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CORRELATION BETWEEN SOIL NUTRIENTS AND SOIL-BORNE MYCOFLORA IN WHEAT-RICE CROPPING SYSTEM OF PUNJAB, PAKISTAN

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ABSTRACT

Soil samples were collected from 12 wheat fields with wheat-rice cropping history, from Lahore, Sheikhupura and Sialkot districts. Electrical conductivity (EC), pH, organic matter, nitrogen (N), phosphorus (P) and potassium (K) of soil samples ranged from 533–2360 µS cm^{-1}, 6.9–9.0, 0.69–2.02%, 0.03–0.10%, 4–225 ppm and 100–650 ppm, respectively. Eighteen fungal species belonging to *Aspergilus, Alternaria, Cladosporium, Drechslera, Emericella, Fusarium, Mortierella, Mucor* and *Penicillium* genera were isolated from soil samples. There was a significant variation in number of colonies of total, saprophytic and pathogenic fungi among the soil samples. Total number of fungal colonies, and number of colonies of saprophytic and pathogenic fungi ranged from 414–1872 g^{-1}, 412–1728 g^{-1} and 36–234 g^{-1} of soil, respectively, in different soil samples. Total number of fungal colonies and number of colonies of saprophytic fungi were significantly and negatively correlated with organic matter and N, and significantly and positively correlated with EC and K, respectively. Correlation between number of colonies of pathogenic fungi and different soil characteristics was insignificant. This study concludes that saprophytic fungi are adversely affected by organic matter and N, and are stimulated by K in rice wheat cropping system in fertile lands of central Punjab.

Keywords: Correlation, soil-borne mycoflora, soil nutrients, wheat-rice cropping.

INTRODUCTION

The wheat-rice cropping is among the most extensive agricultural production systems not only in Pakistan but also in all South Asian countries (Timsina and Connor, 2001; Hussain *et al.,* 2012). In Pakistan, 2.1 million hectares of agricultural land is under the wheat-rice cropping practice and out of which 60% is being carried out in Punjab province. Although this cropping system ensures national food security but the actual productivity of these crops is less than their potential productivity due to continuous wheat-rice rotation (Ladha *et al.,* 2003, Bhatt *et al.,* 2016). Besides, many other factors such as low soil fertility, insect pests, weed infestation and emerging challenges of climatic changes are the major reasons for low productivity of these two food crops. The rice-wheat cropping system causes a considerable decline in soil nutrients due to depletion of macro and micro-nutrients (Kukal and Aggarwal, 2003). To improve the stagnating yields in the wheat-rice system, farmers apply more fertilizer and select short duration varieties (Sheikh and Abbas, 2007).

Both rice and wheat crops require an ideal soil nutrient and moisture conditions to grow which in turn provide perfect conditions for soil pests and welcome disease complexes (Six *et al.,* 2004). Soil-borne pathogens particularly fungi and their associated diseases, which are not studied well in view point of wheat-rice cropping are emerging threat for this system as they share the soil nutrients and increase soil respiration (Liang *et al.,* 2015). Foliar blight is one of the most prevalent fungal diseases in the wheat-rice system. This disease is a combination of spot blotch caused by *Cochliobolus sativus* and tan spot by *Pyrenophora tritici-repentis* (Kariyawasam *et al.,* 2016; Villa-Rodriguez *et al.,* 2016). In addition, *Alternaria alternata, Bipolaris sorokiniana, Stemphylium* spp. and *Cladosporium* spp. are also known to infect both the crops (Taheri *et al.,* 2014; Ali *et al.,* 2016). *Rhizoctonia solani* is responsible for sheath blight in rice and foot rot in wheat (Almasudy *et al.,* 2015). *Fusarium* spp., *Nigrospora* spp., *Curvularia* spp., *Phytophthora* spp., *Aspergillus* spp., *Chaetomium* spp., *Acremonium* spp., *Trichocladium* spp. and *Stachybotrys* spp. have also been isolated from the soil of wheat-rice cropping area of Punjab (Iram *et al.,* 2003). Most of the fungal pathogens survive in crop debris and difficult to eliminate as they form resting spores. These spores become active when the same crop is grown in the same field (Iram and Ahmad, 2005). Their wide host range and ability to survive in harsh environment make their management difficult, however, improvement in soil health and crop rotation to non-host crops can reduce the pathogen populations in the soil (Ali *et al.,* 1998). In spite of impact of soil nutrients on soil-borne fungi in wheat-rice cropping systems, no planned study in this regard so far has been conducted in Punjab, Pakistan. Therefore, the present study was carried out to investigate the correlation between soil nutrients and soil-borne fungi in wheat-rice cropping system in three districts of Punjab namely Lahore, Sheikhupura and Sialkot.

MATERIALS AND METHODS

Sample collection: For soil sampling, twelve fields under wheat-rice cropping system were randomly selected from different districts of Punjab viz. Lahore, Sialkot and Sheikhupura. Five soil samples from each field were collected in February, 2013 when the wheat crop was on its booting stage. A core sampler (7.5 cm × 4.5 cm) was used to collect five sub-samples from each field which was then thoroughly mixed to form a composite. The sample soils were transported to laboratory in sterile polythene bags and were assessed for associated mycoflora on the next day. A part of each sample was ground and air dried under shade and used for chemical analysis.

Isolation and identification of soil mycoflora: Fungal strains were isolated from soil on 2% malt extract agar (MEA) medium as described by Dhingra and Sinclair (1993) by employing dilution plate method. Soil suspension from each sample was prepared by mixing 1 g of soil in 10 ml sterilized water. This suspension was then serially diluted to obtain 10^{-4} dilution. From each suspension, 100 µl were spread uniformly onto the MEA medium with the help of sterilized glass spreader. Five plates from each soil samples were incubated at 25 ºC for 2-3 days and monitored for the emerging fungal colonies. Number of each morphologically distinct fungal colony as well as total colony count was recorded from each Petri plate and then per gram of soil. All fungal isolates were sub-cultured for purification by single spore isolation technique (Choi *et al.,* 1999).

Pure fungal cultures were identified conventionally on the basis of macro (observable colony features like colour, diameter, pigmentation etc) as well as microscopic features. Complete description of each isolate based on macro and micro morphological characters was prepared. Species were keyed out by comparing their description with published authentic literature (Raper and Fennell 1965; Domsch *et al.,* 1980; Barnett and Hunter, 1998; Klich, 2002; Simmons, 2007; Bennett, 2010).

Chemical analysis: Air dried soil was ground to pass through 2 mm sieve. Electrical conductivity of saturated soil paste extract (EC_e) and pH of saturated soil paste (pH_s) were determined by digital conductivity and pH meters, respectively. Soil organic matter was estimated by oxidation with known volume of acidified potassium dichromate, the residual of which was titrated against 1 N ferrous sulfate by using diphenylamine indicator (Moodie *et al*., 1959). For nitrogen determination, a known weight of soil was acid-digested followed by distillation on Kjeldahl apparatus. During distillation evolved ammonia was trapped in saturated boric acid solution and assessed by titration against 0.01 N H_2SO_4 (Jackson, 1967). Olsen method was used to determine $NaHCO_3$-extractable phosphorus. In the method blue colour was developed with potassium antimony tartrate and ascorbic acid, and optical density was measured at 880 nm (Watanabe and Olsen, 1965). Ammonium acetate-extractable potassium was determined on flame photometer by comparing with a standard curve of known potassium concentrations (USSLS, 1954).

Statistical analysis: Data regarding number of pathogenic and saprophytic fungal colonies were analyzed by analysis of variance followed by LSD test ($P \leq 0.05$) to separate treatment means using computer software Statistics 8.1. Correlations of number of fungal colonies with various soil characteristics were calculated using MS Excel.

RESULTS

Soil Characteristics: A great variation was recorded in different soil characteristics among the fields. EC and pH ranged from 533–2360 µS cm^{-1} and 6.9–9.0. In most of the soils, organic matter was generally higher than average organic matter in Pakistani soils. Its range in different soils was 0.69–2.02%. Likewise, nitrogen in different soil samples was 0.03–0.10%. Range of phosphorus and potassium was 4–225 ppm and 100–650 ppm, respectively (Table 1).

Soil mycoflora: Eighteen different fungal species belonging to eight genera were isolated from the soil samples (Table 2). These include six species of genus *Aspergillus* (*A. niger*, *A. fumigatus*, *A. penecilloides*, *A. terreus*, *A. flavus* and *Emericella. nidulans*), four of genus *Penicillium* (*P. italicum*, *P. expansum*, *P. restrictum* and *P. griseofulvum*), three of genus *Mucor* (*M. flavus*, *Mucor* sp. 1 and *Mucor* sp. 2), two of genus *Cladosporium* (*C. cladosporoides* and *C. herbarum*) and one each of *Alternaria* (*A. alternata*) *Drechslera*, *Fusarium* (*F. oxysporm*), and *Mortierella* (*M. chlamydospora*). *A. niger* showed the highest number of colonies per gram of soil sample (5346) followed by *A. terreus* (1476), *E. nidulans* (1458), *A. fumigatus* (1422), *P. italicum* (1360), *M. chlamydospora* (1152), *C. herbarum* (810), *Mucor* sp. 1 (540) and *F. oxysporm* (342). Number of colonies of rest species was generally low and was in the range of 36–270 per gram of soil sample.

Total number of fungal colonies varied significantly among the soil samples from different wheat fields. It ranged from 414–2448 per gram soil sample in different fields. However, number of fungal colonies was 774 or higher in nine out of 12 soil samples (Fig. 1A). Among the total, number of colonies of pathogenic and saprophytic fungi also varied significantly among the soil samples taken from different wheat fields. Generally, number of colonies of saprophytic fungi was much higher than number of colonies of saprophytic fungi in all the

soil samples. There were 360–2304 colonies of saprophytic fungi as compared to 36–234 colonies of pathogenic fungi per gram of soil sample in different wheat fields (Fig. 1B&C).

Correlation between soil characteristics and soil mycoflora: There was a marked variation between various soil characteristics and the isolated soil mycoflora. Total number of fungal colonies as well as number of colonies of saprophytic fungi were significantly ($P \leq 0.05$) and negatively correlated with soil organic matter and nitrogen. By contrast, number of colonies of total and saprophytic fungi was significantly ($P \leq 0.05$ or 0.01) and positively correlated with EC and soil potassium. Relationship of number of colonies of pathogenic fungi with all the studied soil chemical characteristics was insignificant. Likewise, soil pH and phosphorus exhibited non-significant correlation with number of colonies of saprophytic fungi (Table 3).

Table 1. Chemical characteristics of soil samples collected from wheat fields.

Samples	Soil pH	Soil EC (µS cm^{-1})	Soil organic matter (%)	Soil nitrogen (%)	Soil phosphorus (ppm)	Soil potassium (ppm)
1	7.9	633	1.75	0.09	24	172
2	7.8	534	1.60	0.08	225	138
3	7.7	871	1.88	0.09	54	186
4	7.9	610	1.61	0.08	137	150
5	7.9	664	2.02	0.10	5	104
6	7.8	581	1.74	0.09	27	198
7	6.9	587	0.91	0.04	16	128
8	7.7	533	0.83	0.04	28	100
9	8.5	1757	1.25	0.06	53	602
10	8.6	2360	1.25	0.06	200	606
11	9.0	913	1.11	0.06	4	650
12	8.3	737	0.69	0.03	8	303

Table 2. Number of colonies of different fungi isolated from soils of wheat fields.

Field No.	No. of fungal colonies per gram of soil																		Total colonies/field
	AN	AF	AP	AT	ASF	AA	CC	CH	DR	EN	FO	MC	MF	MS1	MS2	PI	PE	PR	
1	108	36	0	144	0	18	0	72	0	72	0	72	0	0	0	126	126	0	774
2	198	36	0	180	90	0	36	162	0	90	0	72	0	0	0	108	144	18	1134
3	0	0	0	216	0	0	0	54	0	0	0	90	0	0	0	54	0	0	414
4	144	0	0	18	0	0	0	36	0	72	0	198	0	0	0	144	0	36	648
5	108	18	0	126	0	0	54	144	0	0	0	108	0	0	0	54	0	0	612
6	810	0	0	18	0	0	0	72	0	54	0	90	0	18	0	162	0	0	1224
7	864	72	0	36	0	0	0	180	0	144	0	126	0	0	0	36	0	0	1458
8	756	162	0	0	0	108	0	72	0	108	54	126	0	90	0	126	0	0	1602
9	864	126	36	324	0	0	36	0	0	198	108	198	0	234	0	324	0	0	2448
10	756	180	0	144	0	0	0	18	0	324	126	36	72	90	0	126	0	0	2898
11	702	180	0	0	0	36	0	0	0	378	18	36	0	72	0	90	0	0	1512
12	36	612	0	270	36	0	0	0	72	18	36	0	0	36	162	0	0	36	1314
Total	**5346**	**1422**	**36**	**1476**	**126**	**162**	**126**	**810**	**72**	**1458**	**342**	**1152**	**72**	**540**	**162**	**1360**	**270**	**90**	

AN: *Aspergilus niger;* **AF:** *A. fumigatus;* **AP:** *A. penecilloides;* **AT:** *A. terreus;* **ASF**: *A. flavus*; **AA:** *Alternaria alternata;* **CC:** *Cladosporium cladosporoides;* **CH:** *Cladosporium herbarum;* **DR:** *Drechslera* sp.; **EN:** *Emericella nidulans;* **FO:** *Fusarium oxysporm;* **MC:** *Mortierella chlamydospora;* **MF:** *Mucor flavus*; **MS1:** *Mucor* sp. 1; **MS2:** *Mucor* sp. 2; **PI:** *Penicillium italicum;* **PE:** *P. expansum*; **PR:** *P. restrictum.*

Table 3. Correlation between soil characteristics and number of fungal colonies of soil of wheat fields.

	pH	EC	Organic matter	Nitrogen	Phosphorus	Potassium
Total No. of colonies	0.39	0.62*	-0.64*	-0.62*	0.065	0.68*
No. of colonies of saprophytic fungi	0.44	0.64*	-0.63*	-0.61*	0.055	0.74**
No. of colonies of pathogenic fungi	-0.30	-0.01	-0.26	-0.29	0.105	-0.25

*' **' Significant at P≤0.05 and P≤0.01, respectively.

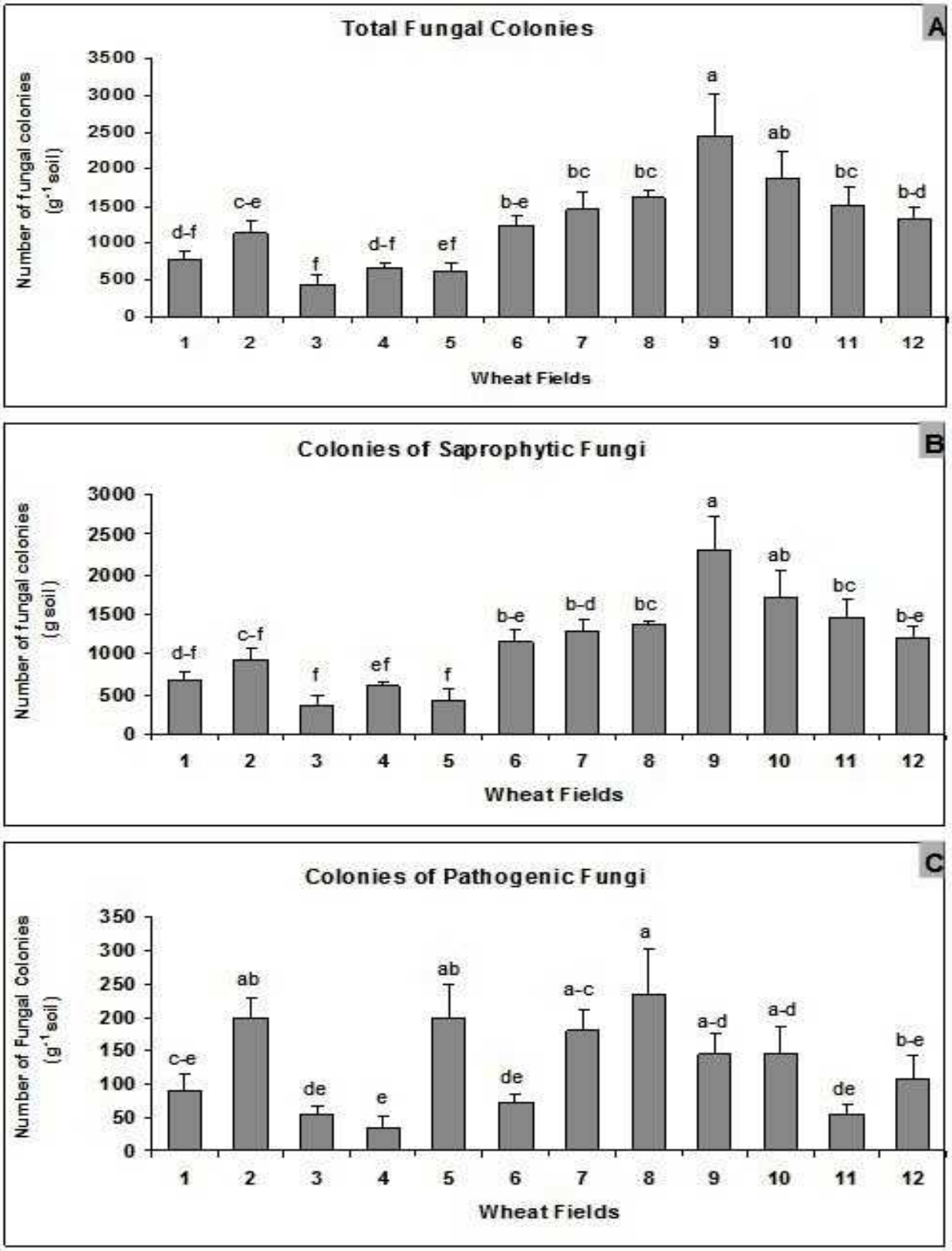

Fig. 1. Total number of fungal colonies, and number of saprophytic and pathogenic fungal colonies in soils of different wheat fields. Vertical bars show standard error of means of five replicates. Bars with different letters show significant difference (P≤0.05) as determined by LSD method.

DISCUSSION

Soil organic matter, nitrogen, phosphorous and potassium are the fertility parameters of a cultivated land. In cereal-cereal based cropping systems, soil fertility reduces gradually which results in continuous addition of chemical fertilizers (Shah *et al.,* 2003). In such cropping system, soil nitrogen is the most exhausted nutrient which is never sufficient in soil even when recommended amount of chemical fertilizers are provided (Shah *et al.,* 2011).. It has been widely established that plant growth and productivity are highly affected by amount and type of soil organic and inorganic nutrients (Eifediya and Remison, 2010; Nwofia *et al.,* 2015).. Rice-wheat cropping system, which is the most popular cropping system of Pakistan, causes high depletion of nitrogen, potassium, phosphorous, zinc, iron and manganese etc from the soil (Ladha *et al*., 2003). In this system, the productivity of both of rice and wheat crops is threatening in long term and also results in higher use of chemical fertilizers (Suman, 2004; Akhtar *et al.,* 2009). In general, 90% agricultural soil of Pakistan is deficient in nitrogen and phosphorous, and 50% of soil has lesser potassium and other micronutrients than the recommended value. Results of present study suggested that amount of nitrogen in all soil samples was less than the recommended amount for healthy growth of plants. The amount of nitrogen in soil samples ranged between 0.03-0.10%. Zia *et al.* (1998) reported that irrigated land of Pakistan is 68-88% is deficient in phosphorous, 5-52% deficient in potassium. Current analysis of soil samples for potassium and phosphorous content gave a range of 4-25 ppm and 100-650 ppm, respectively. These results indicated that most of the studied fields were phosphorous deficient.

One of the main reasons of the low productivity of rice-wheat cropping system is decline in relative amount of soil organic matter (Grace *et al*., 2003; Kukal *et al*., 2009). Agricultural soil of Pakistan is extremely deficient in organic matter (Zia *et al.,* 1998; Bhatti, 1999). A good cultivated land should have at least 1.29% organic matter. However, in highly productive agricultural lands, for example in Australia, soils have 15-30% organic matter (Kirkbey and Mengel, 1987). But in the present study, soil organic matter was in the range of 0.69–2.02%. During a study conducted to determine the fertility parameters of agricultural soil of Pakistan, Azam *et al.* (2001) reported approximately 0.52-1.38% organic matter in different soils and most of the samples showed less than 1% organic matter.

Soil pH and electric conductivity (EC) are the important characteristics that describe the suitability of the soil environment for growing plants. Both soil pH and EC are good indicators of available nutrients to plants (Alpaslan and Gunes, 2001). Salt affected soils show increase in soil pH and dis-balance of cations (Muhammad, 1986). Results of the present study showed that soil pH of all the samples collected from different agricultural lands ranged from 7.7-9.0. These results show that these soils are slightly to moderately saline. In a similar work, it was noticed that 92% studied fields had soil with pH 8.3 or above (Bhatti, 1999; Arain *et al.,* 2000).

Present study shows that the amount of organic matter and nitrogen in soil is negatively correlated with the fungal population of the soil. Working on the similar lines, Bibekanda and coworkers (1993) suggested that resistance of rice plants against *Rhizoctonia solani* can be increased considerably by proper fertilization management of potassium and nitrogen. The severity of Aggregate Sheath Spot of rice caused by *Rhizoctonia orzae-sativae* can also be considerably reduced by increasing fertilization of nitrogen and potassium (Linquist *et al.,* 2008). General trend regarding the population of soil saprophytic fungi was such that, it was directly related to the amount of organic matter and nitrogen content, however, results of the present study are conversely showing significant decline in saprophytic fungal flora as the amount of organic matter and nitrogen increased in the soil samples. This was probabily due to the excessive and misuse of chemical fungicides which significantly affect the non-target soil microbial community as well as nitrogen dynamics (Chen *et al.,* 2001; Akhtar *et al.,* 2015). In the present study, the number of pathogenic fungi is inversely related with the soil pH. In parallel lines, Trolldenier (1973) suggested that at pH 5.5, soil provides optimum environment for fungal growth, however, at higher pH bacteria dominate over fungi.

It is concluded from the present study that soil of the fields under rice-wheat cropping system in Punjab, Pakistan is mostly below the recommended level of nutrients. Also the other soil fertility factors are not up to the mark in this huge and economically important cropping system. Under the resent nutrient status and agricultural practices, pathogenic fungal population has insignificant relation with soil nutrition while saprophytic fungi are being adversely affected by soil organic matter and nitrogen and are stimulated by K. This study can further be extrapolated by measuring other soil physiochemical properties and sampling sites for better understanding of this specific field of research.

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