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ISOLATION OF FUNGI FROM VARIOUS AGRICULATURAL FIELDS AS A FUNCTION OF SOIL DEPTH AND SEASONAL VARIABILITY

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ABSTRACT

Study of population and species occurrence of fungi as a function of soil depth and seasons was conducted during 2009 and 10. The samples were collected from ten agricultural fields at Quetta district. Soil sampling was conducted during four seasons such as February, 2009 and 10 (S1 and S3) and August, 2009 and 10 (S2 and S4) across soil depth of 0-06 and 06-12 inches. The serial dilution plate technique was used for isolation of fungi which were significantly affected by seasonal variability and soil depth. The results exhibited higher fungal (18.30 cfu g^{-1}soil) population in location 9 at upper depth in S_1 and lower (6.50 cfu g^{-1}soil) in location 8 at lower depth. From each location 18 fungal species were isolated and *Aspergillus niger* was found dominant fungal species in all locations. From this study it was suggested that less perturbed soil like orchards showed more O.M. contents indicating higher available nutrients and high fungal population than field crops which were subjected to more perturbation.

Keywords: Agricultural fields, fungal population, species, soil depth, seasons

INTRODUCTION

A single gram of fertile soil reveal the biological universe having all microbiota such as bacteria, archaea and eukarya with life indispensible elements including H, C,N,P,O and S. These elements are transformed through biogeochemical process between abiotic and biotic matter across the interfaces of soil, air and water (Madsen, 2008). The biogeochemical cycling of the nutrients is depending on soil microorganisms. All the soil microorganisms such as bacteria, fungi, archaea and algae, soil animals including protozoa, nematodes, mites, springtails, spiders, insects and earthworms as well as plants form the soil biota (SQI, 2001) that are living entirely in soil or part of their lives in soil that affected soil use and management (Brady and Weil, 2002). Soil organisms play a major role in the pedosphere and their prominent task is regulation of biogeochemical transformations like mineralization and immobilization of organic matter, nutrient cycling, transmission and prevention of diseases, decomposition of pollutant and improvement in soil aggregation (Gupta *et al.*, 1997). In addition, the soil micro biota is also responsible for regulation of greenhouse gases (CO_2, CH_4 and N_2O) during the metabolic oxidation or reduction of carbon and nitrogen compounds in soil which are further stimulated by soil management practices like N fertilization and tillage operations (Madsen, 2008; GGWG, 2010).

Fungi are an indispensible part of soil micro biota and its microbial biomass is greater than bacteria (Ainsworth and Bisby, 1995) depending on soil depth and nutrient conditions. in soil, its exist in the form of actively growing organisms and as latent particles (Warcup, 1957). The function of fungi in soil is tremendously multifarious and vital to the whole soil ecosystem (Bridge and Spooner, 2001) because micro-fungi have major contribution in the global nutrient cycle and they decompose complex structures of plant origin like cellulose, hemicellulose and lignin in soil and mineralized nutrients in these plant debris (Christensen, 1989). The multiplicity of soil micro fungi is indispensible for the stability and productivity of ecosystem and many of them are the potential source of various chemicals of industrial importance like pharmaceutics and agrochemical (Petersen and Hughes, 1999.). The flora of fungi is characterized to innate soil and is extensively distribution in soil which is affected by the levels of soil organic carbon, environmental conditions, soil physical and chemical nature, surface cover with plants and vegetation (Devi *et al.,* 2012). The population of micro fungi in forest soil is comparatively higher than those in agricultural soil where the physical, chemical and biological environ is remain undisturbed and not subjected to land use management. On the other hand, low fungal population in agriculture soil is due to low soil organic carbon and also

because of the pesticides and fungicides contamination for a long time (Bissett and Parkinson, 1979; Osono and Hirose, 2009). According to Song *et al.,* (2004) that the fungal population and diversity is also affected by some physical and chemical characteristics of soil like pH, temperature, moisture and texture along with land topography such as altitude. In a study conducted by Devi *et al.,* (2012) that the number of fungal isolates and CFU counts were found to decrease with increase in altitude and opposite was recorded at lower latitude. The identification and classification of fungi is generally carried out by using the morphological characteristics particularly their sexual structures showing distinct variability (Burnett, 2003).

Soil is the natural habitat of soil microorganisms and is vital component of ecosystem that includes soil fungi, bacteria, actinomycetes and others. Soil fungi are responsible for the decomposition of organic matter, nutrients availability and improve soil quality sustainably (Hackel *et al.,* 2004). Soil physical, chemical and biological properties are controlled by soil microorganisms (Tangjang and Arunachalam, 2009) and its soil biomass is greater than bacteria (Ainsworth and Bisby, 1995). For the study of soil microbial communities in different agricultural soils, Franklin and Mills (2003) used sampling depth from 2.5 to 11 cm and indicated that soil microbial communities makes different organizational levels which are directly dependent on soil properties. Similarly, the soil microbial population in agricultural soil is also affected by seasonal variability (Grantina *et al.,* 2012). Keeping in view of the importance of fungi in agriculture soil system, the present study was conducted to evaluate the assessment of fungi in different agricultural soil as affected by seasonal and spatial variation.

MATERIALS AND METHODS

The study area: Study was conducted in area encompassed the district Quetta, the capital of Balochistan province (Pakistan), where 10 different agricultural fields comprising of five apple orchards (location 6-10), one grape vineyard (location 5) and four different crop fields such as wheat crop field (location 3), vegetable field (location 4), floriculture field (location 1) and botanical garden (location 2) were selected. The latitude and longitude were recorded as sampling points using GPS and details pertaining to all ten locations along with their respective location name, latitude, longitude and with more detail of locations are given in **Table 1**.

Table - 1: Ten agricultural fields/orchards selected for sampling to study fungi as a function of soil depth and seasonal variability during 2009-10

S.No.	Name of location	Latitude	Longitude	Detail
1	Rani Bagh	30°10'19.11"N	66°59'41.69"E	Floriculture field, Agriculture Extension, Rani Bagh Quetta
2	Botanical garden	30°9′54.57"N	66°59′30.29"E	University of Balochistan
3	Exp. field of Agronomy	30° 6'50.27"N	66°58'44.17"E	Agriculture Research Institute (ARI) Quetta
4	Vegetable Seed farm	30°6'26.99"N	66°58'18.92"E	Agriculture Research Institute (ARI) Quetta
5	Grape vine	30°9'53.10"N	66°58'21.24"E	Qirani road, Quetta.
6	Chisma Achozai	30°16'36.67"N	66°58'46.49"E	Apple orchard Chisma Achozai, Quetta.
7	Agriculture College	30°15'58.99"N	66°55'47.90"E	Apple orchard, Agriculture College Baleli Quetta.
8	SAKA	30°11'48.12"N	66°57'41.33"E	Apple orchard, SAKA cold storage Barori, Quetta.
9	Hanna urak	30°16'31.95"N	67°10'23.28"E	Apple orchard Hanna Quetta
10	Kuchlak	30°24'12.04"N	66°58'59.73"E	Sahibzada apple orchard kuchlak, Quetta.

Soil sampling and analysis: Soil sampling was collected during four seasons such as S1 (February, 2009), S2 (August, 2009), S3 (February 2010), S4 (August 2010) with two depths (0-02 and 02-04 inches) from all locations at three position 20 feet apart as replicates. During sampling, Soil auger was disinfected with ethanol for each depth and location. The samples were kept in sterilized polythene bags and delivered to Laboratory of CASVEB University of Balochistan in boxes having small ice blocks

enclosed in polythene bags and stored in the refrigerator at 4°C for microbial study.

Pre-soil analysis of ten agriculture fields in Quetta district was carried out at Soil and Water Testing Laboratory, ARI, Sariab Quetta for determination of the physico-chemical properties and nutrient status of soil (Table 2). The ratio of three soil separates was found by hydrometer method (Bouyoucos, 1962), pH and EC in 1:5 ratio of soil water suspension (McKeague, 1978; McLean, 1982), organic matter by Walkley and Black (1934) method, total nitrogen by Kjeldhal method (Jones, 1991). AB-DTPA method was followed for extracting phosphorus, potassium, copper, iron, manganese and zinc from soil (Soltanpur and Schwab, 1977).

Isolation of fungi: Fungi in ten different agricultural soils were assessed by dilution plate method (Johnson and Curl, 1972; Frankland *et al.,* 1990; Parkinson *et al.,* 1971; Trevors, 1998; Tabacchioni *et al.,* 2000). One gram soil was serially diluted from 10^{-1} to 10^{-6} with sterilized distilled water using 250 ml flasks. 1 ml from the soil water suspension of 10^{-3} to 10^{-6} dilution was taken and used in different media plates. Three types of media were used for isolation of fungi viz, Potato Dextrose Agar, Dextrose-peptone-yeast extract Agar (DPYA) and V-8 Agar (Smith and Onions, 1994). These media were freshly prepared according to their respective recipes given in the mycological laboratory manual (Anon*.,* 1984; Smith and Onions, 1994). After sterilization in autoclave at 121 C° with 15 lb in^{-2} for 20 minutes and cooling at 45 °C, the pH of the media was adjusted to neutral and slightly alkaline using sterilized potassium hydroxides or hydrochloric acid (Penn, 1991). All the petri dishes, test tubes, inoculating needle, and other materials were sterilized in oven at 230 °C for 60 minutes before its usage in this study. Approximately 14 ml media was poured in the petri dishes following the standard laboratory procedure and then 1 ml of soil water suspension (from 10^{-1} to 10^{-6}) were added in the form of streak. Finally, the petri dishes containing media and soil suspension were incubated at 25 C° for 5 days. The total number of colonies developed during incubation were counted using colony counter as per location site and its replication. These different colonies were identified with respect to specific fungal species using macro and microscopic technique following the method of Booth (1971) and Onions *et al.* (1981). Lactophenol was used as mountant and aniline blue and trypan blue were used for staining (Parkinson *et al.,* 1971; Frankland *et al.,* 1990).

Statistical analysis: Three way factorial analysis was carried out for fungal population while descriptive statistics was adopted for species occurrence using Statistix 8.1 computer software. The LSD value for mean comparison was calculated only if the general treatment F test was significant at probability of ≤0.05 (Gomez and Gomez, 1984).

RESULT AND DISCUSSION

This study was conducted during 2009 and 2010 at Quetta district to evaluate fungal population and species occurrence of ten different agriculture fields as affected by various seasons and soil depths. The soil of the sampling points were also analyzed for physicochemical properties and nutrient status so that its effect on soil fungi can be studied. The soil physical properties were investigated in the form of three soil separates such as sand, silt and clay, Textural class and O.M. contents. Chemical properties were analyzed for electrical conductivity (EC), pH, total N, available P, K, Cu, Fe, Mn and Zn. While soil fungi which were isolated from bulk soil and their respective population were determined in the form of colony forming unit per gram soil (cfu g^{-1} soil). Similar microbial isolation such as soil bacteria, fungi and actinomycetes counts was also investigated by Sonya *et al.* (2012).

The Pre-soil analysis of ten agricultural fields as given in Table -2 revealed that out of 10 locations, 6 were found sandy loam (i.e. location 1-5 and 8), 2 were clay loam (location 6 and 9) and the other two were sandy clay loam (location 7 and 10) in texture and were alkaline in nature having pH from 7.88 to 8.09, 8.10, 8.01, 8.16, 8.05, 8.00, 8.30, 8.23 and 8.21. The electrical conductivity of all locations was less than 4 dSm^{-1}. The organic matter contents of these locations were from 0.82 to 0.94, 1.26, 0.63, 0.57, 0.79, 1.01, 0.46, 1.25 and 1.08%. The fertility status of these agricultural fields indicated that the nitrogen and phosphorus were deficient whereas, iron and zinc were in low range but copper and manganese were in sufficient range. However, the potassium level of all locations was high (**Table 2**).

Table - 2: Pre soil analysis of ten agriculture fields for the determination of physicochemical properties and nutrient status

Soil properties	Units	Agricultural fields									
		1	2	3	4	5	6	7	8	9	10
Sand	%	66.0	60.0	65.0	59.1	63.0	40.0	55.0	67.5	36.0	58.0
Silt	%	18.0	27.7	17.0	21.9	19.0	29.0	20.0	16.3	29.4	15.6
Clay	%	16.0	12.3	18.0	19.0	18.0	31.0	25.0	16.2	34.6	26.4
Textural class		Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Clay loam	Sandy clay loam	Sandy loam	Clay loam	Sandy clay loam
pH		7.88	8.09	8.10	8.01	8.16	8.05	8.00	8.30	8.23	8.21
EC	dSm^{-1}	2.92	4.11	3.68	3.73	3.09	3.71	2.58	3.79	4.55	3.69
Organic matter	%	0.82	0.94	1.26	0.63	0.57	0.79	1.01	0.46	1.25	1.08
Total nitrogen	%	0.050	0.054	0.071	0.040	0.035	0.055	0.059	0.031	0.074	0.061
AB-DTPA extractable P,K, Cu, Fe, Mn and Zn											
Phosphorus	ppm	0.58	0.67	0.68	0.66	1.93	2.37	1.40	0.77	2.00	1.92
Potassium	ppm	185	172	160	170	226	136	157	240	335	279
Copper	ppm	0.47	0.54	0.56	0.33	0.46	0.42	0.60	0.44	0.74	0.51
Iron	ppm	3.17	2.97	3.87	3.24	2.64	2.48	3.45	2.67	3.24	2.93
Mn	ppm	0.80	0.93	1.20	0.82	0.72	0.86	1.10	0.69	1.29	0.86
Zn	ppm	1.05	0.78	1.07	0.59	0.68	0.88	1.04	0.53	1.30	0.99

The three way factorial analysis used to check the effect of seasonal variability and soil depth on soil fungi (cfu g^{-1} soil) in ten agricultural fields revealed significant ($p<0.05$) differences. The overall fungal population in 10 locations showed maximum cfu from 21 to 25, 25, 16, 17, 19, 26, 11, 29 and 27 while minimum was ranged from 6 to 6, 8, 5, 5, 5, 7, 3, 7, and 7 cfu g^{-1} soil respectively. The LSD test ($p<0.05$) for mean comparison (Table 3) showed highest (16.88 cfu g^{-1} soil) fungal population in location 9 whereas location 8 indicated the lowest (6.83) number of cfu. Location 1 and 6, 2 and 3, 4 and 5, 10 and 7 were statistically same from one another but were differed over other locations. The causes of such fluctuation in their population dynamics might be due to its association with soil nutrient status and soil O.M. The variation in microbial population dynamics across different agricultural fields within an area is maneuvered by variety of environmental elements such as soil texture, moisture, temperature, pH, O.M., nutrients and other physic-chemical properties of soil (Kennedy *et al.,* 2005). The variation in soil fungal and bacterial population in ten different agricultural fields supports the observations of Laverman *et al.,* (2002) who recorded a greater spatial variability in nitrifying bacterial population using one meter spatial scale. In another study conducted by Klironomos *et al.,* (1999) indicated that soil organisms vary even at smaller spatial scale of 0.2 m. The results of this study is supported by the findings of Vasanthakumari and Shivanna (2011) who observed variation in fungal communities across seasons, plant types and nutrient status of soil. The effect of soil depth on fungal cfu was also significant and the upper depth of 0-6 inch showed highest fungal population of 13.99 cfu g^{-1} soil as compared to the soil depth of 06-12 inch which is account for 17.56% increase over soil depth of 06-12 inch (Table 3). Like soil depth, significant variability was noted among the seasons with respect to fungal cfu g^{-1}soil. Soil sampling during 2009 and 10 in the month of February revealed highest significant fungal population (16.13 and 14.28 cfu g^{-1} soil) when compared to August sampling. The maximum number of cfu during the four seasons (S1, S2, S3 and S4) was from 29 to 22, 26 and 16 while the minimum was from 6 to 2, 3 and 3. The seasonal variability was recorded in the order like S1>S3>S2>S4. But the interaction between location x season, location x depth, Season x Depth and Location x Season x Depth were statistically found non-significant. Such explanation coincides with the observations noted by Berg and Verhoef (1998) who reported that the seasonal fluctuation in the population of soil flora corresponds to changing pattern in soil moisture and temperature regime. The findings of Das *et al.,* (2013) are resemble with our results who observed higher bacterial count in the surface 0-10 cm depth during spring season and some other researcher like Jha *et al.,* (1992) who noted higher bacterial population in spring season and after rainy seasons.

Table - 3: Fungal colony forming units (cfu/g soil) of ten agricultural fields as affected by seasonal variability at two different depths

Study factors	Fungal colony forming unit (cfu/g soil)
Locations	
1	12.75 d
2	14.54 c
3	14.83 bc
4	9.92 f
5	10.38 ef
6	11.54 de
7	15.79 ab
8	6.83 g
9	16.87 a
10	16.0 ab
S.E. ±	0.63
LSD at 5% probability	1.24
Seasons	
S1	16.13 a
S2	12.48 c
S3	14.28 b
S4	8.88 d
S.E. ±	0.40
LSD at 5% probability	0.78
Depths	
0-06 inch	13.99 a
06-12 inch	11.90 b
S.E. ±	0.28
LSD at 5% probability	0.55
Interactions	**F value**
Locations	53.33 **
Seasons	121.80**
Depths	55.76 **
Locations x seasons	0.94 NS
Locations x depths	0.51 NS
Seasons x Depths	1.57 NS
Locations x seasons x depths	0.12 NS

Means followed by common letter are not significantly different at 5% probability level.
**Highly significant NS Non-significant

The number of fungal species and number of fungi per gram soil as affected by seasonal variability with respect to soil depths are given in Table **4-6**. The soils of three apple orchards i.e. location 7, 9 and 10 indicated maximum number of cfu in all seasons and in both depths as compared to other locations, while the minimum number of cfu were noted in location 4, 5 and 8. In the month of February as season one (S1) and three (S3) during both years the cfu in all location were high but low in the month of August (S2 and S4). The results regarding seasonal changes in fungal (cfu g^{-1}soil) were found contradicted with the results of Blume *et al.,* (2002) who observed no significant differences in microbial biomass for upper and lower depth across winter and summer samples from two soils and similar seasonal influence was recorded by Holmes and Zac (1994). However, in both months (February and August) the surface soil sampling (0-6 inch) indicated more cfu than below the surface (06-12 inch). The seasonal effects on soil microbial biomass in the surface and shallow subsurface as studied by other scientists are contradictory. Greater microbial biomass was observed in summer than in winter according to the study of Buchanan and King (1992) who explained that this high microbial biomass is might be due to high temperature in summer. But the study of Baath and Soderstror (1982) and Sarathchandra *et al.,* (1989) showed that the soil microbial biomass was high in spring and lower in summer and winter.

Those agricultural field having organic matter more than 1% and nutrient concentration in sufficient range showed high number of cfu. The total number cfu in all locations at 0-6 inch depth was found higher (556 cfu) in comparison to the soil depth of 06-12 inch which was 464 cfu (Table 4 and 5). From each location 18 fungal species were isolated that representing nine genera. Five species belonged to genus *Aspergillus*, two species belonged is each genus of *Cladosporium, Penicillum* and *Trichoderma*. Three species to *Glomus*, while the rest of genera *Mucor*, *Fusarium*, *Rhizopus* and *Scutello spora* were represented by one species. In the upper 6 inch soil, total number of cfu of 18 fungal species in 1-10 locations from 54 to 62, 62, 44, 44, 51, 26, 73 and 69 (Table 4). Among these species, the *Aspergillus niger* was found the most dominant species in all locations whose percent occurrence was from 13.0 to 14.5, 9.7, 11.4, 9.1, 13.7, 7.7, 13.7 and 15.9 % followed by *Penicillum rubrum* whose percent occurrence was from 11.1 to 9.7, 6.5, 6.8, 11.4, 7.8, 9.9, 11.5, 6.8 and 5.8 %. Whereas, the percent occurrence of *Fusarium oxysporium* and *Scutello spora pellucid* was lowest in all locations. *Fusarium* was from 1.9 to 1.6, 4.8, 4.5, 2.3, 3.9, 5.6, 3.8, 2.7 and 2.9 % while, *Scutellospora* was from 1.9 to 4.8, 3.2, 2.3, 4.5, 3.9, 4.2, 3.8, 2.7 and 2.9%.

Table - 4: Assessment of fungi in ten different agricultural fields with soil depth of 0-06 inch as number of colonies and percent occurrence of fungal species

Fungal species	Agricultural fields																			
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
	No. of colonies										Occurrence (%)									
Aspergillus niger	7	9	6	5	4	7	8	2	10	11	13.0	14.5	9.7	11.4	9.1	13.7	11.3	7.7	13.7	15.9
A. ridulens	5	6	4	3	2	6	7	2	5	8	9.3	9.7	6.5	6.8	4.5	11.8	9.9	7.7	6.8	11.6
A. flavus	2	3	4	1	1	1	3	1	4	3	3.7	4.8	6.5	2.3	2.3	2.0	4.2	3.8	5.5	4.3
A. candidus	2	4	5	1	3	2	4	1	3	2	3.7	6.5	8.1	2.3	6.8	3.9	5.6	3.8	4.1	2.9
A. ustus	1	2	3	1	3	3	2	1	2	3	1.9	3.2	4.8	2.3	6.8	5.9	2.8	3.8	2.7	4.3
Cladosporium cladosporoides	3	5	4	2	2	5	6	2	5	4	5.6	8.1	6.5	4.5	4.5	9.8	8.5	7.7	6.8	5.8
C. lunata	2	1	3	1	3	1	3	1	3	2	3.7	1.6	4.8	2.3	6.8	2.0	4.2	3.8	4.1	2.9
Fusarium oxysporium	1	1	3	2	1	2	4	1	2	2	1.9	1.6	4.8	4.5	2.3	3.9	5.6	3.8	2.7	2.9
Glomus mosseae	4	2	5	4	2	3	2	1	5	4	7.4	3.2	8.1	9.1	4.5	5.9	2.8	3.8	6.8	5.8
G. ambisporium	2	1	1	3	1	2	3	2	2	2	3.7	1.6	1.6	6.8	2.3	3.9	4.2	7.7	2.7	2.9
G. formosanum	1	4	1	3	2	2	3	1	2	1	1.9	6.5	1.6	6.8	4.5	3.9	4.2	3.8	2.7	1.4
Mucor mucido	3	5	3	4	3	2	4	2	5	6	5.6	8.1	4.8	9.1	6.8	3.9	5.6	7.7	6.8	8.7
Pencillum rubrum	6	6	4	3	5	4	7	3	5	4	11.1	9.7	6.5	6.8	11.4	7.8	9.9	11.5	6.8	5.8
P. purprogerum	2	1	2	3	2	1	3	1	4	3	3.7	1.6	3.2	6.8	4.5	2.0	4.2	3.8	5.5	4.3
Rhizopus arrhizus	3	2	6	2	4	5	4	1	4	3	5.6	3.2	9.7	4.5	9.1	9.8	5.6	3.8	5.5	4.3
Scutellospora pellucid	1	3	2	1	2	2	3	1	2	2	1.9	4.8	3.2	2.3	4.5	3.9	4.2	3.8	2.7	2.9
Trichoderma lignorum	5	5	3	4	2	2	3	2	6	5	9.3	8.1	4.8	9.1	4.5	3.9	4.2	7.7	8.2	7.2
T. viride	4	2	3	1	2	1	2	1	4	4	7.4	3.2	4.8	2.3	4.5	2.0	2.8	3.8	5.5	5.8
Total	54	62	64	44	45	51	71	26	73	69										

However, in the soil depth of 06-2 inch the total cfu of fungal species were decreased in comparison to the upper 0-6 inch soil and enumerated from 47 to 52, 55, 36, 33, 40, 57, 25, 60 and 59 cfu (**Table 5**). But percent occurrence of some species were found higher than the upper 6 inch soil. This might be due to some favourable conditions of physical and chemical nature for those fungal species. Blue *et al.,* (2002) observed that due to localized carbonate deposit increased pH at shallow subsurface which might be resulted in increased fungal species. His findings further revealed that the microbial activity was noted higher in winter at both surface and subsurface but the microbial structure was increased at subsurface and suggested that high temperature increased microbial population resulting in more nutrient utilization as well as organic matter which enter into the soil as inputs. The reasons for these variations in the percent occurrence of different fungal and bacterial species across soil depths and locations can't be explained through this study because most of the species whether fungal or bacterial showed percent dominancy in those soil where other soil physical, chemical and biological properties do not seem conducive as exhibited in the results. And the some species percent occurrence was observed higher at lower soil depth of 06-12 inch over the upper depth (0-06 inch). According to Stagnari *et al.,* (2014) that accurate demarcation of soil microbial community organization, diversity and their functions still remains a black box. Fierer and Jackson (2006) narrated that limited number of soil microbes can be isolated and identified through culturing techniques and their role in agro-ecosystem. Researchers like Vogel *et al.,* (2009) reported about the project known as TerraGenoma which highlighted the enormous unevenness in soil microbial richness and distribution under the influence of space, time and management and ratifying the complex nature of organic and conventional ecosystem. These results are supported by the findings of Griffiths *et al.,* (1999) who revealed that the addition of organic C to soil will increase the magnitudes of fungi.

Table -5: Assessment of fungi in ten different agricultural fields with soil depth of 06-12 inch as number of colonies and fungal pecies percent occurrence

Fungal species	Agricultural fields																			
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
	No. of colonies										Occurrence (%)									
Aspergillus niger	5	7	6	3	2	4	6	3	7	7	10.6	13.5	10.9	8.3	6.1	10.0	10.5	12.0	11.7	11.9
A. ridulens	3	4	5	2	2	3	4	1	4	6	6.4	7.7	9.1	5.6	6.1	7.5	7.0	4.0	6.7	10.2
A. flavus	1	2	3	1	1	1	2	1	2	2	2.1	3.8	5.5	2.8	3.0	2.5	3.5	4.0	3.3	3.4
A. candidus	1	2	2	1	2	1	3	1	3	2	2.1	3.8	3.6	2.8	6.1	2.5	5.3	4.0	5.0	3.4
A. ustus	2	3	2	1	2	1	3	1	3	3	4.3	5.8	3.6	2.8	6.1	2.5	5.3	4.0	5.0	5.1
Cladosporium cladosporoides	3	5	4	3	1	4	5	1	6	3	6.4	9.6	7.3	8.3	3.0	10.0	8.8	4.0	10.0	5.1
C. lunata	2	2	1	1	2	1	3	1	3	3	4.3	3.8	1.8	2.8	6.1	2.5	5.3	4.0	5.0	5.1
Fusarium oxysporium	1	1	1	3	1	1	3	1	3	1	2.1	1.9	1.8	8.3	3.0	2.5	5.3	4.0	5.0	1.7
Glomus mosseae	3	2	4	3	2	4	4	2	5	3	6.4	3.8	7.3	8.3	6.1	10.0	7.0	8.0	8.3	5.1
G. ambisporium	1	1	2	2	1	2	1	2	2	3	2.1	1.9	3.6	5.6	3.0	5.0	1.8	8.0	3.3	5.1
G. formosanum	1	2	2	2	1	2	1	1	2	2	2.1	3.8	3.6	5.6	3.0	5.0	1.8	4.0	3.3	3.4
Mucor mucido	5	3	4	3	3	2	3	2	3	5	10.6	5.8	7.3	8.3	9.1	5.0	5.3	8.0	5.0	8.5
Pencillum rubrum	5	4	3	2	3	5	4	3	3	3	10.6	7.7	5.5	5.6	9.1	12.5	7.0	12.0	5.0	5.1
P. purprogerum	1	1	2	2	2	2	3	1	3	3	2.1	1.9	3.6	5.6	6.1	5.0	5.3	4.0	5.0	5.1
Rhizopus arrhizus	3	4	5	3	2	4	4	1	5	3	6.4	7.7	9.1	8.3	6.1	10.0	7.0	4.0	8.3	5.1
Scutellospora pellucid	1	2	2	1	2	1	3	1	1	3	2.1	3.8	3.6	2.8	6.1	2.5	5.3	4.0	1.7	5.1
Trichoderma lignorum	5	5	4	2	3	1	3	1	3	4	10.6	9.6	7.3	5.6	9.1	2.5	5.3	4.0	5.0	6.8
T. viride	4	2	3	1	1	1	2	1	2	3	8.5	3.8	5.5	2.8	3.0	2.5	3.5	4.0	3.3	5.1
Total	47	52	53	36	34	40	57	25	60	59										

Table- 6: Assessment of fungi in ten agricultural fields as number of colonies in serial dilution plate method and number of fungi per gram soil influencing by seasonal variability

Seasons	Dilution	No. of colonies in serial dilution										No. of organism per gram of soil									
		Different agricultural field										Different agricultural field									
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
		0-06 inch depth																			
S1	10^{-3}	9	10	11	7	7	8	11	5	12	11										
	10^{-4}	5	6	6	4	4	5	7	3	7	7										
	10^{-5}	2	2	2	2	2	2	3	1	3	3										
	10^{-6}	1	1	1	1	1	1	1	0	1	1	$17x10^6$	$19x10^6$	$20x10^6$	$14x10^6$	$14x10^6$	$16x10^6$	$22x10^6$	$9x10^6$	$23x10^6$	$22x10^6$
S2	10^{-3}	8	9	9	6	6	7	10	4	10	9										
	10^{-4}	4	4	4	3	3	3	5	2	5	5										
	10^{-5}	2	2	2	2	2	2	2	1	1	2										
	10^{-6}	0	0	0	0	0	0	1	0	1	0	$13x10^6$	$15x10^6$	$15x10^6$	$11x10^6$	$11x10^6$	$12x10^6$	$18x10^6$	$7x10^6$	$16x10^6$	$16x10^6$
S3	10^{-3}	8	9	9	6	6	7	10	4	10	10										
	10^{-4}	4	5	5	3	4	4	6	2	6	6										
	10^{-5}	2	2	2	2	1	2	2	0	3	3										
	10^{-6}	1	1	1	1	0	1	1	0	1	1	$15x10^6$	$17x10^6$	$17x10^6$	$12x10^6$	$11x10^6$	$14x10^6$	$19x10^6$	$6x10^6$	$19x10^6$	$20x10^6$
S4	10^{-3}	6	7	6	4	5	5	7	3	8	7										
	10^{-4}	3	3	3	2	2	3	4	1	4	3										
	10^{-5}	1	1	1	1	1	1	1	0	2	1										
	10^{-6}	0	0	0	0	0	0	0	0	1	0	$9x10^6$	$11x10^6$	$10x10^6$	$7x10^6$	$8x10^6$	$9x10^6$	$12x10^6$	$4x10^6$	$15x10^6$	$11x10^6$
		06-12 inch depth																			
S1	10^{-3}	8	9	9	6	6	7	9	5	10	10										
	10^{-4}	4	5	5	3	4	4	5	3	6	6										
	10^{-5}	2	2	2	1	1	1	2	1	2	2										
	10^{-6}	0	1	1	0	0	1	1	0	1	1	$14x10^6$	$17x10^6$	$17x10^6$	$10x10^6$	$11x10^6$	$13x10^6$	$17x10^6$	$9x10^6$	$19x10^6$	$19x10^6$
S2	10^{-3}	6	7	7	5	5	6	8	4	8	8										
	10^{-4}	3	4	4	3	3	3	4	2	4	4										
	10^{-5}	2	2	2	1	1	1	2	1	2	2										
	10^{-6}	0	0	0	0	0	0	0	0	0	0	$11x10^6$	$13x10^6$	$13x10^6$	$9x10^6$	$7x10^6$	$10x10^6$	$14x10^6$	$7x10^6$	$14x10^6$	$14x10^6$
S3	10^{-3}	6	7	8	5	5	6	8	3	9	8										
	10^{-4}	4	4	4	3	2	3	5	2	5	5										
	10^{-5}	2	2	2	1	0	1	2	1	2	2										
	10^{-6}	1	1	1	1	0	1	1	0	1	1	$13x10^6$	$14x10^6$	$15x10^6$	$10x10^6$	$9x10^6$	$11x10^6$	$16x10^6$	$6x10^6$	$17x10^6$	$16x10^6$
S4	10^{-3}	5	5	6	4	4	4	6	2	6	6										
	10^{-4}	3	3	3	2	1	2	3	1	3	3										
	10^{-5}	1	0	1	1	1	0	1	0	1	1										
	10^{-6}	0	0	0	0	0	0	0	0	0	0	$9x10^6$	$8x10^6$	$10x10^6$	$7x10^6$	$6x10^6$	$6x10^6$	$10x10^6$	$3x10^6$	$10x10^6$	$10x10^6$

CONCLUSION

The pooled data suggested that the ten agriculture fields varied significantly in soil fungal population associated with soil physicochemical properties that were affected by seasonal variability and soil depth. The soil of medium texture with optimum level of organic matter and available nutrients manifested higher fungal population as noted in location 9 at upper depth in spring season and minimum in location 8 at subsurface in summer season. From each location 18 fungal species were isolated and *Aspergillus niger* was found as a dominant fungal species in all locations. So, it is concluded that the soil with medium texture retain more O.M. than light texture soils and such soils indirectly depicted more available nutrients and high microbial activity provided that other factors like temperature, moisture, pH, texture and others remain conducive.

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