Cooper KM and Tinker PB (1978). Translocation and transfer of nutrients in vesicular mycorrhizas II. Uptake and translocation of phosphorus, zinc and sulphur, *New Phytol*, 81: 43-52.

8. Dubey RC and Maheshwari DK (1992). Soil microbiology. In: Text book of microbiology 1st ed. S. Chand and Company Limited, New Delhi.
9. Evelin H, Kapoor R and Giri B (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress, *Annals of Botany*, 104: 1263-1280.
10. Ghany E1-Abd, Bouthaina F, Arafa, Rhawahia AM, Rahmany, E1, Tomader A and Mona M (2010). Effect of some soil microorganisms on soil properties and wheat production under north Sinai conditions. *Journal of Applied Sciences Research*, 4(5): 559-579.
11. Griffin DM (1972). In. Ecology of soil fungi. Chapman & Hall, London, 193.
12. Hajieghrari B, Torabi-Giglou M, Mohammadi MR and Davari M (2008). Biological potantial of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. Afr. J. Biotechnol, 7: 967-972.
13. Hawksworth DL (1991). Frontiers in Mycology. Honorary and General Lectures from the Fourth International Mycological Congress, Regensburg, Germany.
14. Hiltner L (1904). In. History of microbiology. Prentice hall of India private limited, New Delhi, 11.
15. Huang J, Li H and Yuan H (2006). Effect of organic amendments on *Verticillium* wilt of cotton. Crop Prot., 25: 1167-1173.
16. Ishimoto H, Fukushi Y, Yoshida T and Tahara S (2000). Rhizopus and Fusarium are selected as dominant fungal genera in rhizospheres of Brassicaceae. *Journal of Chemical Ecology*, 26(10): 2387–2399.
17. Jefwa JM, Ohiokpehai O, Kavoo AI and Wasike VW (2010). Soil microbe mediated zinc uptake in soybean. *African Journal of Food Agriculture Nutrition and Development*, 10(11): 4340-4349.
18. Johnson D, Ijdo M, Genney DR, Andreson IC and Alexander IJ (2005). How do plants regulate the function community structure, and diversity of mycorhhizal fungi. *Journal of Experimental Botany*, 56(417): 1751-1760.
19. Joshi BB, Bhatt RP and Bahukhandi D (2010). Antagonistic and plant growth activity of *Trichoderma* isolates of Western Himalayas. *Journal of Environmental Biology*. Lucknow (India), 31(6): 921-928.
20. Kapri A and Tewari L (2010). Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* spp. *Brazilian Journal of Microbiology*. ISSN 1517-8382.
21. Kathiresan K and Manivarman S (2006). α– amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. *African Academic Journal of Biotechnology*, 5(10): 829- 832.
22. Kaushik MP (2004). In: A Text book of Botany 31st ed. Prakash Publications, Muzzaffar nagar, (U.P.).
23. Kloepper JW, Ryu CM and Zhang S (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopath*, 94: 1259-1266.
24. Korkama T (2007). Effect of Norway spruce clones on active needle litter decomposers and the role of endophytic fungi in 2 year decomposition. In: Proceedings of National Seminar on Forest Pathology, Forest Research Institute Dehradun.
25. Kredics L, Antal Z, Manczinger, Szekeres A, Kevei F and Nady E (2003). lnfluence of environmental parameters on *Trichoderma* strains with biocontrol potential. ln: *Trichoderma* strains with biocontrol potential. Food Tech. Biotech, 47: 37- 42.
26. Kremer RJ, Means NE and Kim S (2005). Glyphosate affects soybean root exudation and rhizosphere microorganism, *International Journal of Analitical Environmental Chemistry*. 8, (72): 1- 14.
27. Manka M (2007). Fungal biodersity in rhizosphere of healthy and needle cast cast- affected scots pine transplants. *Acta Mycologica*, 42(2): 199- 202.
28. Mehalawy E1 AA, Hassanein NM, Khater HM, E1-din K, Zahra E1 A and Youssef YA (2004). Influence of Maize root colonization by the rhizosphere actinomycetes and yeast fungi on plant growth and on the biological control of late wilt disease. *International Journal of Agriculture and Biology*, 6(4): 599- 605.
29. Mithusin EN (1957). In: Soil Microbiology. CBS Publishers New Delhi.
30. Mogle UP and Mane RY (2010). Antagonistic effect of biofertilizers against seed borne mycoflora of Tomato (*Lycopersicun esculentum*). *Research Journal of Agricultural Sciences*, 1(3): 255- 258.
31. Motta MS, Cavalcanti MAQ, Fernandes MJS, Lima DMM, Nascimento JP and Laranjeira D (2003). Identification and characterization of filamentous fungi isolated from the sunflower (*Helianthus annus L.*) rhizosphere according to their capacity to hydrolyse inulin. *Brazilian Journal of Microbiology*, 34: 273- 280.
32. Nene YL and Thapliyal PN (1993). Fungicides in Plant Disease Control. International Science Publisher, 1993-691 Pages.
33. Nulton PC and Campbell MI (1977). Mycophenolic acid is produced during balanced growth of *Penicillium brevicompactum*. *Canadian Journal of Microbiology*, 23(1): 20-27.
34. Ogbebor NO, Adekunle TA, Eghafona ON and Ogboghodo IA (2010). *Ganoderma pseudoferreum* : Biological control possibilities with microorganisms isolated from soils of rubber plantation in Nigeria. *African Journal Of Agriculture*, 6(4): 301- 305.
35. O' Sullivan DJ and O'Gara F (1992). Traits of *Fluorescent Pseudomonas* spp. Involved in Suppression of Plant Root Pathogens, 56(4): 662-676.
36. Olsson PA and Johnson NC (2005). Tracking carbon from the atmosphere to the rhizosphere. *Ecology letters*, 8: 1264-1270.
37. Pandey A, Palni LM and Bisht D (2001). Dominant fungi in the rhizosphere of established tea bushes and their interaction with the dominant bacteria

under in situ conditions. Microbiol. Res., 156: 377-382.
38. Pandya U and Saraf M (2010). Application of fungi as a biocontrol agent and biofertilizer potential in agriculture. *Journal adv. Dev. Res*., 1(1): 90- 99.
39. Pandy A and Palni LS (2007). The rhizosphere effect in trees of the Indian Central Himalayas with special reference to altitude. *Applied Ecology and Environmental Research*, 5(1): 93- 102.
40. Rangaswami R and Bayaraj DJ (1993). In: Agricultural Microbiology 2nd ed. Prentice- Hall of India Private limited, New Delhi.
41. Rao SNS (2009). Soil Microbiology. In: soil microorganisms and plant growth 4th ed. Oxford & IBH Publishing Company Pvt. Ltd. 113- B Shahpur Jat, New Delhi.
42. Sabat J and Gupta N (2010). Nutritional factor affecting the antifungal activity of *Penicillium stecki* of mangrove origin. *African Journal of Microbiology Research*, 4(3): 126-135.
43. Saleem AR (2010). Effect of Kocide and Ridomil plus fungicides on the mycoflora of tomato plant in Upper Egypt, 43(17).
44. Saravankumar K and Kaviyarsan V (2010). Seasonal distribution of soil fungi and chemical properties of montain wet temperate forest types of Tamilnadu. *African Journal of Plant Science*, 4(6): 190-196.
45. Saravanakumar K and Kaviyarsan V (2010). Diversity and Distribution of soil microflora of dry dexiduous forest of Tamilnadu Southern India. *Journal of Biosciences Research*, 1(1): 25-23.
46. Sheng WH, Liu YD, Yang XL, Chen XQ, Wang ZH, Kong XY, Liu XX and Yan S (2010). Growth responses of *in vitro Fusarium oxyporum*, *Fusarium* species *niveum* to external supply of tannic acid. *Journal of Environmental Biology*, 31(6): 1017-1022.
47. Singh PK and Sharma KP (2009). Microflora (fungal anf bacteria) of selected terrestrial and marshy species of rhizosphere in response to spent wash treatments. *Indian Journal of Biotechnology*, 8: 240-243.
48. Tamilarasi S, Nanthakumar K, Karthi Keyan K and Lakshmana Perumalsamy P (2008). Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of *Coriandrum sativum*. *Journal of Environmental Biology*, 29(1): 127-134.
49. Tariq M, Dawar S and Mehdi FS (2007). Studies on the rhizosphere mycoflora of mangroves. *Turk Journal Botany*, 32: 97-101.
50. Trivedy RK and Goel PK (1984). In: Chemical and biological method for water pollution studies. Enviro Media Publication, Karad.
51. Vashishtha PC (1992). A text of Book of Fungi (Pradeep Publication).
52. Watanabe T (2002). In: Pictorial atlas of soil and seed fungi. Morphologies of cultured fungi and key to species 2nd ed. CRC Press, Newyork.
53. Weler DM and Thomashow LS (1994). Current challenges in introducing beneficial microorganisms into the rhizosphere. In Molecular Ecology of Rhizosphere microorganism: Biotechnology and Release of GMOs, 1-18.
54. Woo SL, Scala F, Ruocco M, Lorito M (2006). The molecular biology of the interactions between *Trichoderma* spp., phytopathogenic fungi and plants. Phytopathol., 96: 181-185.
55. Zakaria L, Foong MK, Hsuan HM, Zakaria M and Salleh B (2010). *Fusarium* species isolated form mangrove soil in Kampong. Pantai Acheh Balik Pulau, Pulau Pinang Malaysia, *Tropical life Sciences Research*, 21(1): 21-29.

Websites

www.en.wikkipedia.org2011
www.physicalgeography.net
www.plantphysiol.org.
www.dpi.nsw.gov