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EVALUATION OF SOILBORNE PLANT PATHOGENIC FUNGAL COUNT IN VARIOUS LAND USE TYPES OF DISTRICT OKARA

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ABSTRACT

A field survey was conducted in district Okara to determine existing plant pathogenic fungal count in various land use types. The target sites were classified as orchard, barren land, fallow land, cultivated land and forest land use types. Field soil samples were collected from these land use types at different depths of 15, 30 and 45 cm respectively. Dilution of soil samples was made in sterilized distilled water and 1ml was poured on to the malt extract agar (MEA) medium in 90 mm diameter Petri plates incubated at 25±2°C for 5-7 days. Purified fungal colonies of *Mucor* spp., *Fusarium solani*, *Aspergillus niger*, *Penicillium* spp., *Alternaria alternata*, *Curvularia* spp., *Rhizopus* spp., and *Helminthosporium* spp. on potato dextrose agar (PDA) medium were identified based on their morphological descriptions. These fungal counts were recovered from the soil at 30 cm depth which indicates an alarming situation for deep-rooted crops to soilborne fungal pathogens infestation in future.

Keywords: Soilborne fungus, fungal counts, land use types, soil microbes.

INTRODUCTION

Okara District is playing a significant role in crop productivity as it is renowned in producing sugarcane, wheat, rice and maize crops and dairy products. Oranges and Mangoes orchards are famous. It is known as Pakistan's largest city for production of these commodities (GOP, 2016). Most of the cultivated soils of this district have undergone major changes due to the cultivation of exhaustive crops resulting in land deterioration. Non-judicious input application to increase crop productivity is also a major problem. There is an increasing concern that herbicides not only affect the target organisms (weeds) but also the microbial communities present in soils, and these non-target effects may reduce the performance of important soil functions (Hütsch, 2001). Improved soil quality often indicated by increased infiltration, aeration,

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macropores, aggregate size, aggregate stability and soil organic matter, decreased bulk density, soil resistance, erosion and nutrient runoff and presence of soil microorganisms (Parr *et al.*, 1992). One of its important aspects is the microbial status of the soil especially the dominance of pathogenic microbes including fungi, bacteria and viruses. Soil microorganisms play pivotal role in various biogeochemical cycles and are considered to be important for soil quality and crop productivity (Wall and Virginia, 1999). The soil, like air and water, is an integral component of our environment and together with water constitutes the most important natural resource. The wise use of this vital resource is essential for sustainable development and feeding the growing world population. Information about soil microbes helps in determining soil health and its compatibility with plant ecosystem and availability of nutrients to plants (Azam *et al.*, 2003; DeLorenzo *et al.*, 2001). The development of effective methods for studying the diversity, distribution, and behavior of microorganisms in soil habitats is essential for a broader understanding of soil health (Nandhini and Josephine, 2013). Soil microorganisms also influence aboveground ecosystems

by contributing to plant health and nutrition, soil fertility and its structure (Filion Cassel and Wollum, 1999; O'donnell *et al.*, 2001).

In the past decades, several studies have dealt with the selection of suitable criteria for assessment of soil microbes (Haney *et al.*, 2001). Literature reports several studies on soil bacterial communities, very little research has been undertaken for soil fungus (Duarte *et al.*, 1998).

Considering the vital role of district Okara in agriculture economy of the province a baseline was designed to report soilborne plant pathogenic fungal count in different land use types. The studies were conducted at Institute of Agricultural Sciences (IAGS) and College of Earth and Environmental Sciences, University of the Punjab, Lahore.

MATERIAL AND METHODS

Sample collection: Okara district features a humid subtropical climate with hot in summer and cold in winter. The climate of the region has considerable temperature May and June is the hottest months with the maximum temperature reaching 44 °C. January is the coldest month with minimum temperature falling to 2C. The average annual rainfall is 200 mm (GOP, 2016). A two years survey was conducted during November – December 2014 and 2015 in three tehsils; Okara, Pakpattan and Sahiwal. Soil samples were collected with the help of hard steel type of auger of 91cm. The length and diameter of borer was 13 and 4 cm respectively. Samples were taken out of 15,30 and 45 cm soil depths that is sowing, ploughing and at root depth respectively from barren land, cultivated land, forest, orchard and fallow land. A total of seventy-five samples were taken from five different land use types, fifteen samples per site and five samples at different depths of 15, 30 and 45 cm respectively. Collected samples were kept in dry condition and packed in 18×24 cm cellulose bags labelled with information including sampling date, place and source of collection and were designated with the special code of identification.

Samples were uniformly mixed air dried, homogenized and passed through 2mm mesh sieve followed by transferring to wide-mouthed screw-topped numbered plastic bottles/jars. The bulk sample was divided into subsamples or working samples weighing 300-500g.

Isolation of Fungal Colonies: Five grams of sieved soil was added to 500 ml sterilized distilled water in a commercial blender mixed at medium revolutions. The

solution was blended three times for 30 sec with a 3 min interval between the two blendings. The blended mixture was passed through a 45µm sieve in gently running tap water. Finally, 10ml of the mixture was added in 15 ml cultural tubes containing MEA medium. 1 ml of soil suspension was aseptically poured in Petri plates containing PDA media. The plates were gently rotated so as to spread the suspension on medium. These plates were incubated at 25±2 °C for 5-7 days (Nandhini and Josephine, 2013).

Identification of Fungal Colonies: Pure cultures were obtained by transferring a media plugs (4 mm diameter) from the margins of actively growing fungal colonies to a new Petri plate containing PDA media. The fungal colonies were identified based on their microscopic studies and morphological descriptions with the help of the key developed by Barnett and Hunter (2003).

RESULTS AND DISCUSSION

Mucor spp., *Fusarium solani*, *Aspergillus niger*, *Penicillium* spp., *Alternaria alternata*, *Curvularia* spp., *Rhizopus* spp., and *Helminthosporium* spp. were recovered from soil samples collected from different land use types. A colony of *Mucor* spp. was typically white to grey. Fine threads crowned with ball-shaped clusters of spores. Sporangiohores formed globular sporangia that were supported and elevated by a column-shaped columella. Morphological descriptions of the *Mucor* spp. were in line with those described by Konig and Jurgen (2009) and Saito *et al.* (2016).

Fungal colonies of black coloration and consisted of mats of hyphae that made up a mycelium with septate and hyaline hyphae, conidiophores originated from the basal foot cell located on the supporting hyphae and terminated in a vesicle at the apex confirmed the descriptions of *Aspergillus niger* as described by Altieri *et al.* (2007) and Pawar *et al.* (2008).

Fungal colonies identified as *Alternaria alternata* were brownish grey to black in colouration with white edges. Spores were large, brown, multicelled, obclavate and beaked as described by Picco and Rodolfi (2004) and Akhtar *et al.* (2004).

Cells of the *Penicillium* spp. were multicellular and ellipsoid. Spores were present in the form of conidia and phialidies. The conidiospores were branched while conidia were round and unicellular. Fungal colonies were initially white on PDA medium that later turned blue-green to grey-green. Similar descriptions of the *Penicillium* spp. were also reported by Torelli *et al.*

(2006) and Ghuffar *et al.* (2017).

Colonies of the *Rhizopus* spp. were white to greyish in colouration. Coenocytic cells in hyphae with septa were only present where gametes formed. Many haploid sporangiospores were held within the sporangia structure, first as a cottony white structure and then turned black on the surface. These descriptions for the identification of *Rhizopus* spp. were in line with those reported by Corison *et al.* (1979) and Kim and Baek (2011).

Fungal colonies of colony texture with velvety to woolly appearance and olive green to black from the front and black in colour from the reverse confirmed as *Helminthosporium* spp. Fungal hyphae were septate, conidiophores were brown to dark brown, erect while conidia were club-shaped, pale to dark brown in colour as described by Larone (1995) and Alcorn (1988).

Curvularia spp. produced woolly colonies. The colour of the colony was white to pinkish grey initially that later

turned olive brown to black. Septate, brown conidiophores, brown hyphae, and conidia were visualized. Conidiophores were simple or branched, bent from where the conidia originated. These descriptions were in line with the findings of Coombe *et al.* (1968) and Ashwathi *et al.* (2017).

Table 1 shows the presence of fungi at different depths of land use types. *Helminthosporium* spp. was found at the depth of 15 cm only mostly in forest, orchard and vegetable fields. It is basically a surface contaminant. *Rhizopus* spp. was only found at 15cm depth in vegetable fields and in the forest while absent at other depths in these lands and other land use types. *Curvularia* spp. was also found at 15cm and 30 cm in the vegetable fields, however not found in other types of the lands. *A. alternata* was predominant only in vegetable fields at 15cm depth. *Penicillium* spp. and *A. niger* was present at both depths 15 cm as well as at 30 cm in forest and vegetable fields.

Table 1. Isolated Fungi from different Land use types at different depths.

Land types	Depth cm	Fungi isolated from soil (no. of spores per gram of soil)							
		<i>Mucor</i> spp.	<i>Fusarium solani</i>	<i>Penicillium</i> spp.	<i>Alternaria alternata</i>	<i>Curvularia</i>	<i>Rhizopus</i> spp.	<i>Aspergillus niger</i>	<i>Helminthosporium</i>
Forest	15	9	5	6	0	0	5	7	4
	30	5	2	3	0	0	0	5	0
	45	2	0	0	0	0	0	0	0
Orchard	15	7	0	0	0	0	0	0	3
	30	5	0	0	0	0	0	0	0
	45	3	0	0	0	0	0	0	0
Vegetable fields	15	0	8	5	3	4	3	5	3
	30	4	8	2	0	5	0	4	0
	45	2	3	0	0	0	0	0	0
Barren lands	15	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0
Fallow lands	15	0	6	0	0	0	0	0	0
	30	0	6	0	0	0	0	0	0
	45	0	2	0	0	0	0	0	0

Mucor spp. and *F. solani* were found frequently occurring almost at all the depths with a different number of spores per gram of soil. *F. solani* was not found in orchards as well as in barren land while isolated from all depths in fallow and vegetable fields and in the forest

only found at 15 and 30 cm.

Different isolates of *Fusarium* spp. were isolated from soil samples collected from a mangrove forest Malaysia were isolated using soil dilution technique. Based on identification using morphological characteristics, *F.*

solani was the most common species recovered from soil (Zakaria *et al.*, 2010). *Mucor* spp. was absent in barren and fallow lands while prevailing in other lands types. *Mucor* spp. was isolated in maximum number in forest, orchard and vegetable fields except for 15 cm depth of vegetable fields mentioned.

The presence of surface contaminants was not observed at the plough and root depths. This is primarily linked with the physical and chemical properties of the soil at root depth. It links stronger with the nature of the dead plant material and soil moisture percentage. *Mucor* spp., *F. solani*, *A. niger*, *Penicillium* spp., *A. alternata*, *Curvularia* spp., *Rhizopus* spp., and *Helminthosporium* spp. are soilborne as well as seed borne with respect to their count on feeding roots in the soil and has the potential to become problematic for forest and orchards. Mostly saprophytic fungi like *Mucor* and *Rhizopus* spp., grow on organic substances including dead plant debris or animal waste materials in soil (Kim *et al.*, 2014). Travelling of plant pathogenic fungi below ploughing depth creates an alarming situation for deep-rooted crops infestation in future. Barren and fallow lands have lack of organic matter in these land use types (Allison, 1973). In the present studies, these land use types remained free from fungi at all depths.

Measuring microbial biomass is a valuable tool for understanding and predicting long term effects on changes in land use and associated soil conditions (Sharma *et al.*, 2004).

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