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Isolation and Identification of Soil Mycoflora from Two Different *Brassica campestris* L. Fields

Seemal Vehra[1], Nazish Noor[1], Roheena Abdullah[2], Nafeesa Farooq[1] and Ejaz Mahmood Ahmad Qureshi[3]

[1]Department of Botany, Government Postgraduate College for Women, Samanabad, Lahore.
[2]Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan.
[3]Institute of Public Health, Lahore.

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Soil mycoflora was isolated and identified from two different *Brassica campestris* fields along Multan Road and Raiwind Road, Lahore, Pakistan, to assess the common occurring species. From both sites, a total of 8 species belonging to 3 genera of fungi were isolated. The soil dilution technique was used for the isolation of fungal species from the soil samples and culturing was carried out on Potato Dextrose Agar and Malt Extract Agar medium supplemented with suitable antibiotics such as chloromycitin in order to avoid bacterial contamination. Identification and characterization of the mycoflora up to species level were made on the basis of macroscopic and microscopic morphology such as colony color and texture, and shape of conidia and conidiophores. The most commonly found species which were isolated and identified included *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Aspergillus oryzae, Fusarium oxysporum* and *Candida albicans*. *Aspergillus* was found to be the dominating genus in this study. The results obtained from the comparison of mycoflora from the two sites showed that *Aspergillus* species and *Candida albicans* were common in both *Brassica campestris* fields.

Key words: Mycoflora, soil, *Brassica campestris*.

Soil is the top layer of earth surface consisting of a mixture of rocks and mineral particles with organic matter, gases, liquids as well as diverse group of microorganisms. The fertility of soil is influenced by the presence of microflora and its biological activity such as symbiotic and non-symbiotic atmospheric, nitrogen fixation, denitrification, aggregation, turn-over of organic matter, etc[1]. It is a complex environment, containing anywhere between 5,000 and 50,000 species of microorganisms in each gram of soil[2, 3]. Estimated numbers of soil species include 30,000 bacteria, 1,500,000 fungi, 60,000 algae, 10,000 protozoa, 500,000 nematodes and 3,000 earthworms[4]. The population and diversity of soil microflora depend on several environmental factors such as the type and amount of nutrients, light, moisture, degree of aeration, pH and temperature etc. the main factors being soil pH, organic contents and water contents[5]. Distribution of fungi is also affected by sources and amounts of carbon, nitrogen, vitamins and trace elements as it influences establishment of spore development in soil[6]. High density of fungal population has been reported during the monsoon (rainy) season when the soil moisture is significantly high[7].

Fungi are ubiquitous organisms found in nature as they occur in almost all the types of habitats because they possess wide adaptation

* To whom all correspondence should be addressed.
E-mail: svehra@hotmail.com

abilities and can utilize large spectrum of nutrient sources[6]. Furthermore, largest number of genera as well as species of fungi are present in soil than in any other environment[8].

Decomposer fungi are highly important components of terrestrial ecosystems as they play significant roles in nutrient cycling, soil stabilization, plant succession and other ecosystem processes[9]. Among all fungi, the saprobic fungi constitute the largest proportion of fungal species present in soil and play an essential role in the decomposition of plant structural polymers, such as, celluloses, hemicelluloses, and lignin and thus, maintain the global carbon cycle. They breakdown metabolic by-products and hence, enhance the bioavailability of nitrates, sulphates, phosphates and essential metals and carry out nitrogen fixation[10]. Fungi can also enhance plant growth by forming mutualistic associations called mycorrhizae with plant roots[11].

Mainly crop species are related to endorrhizal fungi and among them Arbuscular Mycorrhizal (AM) and Dark Septate Endophytic (DSE) fungi are the most widespread[12,13]. The growth and yield of crops increase through efficient nutrient uptake, especially phosphorus[14, 15, 16]. They are components of the soil microbiota normally constituting greater soil biomass as compared to bacteria[17].

In addition to beneficial impacts, fungi also exhibit harmful effects as many are parasites on plants, animals (including humans) and other fungi. Pathogens of many cultivated plants that cause serious damage and significant losses to agriculture and forestry include, the rice blast fungus *Magnaporthe oryzae*[18]. Some tree pathogens are *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* that cause Dutch elm disease[19] and *Cryphonectria parasitica* which is responsible for chestnut blight[20] whereas other phytopathogens of annual plants include the genera *Fusarium*, *Ustilago*, *Alternaria*, and *Cochliobolus*. Carnivorous fungi include *Paecilomyces lilacinus*, which are predators of nematodes, and possess specialized structures such as constriction rings and adhesive nets[21]. Additionally, as reported by El-Tarabily and Sivasithamparam[22] , presence of certain yeasts enhance plant growth. However, fungi, especially ascomycetes (to which yeasts belong), causes about 80% of plant diseases. *Rhizopus stolonifer* (Zygomycete) possesing resistant zygosporangium and ability of rapid growth (Campbell *et al*., 2006), easily infects plants. *Brassica* is one of the most economically important genus of family Brassicaceae[23]. Brassicaceae (Cruciferae) constitutes a diverse group of 350 genera and more than 3,500 species of dicotyledonous wild and cultivated herbs possessing complete, hypogynous and cruciform flowers[24]. *Brassica* crops are reported to be seriously challenged by a variety of fungal pathogens and insects, while bacterial and viral diseases have comparatively little effect on their yield[25].

Soil borne fungal pathogens constitute the main biotic components responsible for yield decline in cultivated areas. Such fungal pathogens remain alive on organic residues in soils and may cause root rot with marked decreased growth rates leading to the death of the plant, depending on prevailing environmental conditions and host susceptibility[26]. The most common soil borne pathogens are *Pythium* spp., *Rhizoctonia* spp., *Fusarium* spp., *Phoma* spp., *Cylindrocarpon* spp., *Sclerotinia sclerotiorum*, *Colletotrichum* spp[27]. and *Pythium tracheiphylum*[28]. Intergovernmental Panel on Climate Change[29] predicts a widespread increase of air temperature in the 21th century. Such increase is expected to elevate soil temperature regimes and consequently soil borne pathogens might increase their biological pressure on crops causing high yield losses.

Furthermore, in agriculture, fungi may prove to be valuable if they actively compete for space and nutrients with prevailing pathogenic microorganisms such as other fungi or bacteria by the competitive exclusion principle or if they parasitize on these pathogens. Thus, keeping in mind innumerable impacts of fungi on crop growth in general and *Brassica campestris* in particular, it becomes imperative to study the soil mycoflora present in the agricultural fields where *B. campestris* is grown.

The outcome of the study will highlight not only the common fungal species present in *B.campestris* fields but will enable the farmer to develop management strategies, such as application of particular fungicides in case pathogenic fungi are identified. Additionally, Farmers will also have a general idea as to which

common beneficial fungal species are present.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from two different *B. campestris* fields, one along Multan Road and the other near Raiwind Road in February, 2014. At the time of collection *B. campestris* crop was at mature stage i.e. flowering and seeding stage. Soil was taken from surface as well as from 10, 20 and 30cm depth and was uniformly mixed before transferring to air-tight, labeled sterile polythene bags which were brought to laboratory of Institute of Agricultural Sciences(IAGS), Punjab University and stored at ambient temperature till further analysis. All the glassware to be used was sterilized in oven at 180°C for 2 hours.

Isolation, Purification and Indentification of soil Mycoflora

For isolation of soil mycoflora, the media namely, Potato Dextrose Agar (PDA)[30] and Malt Extract Agar (MEA)[31] were used. Isolation from the soil samples were carried out by serial dilution method[32]. After the dilution preparation, 200 micro liter of suspension was transferred to sterilized Petri plates. Three replicas were made for each dilution. After solidification of media, all plates were incubated in an inverted position at 25 °C for 3 to 5 days. Purification followed identification on the basis of macroscopic and microscopic features. Fungal morphology was studied macroscopically by observing colony features (Color and Texture) and microscopically by observing conidia and conidiophores under microscope with the help of standard microbiology atlas and manual. [33] Methylene blue stain was used for fungal examination.

RESULTS AND DISCUSSION

From the soil samples taken from two different *Brassica campestris* fields located near Multan Road and Raiwind, a total of 8 species were observed.The isolated fungal species belonged to class Ascomycotina (8 colonies) and genera *Aspergillus, yeast* and *Fusarium*. The fungal species, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus, Aspergillus nidulans, Aspergillus oryzae, Candida albicans and Fusarium oxysporum* were isolated and identified on the basis of morphological characteristics such as macroscopic and microscopic morphology. The commonly occurring species in soils from both sites were *Aspergillus niger, Aspergillus flavus Aspergillus nidulans, Fusarium oxysporom* and *Candida albicans* (Table 1).

Growth on PDA appeared well ahead of that on MEA and this observation was supported by previous reports revealing that for quick and profuse growth of fungi, potato dextrose agar was most favorable[34].

Some *Aspergillus* species such as *A. niger* and *A. oryzae* were isolated from soil of forest ecosystems of Taiwan by Hsu and Agoramoorthy[35]. Sharma[36] studied soil mycoflora of Yumthang valley and observed that maximum contribution belonged to Ascomycotina. The report is similar with the findings of the present study in which almost all the isolated fungi belong to Ascomycotina. Microbial analysis of different soil samples of selected site in Obafemi Awolowo University, Nigeria was investigated by Ogunmwonyi *et al.*,[37]. and they found *A. niger* as a dominating fungi among all. This is in line with the present investigation whereby *A. niger* as well as *A. flavus* and *A. nidulans* were found to be dominant among different fungal species. This finding coincides with the work of Elisane *et al.*,[38] who also isolated similar four strains from the contaminated soil.

Fungi belonging to genera *Aspergillus, Fusarium* and *Trichoderma* were isolated from Antarctic soils by Singh *et al.*,[39] About 90 fungal stains were isolated from the soil of Kotri barrage, Pakistan by Suhail *et al.*,[40] and among the 21 *Aspergillus* species isolated, *A. niger* was found to be dominant.

Asan[41] reported *Penicillium* and *Aspergillus* from different habitat soils in Edirne. He found 23 species and two varieties belonging to *Aspergillus* and 16 species belonging to *Penicillium*. *Penicillium* and *Aspergillus* were isolated from soil in North-east Anatolia[42]. In their research they found 20 species of *Aspergillus* and 22 of *Penicillium*. The results of these studies are in accordance with this work, which suggests that the genus *Aspergillus* is the most commonly occurring genus in soils. It has been reported that

Table 1. Fungal isolates collected from the selected *Brassica campestris* fields

S. no.	Location	Sample no.	Media Used	Fungal Isolates
1.	Field near Multan Road	1.	MEA	*Aspergillus nidulans, A.niger,A.fumigatus, A.flavus,*
		2.		*A.niger, F.oxysporum, A.flavus*
		3.		*A.flavus, F.oxysporum, A.nidulans, A.niger*
		1.	PDA	*Candida albicans, A.oryzae.F.oxysporum, A.flavus,A.niger*
		2.		*A.flavus, A.oryzae, C. albicans, A.niger*
		3.		*A.flavus, C.albicans, A.nidulans, A.niger*
2.	Field near Raiwind Road	1.	MEA	*A.niger, A.flavus*
		2.		*A.niger, A.flavus, A.fumigatus, A.nidulans, A.terreus*
		3.		*A.niger, A.oryzae, A.terreus, F.oxysporum*
		1.	PDA	*Candida albicans*
		2.		*C.albicans,F.oxysporum, A.niger,A.nidulans,A.flavus*
		3.		*F.oxysporum, C.albicans, A.nidulans,A.niger,A.flavus*

the density of fungal population is high during the rainy season when the soil moisture content is significantly high too and that environmental factors such as pH, moisture, temperature, organic carbon play an important role in the distribution of mycoflora[43].

In the present study the isolation *F. oxysporum*, a plant pathogenic fungus from the *B. campestris* fields gives an indication to the farmer about the prevalence of pathogenic potential of the mycoflora, thereby enabling him to consider suitable crop for that field, and he may thus avoid growing those particular crops that are highly susceptible to the pathogens identified.

CONCLUSION

The results of this study showed that the soil from two selected sites (one near Multan Road and the other one near the Raiwind Road) of *B. campestris* fields supported a number of soil-borne mycoflora that were isolated and identified according to their macroscopic and microscopic morphologies. The comparative observation between mycoflora of both the sites revealed that the commonly occurring species were *A. niger, A. flavus, A. nidulans, A. terreus, A. oryzae* and *Candida albicans.*

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REFRENCES

1. Chenu, C. and Stotzky, G. Interactions between microorganisms and soil particles: an overview. *In*: *Interactions between soil particles and microorganisms* (ed P.M. Huang, J.M. Bollag & N. Senesi), 2002; pp 3-40. Wiley, New York.
2. Schloss, P.D and Handelsman, J. Toward a census of bacteria in soil. *PLoS Comput. Biol.*, 2006; 2(7):e92.

3. Dance, A. Soil ecology: What lies beneath? *Nature*, 2008; **455**: 724-725.
4. Pankhurst, C.E. Biodiversity of soil organisms as an indicator of soil health. In: *Biological Indicators of Soil Health*. CAB International, 1997; pp 298-315.
5. Yu, C., Lv, D.G, Qin, S.J, Du, G.D and Liu, G.C. Microbial flora in cerasus sachalinensis rhizosphere. *J.Appl. Ecol.*, 2007; **18**(10): 2277-2281.
6. Mitchell, J.I and Zuccaro, A. Sequences, the environment and fungi. *Mycologist*, 2006; **20**(2), pp. 62–74.
7. Bissett, J. and Parkinson, D. Functional relationship between soil fungi and environmental in alpine tundra. *Can j. Bot*, 1979; **57**:1642-59.
8. Nagmani, A., Kunwar, I.K and Manoharachary, C. Handbook of Soil Fungi. Published by I. K.International Pvt, Ltd.New Delhi. 2005.
9. De Boer, W., Folman, L.B, Summerbell, R.C and Boddy**,** L. Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiol. Rev*, 2005; **29:**795–811.
10. Bridge, P. and Spooner, B. Soil fungi: diversity and detection. *Plant Soil*, 2001; **232**: 147-154.
11. Campbell, N.A., Reece, J.B, Taylor, M.R. and Simon, E.J. *Biology: Concepts and Connections*. (6th Ed) Pearson Education, Inc. /Benjamin Cummings, San Franci. 2006.
12. Muthukumar, T., Senthilkumar, M, Rajangam, M and Udaiyan, K. Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western ghats, Southern India. *Mycorrhiza*, 2006; **17**: 11-24.
13. Muthukumar, T and Prakash, S. Arbuscular mycorrhizal morphology in crops and associated weeds in tropical agroecosystems. *Mycoscience*, 2009; **50**: 233-239
14. Akhtar, M.S. and Siddiqui, Z.A. Arbuscular mycorrhizal fungi as potential bioprotectants against plant pathogens. *In*: *Mycorrhizae: Sustainable Agriculture and Forestry* (ed Z.A Siddiqui, M.S. Akhtar & K. Futai), 2008; pp. 61-98. Springer, Dordrecht, the Netherlands.
15. Giasson, P., Karam, A and Jaouich, A. Arbuscular mycorrhizae and alleviation of soil stresses on plant growth. In: Siddiqui, Z.A., Akhtar, M.S. and Futai, K. (Eds.). *Mycorrhizae: Sustainable Agriculture and Foresrty*. Springer, Dordrecht, the Netherlands. 2008; pp. 99-134.
16. Sawers, R.J.H., Gutjahr, C and Paszkowski, U. Cereal mycorrhiza: an ancient symbiosis in modern agriculture. *Trends in plant sciences*, 2008; **13**(2):93-97.
17. Ainswoth, G.C and Bisby, G.R. Dictionary of the Fungi, World Book Publishing. 1995.
18. Talbot, N.J. On the trail of a serial killer: Exploring the biology of *Magnaporthe grisea*. *Annual Reviews in Microbiology*, 2003; **57**: 177–202.
19. Paoletti, M., Buck, K.W and Brasier, C.M. Selective acquisition of novel mating type and vegetative incompatibility genes via interspecies gene transfer in the globally invading eukaryote *Ophiostoma novo-ulmi*. *Molecular Ecology*, 2006; **15** (1): 249–62.
20. Gryzenhout, M., Wingfield, B.D and Wingfield, M.J. New taxonomic concepts for the important forest pathogen *Cryphonectria parasitica* and related fungi. *FEMS Microbiology Letters*, 2006; **258**(2):161–72.
21. Yang, Y., Yang, E, an, Z and Liu, X. Evolution of nematode-trapping cells of predatory fungi of the Orbiliaceae based on evidence from rRNA-encoding DNA and multiprotein sequences. *Proceedings of the National Academy of Sciences USA*, 2007; **104** (20): 8379–84.
22. El-Tarabily, K. A and Sivasithamparam, K. Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Mycoscience*, 2006; **47**: 25-35.
23. Srivastava, M., Akhoon, B.A and Gupta, S.K. Development of resistance against Blackleg disease in *Brassica oleracea* var. botrytis through in silico methods. *Fungal Genet Biol*, 2010; **47**: 800-808.
24. Warwick, S.I., Francis, A and Mulligan, G.A. *Brassicaceae of Canada*. Government of Canada. 2003.
25. Abdel-Farida, I.B., Jahangira, M, Van den Hondelc, C.A.M.J.J, Kima H.K, Choi Y.H. and Verpoorte, RFungal infection-induced metabolites in *Brassica rapa*. *Plant Sci*., 2009; 176: 608-615.
26. Redman, R.S., Dunigan, D.D and Rodriguez, R.J. Fungal symbiosis: from mutualism to parasitism, who controls the outcome, host or invader? *New Phytologist*, 2001; **151**, 705-716.
27. Lees, A.K and Hilton, A.J. Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. *Plant Pathology*, 2003; **12**: 1-12.
28. Gonzalez, A.J., Tello, J.C and Herrero, M.L. First report of *Pythium tracheiphilum* causing wilt and leaf blight on lettuce (*Lactuca sativa*) in Spain. *Plant Disease*, 2004; 88, 138.
29. Intergovernmental Panel on Climate Change (IPCC). Climate Change. *Synthesis Report*. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the

Intergovernmental Panel on Climate Change. Core Writing Team, Pachauri, R.K., and A. Reisinger (Eds.) IPCC, Geneva, Switzerland, 2007; pp. 104.

30. Harrigan, W.F and McCance, M.E. Laboratory methods of food and diary microbiology. (8th Ed) *Academic Press London,* 1990; pp 452.
31. Johnston, A. and C. Booth. *Plant pathologist's pocketbook*. Commonwealth Agricultural Bureaux. , 1983.
32. Warcup, J.H. The soil plate method for isolation of fungi from soil. *Nature London*, 1950; **166**: 117-118.
33. Pitt, A.D and Hocking, J.I. *Fungi and Food Spoilage* (3rd Ed) SpringerLink: Springer e-Books. 2009.
34. Maheshwari, R., Bharadwaj, G and Bhat, M.K. Thermophilic fungi: Their physiology and enzymes. *Microbiol. Mol. Biol,* Rev. 2000; **64**: 461-488.
35. Hsu, M. J. and Agoramoorthy, G. Occurrence and diversity of thermophilous soil microfungi in forest and cave ecosystems of Taiwan. *Fungal Diversity,* 2001; 7: p.27-33.
36. Sharma, K and Mahish, P. Isolation of soil mycoflora from yumthang valley, Sikkim. Lab to land, 2009; **2**(1), p. 42-45.
37. Ogunmwonyi, I.N., Igbinosa, O.E, Aiyegoro, O.A. and Odjadjare, E.E. Microbial analysis of different top soil samples of selected site in Obafemi Awolowo University, Nigeria. *Scientific Research and Essay,* 2008; **3**(3), p.120-124.
38. Elisane, OdS., Célia, FCdR, Cátia, TdP, Ana, V.L.S, Janaína, FdMB, Susana, J.K and Carlos, A.V.B. Pre-screening of filamentous fungi isolated from a contaminated site in Southern Brazil for bioaugmentation purposes. *African Journal of Biotech*nology, 2008; **7**: 1314-1317.
39. Singh, S. M., Puja, G. and Bhat, D. JPsychrophilic fungi from Schirmacher Oasis, East Antarctica. *Current Science*, 2006; 90(10), p.1388-1392.
40. Suhail, M., Irum, F, Jatt, T, Korejo, F. and Abro, H. *Aspergillus* Mycoflora isolated from soil of Kotri Barrage Sindh, Pakistan. *Pak. J. Bot*, 2007; **39**(3) p. 981-984.
41. Asan, A. Microflora fungus occurance in corn fields of European part of Turkey-I. *Turk J. of Botany,* 1997; **21**:89-101.
42. Sulun, Y and Hasenekoglu, I. A study on Aspergillus Mich.ex. Fr. and Penicillium Link ex. Gray flora of the soils of northeast Anatolia, Turkiye. Doga—*Turkey J. Biol,* 1993; **17**:49–60.
43. Deka, H.K and Mishra, R.R. Distribution of soil mycoflora in jhum fallows in north east india. *Acta Batanica Indica*, 1984; **12**:180-184.