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RELATIONSHIP BETWEEN SOIL CHEMICAL CHARACTERISTICS AND SOIL-BORNE FUNGI IN TOMATO TUNNELS OF PUNJAB, PAKISTAN

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Abstract

Soil pH, EC_e, N, K, P and organic matter in 12 soil samples collected from different tomato (*Solanum lycopersicum* L.) tunnels were in the range of 7.7 - 8.3, 0.40 - 2.45 dS/cm, 0.02 - 0.10%, 40 - 282 ppm, 12 - 123 ppm and 0.42 - 2.02%, respectively. A total of 20 fungal species belonging to ten genera, namely *Aspergilus, Alternaria, Cladosporium, Drechslera, Emericella, Fusarium, Mortierella, Mucor, Penicillium* and *Sclerotium* were isolated from the soil samples using direct and dilution plate techniques. Total number of fungal colonies ranged from 450 - 2700/g soil in different soil samples. Among these, number of colonies of saprophytic and pathogenic fungi were 432 - 2070 and 10 - 954/g soil sample, respectively. Number of pathogenic colonies was significantly and positively correlated with soil organic matter and N. The soil organic matter and nitrogen favoured population of pathogenic fungi in tomato tunnels.

Introduction

Tomato (*Solanum lycopersicum* L.), a rich source of vitamin A and C, is the major vegetable crop grown in almost every part of the world. Growing tomato is preferred over other crops as it is a short duration crop and gives larger economic output. Tomatoes can be grown off-season by high tunnel farming. High tunnels being relatively inexpensive are temporary structures covered with plastic sheets and make the plantation of tomatoes approximately one month earlier and six weeks later than the normal cropping time (Jett 2009 and Conner *et al.* 2010).

As compared to normal field crops, the crops grown under high tunnels as well as soils under their cultivation are more intensively managed that results in soil nutrition depletion as well as buildup of pests, pathogens and salts content (Coleman 1999). For this purpose, comparison of soil characteristics of tunnel and adjacent fields could be the one strategy to provide healthy growth medium to tunnel crops. Soil quality can be quantified by collecting data on appropriate chemical, physical or biological indicators over a number of years (Lal 1994, Dumanski and Pieri 2000). Measure of salinity and nutrient analysis are the major chemical indicators of soil quality (Brady and Weil 1999). Organic matter which influences a number of other soil characteristics such as soil structure and mineralization, water holding capacity, air infiltration and biological activity, is a commonly used biological indicator of soil quality (Wander and Bidart 2000).

Soil microbial diversity is highly important for sustainable agriculture. Mono-culturing of crops under control environment shifts the natural communities of soil microorganisms and results in replanting of diseases (, Huang *et al.* 2013). Soil fungi, a diverse microbial group present in soil play several important functions that affect soil characteristics (Burke *et al.* 2012). So far, studies regarding the effect of tomato cultivation in tunnels on soil nutrition and soil microbial communities are lacking. Objective of the present study was to evaluate and compare soil chemical characteristics of tomato tunnels in different areas of Punjab and to correlate it with soil saprophytic and pathogenic mycoflora.

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Materials and Methods

Twelve soil samples were collected from tomato tunnels located in different districts of Punjab, Pakistan *viz.* Sheikhupura, Okara, Faisalabad, Gujranwala and Lahore. About 500 g of soil were taken at the depth of 15 cm from the rhizosphere of tomato plants and collected in polythene bags. Soil of five sub-samples taken from each study site was mixed to make a composite sample. Ten grams of soil from each composite sample was separated for isolation of soil mycoflora while rest of soil was air dried under shade for one week. All the dried samples were grinded, sieved and stored in air dried stock bottles.

Soil parameters analyzed were total nitrogen (N), phosphorous (P), potassium (K), organic matter content, pH and electrical conductivity (EC_e). Organic matter of soil was determined by acidified potassium dichromate method and the excess of potassium dichromate was reduced with ferrous sulphate ($FeSO_4$) by using dimethyl amine indicator. For pH, saturated soil paste was prepared and pH meter was used after standardization with buffer to determine the pH of soil. Kjeldahl method was used to determine the total nitrogen content of soil by digesting the soil samples with concentrated H_2SO_4 and digestion mixture (K_2SO_4: $FeSO_4$: $CuSO_4$ = 10: 1: 0.5) (Jackson 1962). Potassium was extracted with ammonium acetate and its quantity was determined on flame photometer. Olsen's method was used to determine the extractable phosphorous by developing colour with antimony potassium tartrate and measuring the absorbance at 880 nm by spectrophotometer. Intensity of blue colour determined the concentration of available phosphorous in soil (Watanabe and Olsen 1965). To determine the electrical conductivity of soil, 10 g of soil was mixed in 100 ml of distilled water and EC_e was recorded by using an EC_e meter.

For isolation of the fungi from soil on malt extract agar (MEA) medium, dilution plate method was used for isolation. One gram of sieved soil was suspended in 10 ml of sterilized water and 10^{-1} and 10^{-5} dilutions were prepared. A volume of 100 µl of each dilution was inoculated on MEA Petri plates under aseptic conditions and incubated at 25°C for seven days. Five replicates of each treatment were made. After one week of incubation, total number of isolated fungal colonies in one gram of soil was counted from each sample. All the isolated fungal colonies were identified on the basis of cultural and morphological characteristics. All the cultural characteristics i.e. texture of colony, type of margins, culture smell, front and reverse colour of colony, colony size and elevation, any structure formation and pigment exudes were observed under stereoscope. Microscopic characteristics i.e. shape, colour and, size of conidia, vesicle, phialides and metulae, branching in conidiophores, thickness and colour of conidiophores and septation in hyphae were recorded.

Data on number of saprophytic and pathogenic fungi were subjected to analysis of variance followed by mean separation by LSD method ($p \leq 0.05$) using computer software Statistics 8.1. Correlation between number of fungal colonies and different soil characteristics was computed using MS Excel program.

Results and Discussion

All the soils were slightly alkaline within the narrow range of pH 7.7 - 8.3. In contrast, range of electrical conductivity (EC_e) was much wider in different soil samples i.e. 0.40 - 2.45 dS/m. Proportion of organic matter in different soil samples ranged from 0.42 to 2.02% while that of soil nitrogen was 0.02 - 0.10%. Soil phosphorus and potassium varied from 12 - 123 ppm and 40 - 282 ppm, respectively, in different soil samples (Table 1).

Maximum taxonomic diversity was observed among the isolated mycoflora of different soil samples. Members of all the four major fungal groups *viz.* Zygomycota, Deutereomycota, Ascomycota and Basidiomycota were isolated from the soil samples. The fungal community

contained both saprophytic as well as pathogenic strains. Three strains of Zygomycota, namely *Mortierella chlamydospora*, *Mucor flavus*, and *Mucor* sp. were isolated. *Mortierella chlamydospora* (order Mortierellales) was consistently present in each soil sample tested in a range of 72 - 468 colonies/g soil. Other two strains, namely *Mucor flavus* and *Mucor* sp. belong to order Mucorales. *Mucor flavus* was isolated from all the soil samples except one while *Mucor* sp. was absent in only two soil samples with their occurrence ranging from 18 - 162 and 36 - 234 colonies/g soil, respectively (Table 2).

Table 1. Chemical characteristics of soil samples collected from tomato tunnels.

Samples	pH	EC (dS/m)	Organic matter (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)
1	7.8	1.51	0.91	0.04	57	40
2	8.1	0.80	1.47	0.06	38	169
3	7.9	0.69	2.02	0.10	26	165
4	7.9	0.79	0.98	0.05	35	112
5	8.1	0.61	1.40	0.07	16	133
6	8.2	1.10	0.98	0.05	33	282
7	8.2	0.69	0.42	0.02	123	204
8	8.0	0.47	0.42	0.02	13	126
9	8.3	0.98	1.13	0.06	12	214
10	7.7	0.40	1.05	0.05	12	229
11	7.9	2.45	1.60	0.08	31	190
12	7.8	1.31	0.98	0.05	122	250

Table 2. Number of colonies of different fungi isolated from soils of tomato tunnels.

	No. of fungal colonies per g of soil									
Field No.	AN	AF	AP	AT	ASF	AA	CC	CH	DR	EN
1	126	18	0	108	0	0	0	0	0	72
2	90	36	0	90	0	18	90	162	0	108
3	252	36	0	144	0	162	252	468	54	486
4	108	36	0	180	18	36	144	162	54	234
5	774	270	0	54	18	54	0	18	0	72
6	990	306	36	18	36	126	0	36	0	144
7	126	18	0	36	0	0	0	0	126	108
8	198	72	0	756	18	54	0	36	36	54
9	162	36	0	54	18	0	0	0	54	72
10	450	54	0	72	108	18	0	0	18	90
11	0	0	0	54	18	36	18	90	144	0
12	288	126	0	144	72	18	0	0	36	342

(Contd.)

Right side of the table

No. of fungal colonies per g of soil									
ES	FO	MC	MS	MF	PI	PE	PR	PG	SC
0	0	72	0	0	18	36	0	0	0
0	0	432	90	36	0	36	0	0	0
0	18	468	36	162	126	36	0	0	0
0	18	252	90	36	0	36	0	18	0
0	0	360	54	36	126	306	0	0	0
0	108	396	126	36	54	54	18	18	0
0	0	144	108	0	90	0	0	0	0
0	18	324	144	54	0	0	0	0	0
0	18	126	234	36	18	18	18	0	54
0	54	360	36	18	18	0	36	0	0
0	0	72	216	18	36	18	0	0	0
54	18	198	90	54	0	90	90	0	0

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;* ES: *Emericella* sp., FO: *Fusarium oxysporm;* MC: *Mortierella chlamydospora;* MS: *Mucor* sp.; MF: *Mucor flavus*; PI: *Penicillium italicum;* PE: *P. expansum*; PR: *P. restrictum*; PG: *P. griseofulvum;* SC: *Sclerotium rolfsii.*

Members of Deuteromycota (fungi imperfecti) constituted the largest group of fungal community in the present study. Six fungal genera belonging to family Tuberculariaceae (*Fusarium*), Trichocomaceae (*Aspergillus* and *Penecillium*) and Dematiaceae (*Alternaria*, *Cladosporium*, *Drechslera*) were isolated and identified. Five species of genus *Aspergillus,* namely *A. niger*, *A. fumigatus*, *A. penecilloides*, *A. terreus* and *A. flavus* with 0 - 990, 0 - 306, 0 - 36, 18 - 758 and 0 - 108 colonies/g soil, respectively were recorded. Species of genus *Penicillium* constituted the second largest group of the isolated fungi being represented by four species. Reported species of *Penicillium* were *P. italicum*, *P. expensum*, *P. restrictum* and *P. griseofulum* with 0 - 126, 0 - 306, 0 - 90 and 0 - 18 colonies/g soil, respectively. From the family Dematiaceae, one species of *Alternaria* i.e. *A. alternata* with 0 - 162 colonies/g soil; two species of *Cladosporium*, namely *C. cladosporoides* and *C. herbarum* having 0 - 252 and 0 - 468 colonies/g soil, respectively; and one species of *Drechslera* with of 0 - 144 colonies/g soil in different samples were identified. The most notorious pathogen of tomato, *Fusarium oxysporum* was also purified from soil samples with 0 - 108 colonies/g soil (Table 2).

Two species of Ascomycota belonging to genus *Emericella* were isolated. These species included *E. nidulans* and *Emericella* sp. with 0 - 486 and 0 - 52 colonies/g soil, respectively. Only one member from Basidiomycota, namely *Sclerotium rolfsii* was identified from samples and its occurrence was 0 - 54 colonies/g soil (Table 2).

Total number of fungal colonies in investigated soil samples was found to range from 450 - 2700/g soil. Data also showed that recorded number of saprophytic fungal colonies of soil was 432-2232/g soil and that of pathogenic strains was 10 - 954/g soil (Fig. 1).

Results indicated that pH, EC_e as well as amount of organic matter, nitrogen, phosphorous and potassium in probed soil samples had insignificant correlation with the total fungal community as well as saprophytic fungal flora residing in tomato tunnel soils. Although insignificant, out of studied soil characteristics, EC_e and phosphorous content of the soil samples were negatively while pH, organic matter, nitrogen and potassium were positively correlated with

total number of fungal colonies and saprophytic strains of soil fungi. Organic matter content and nitrogen in soil samples were found to be significantly and positively correlated with the prevailing pathogenic fungal community. However, other parameters like pH, EC_e and phosphorous were negatively correlated to number of pathogenic fungi but the relationship was insignificant. The correlation of amount of soil phosphorous and number of pathogenic fungi in samples was negative and statistically insignificant (Table 3).

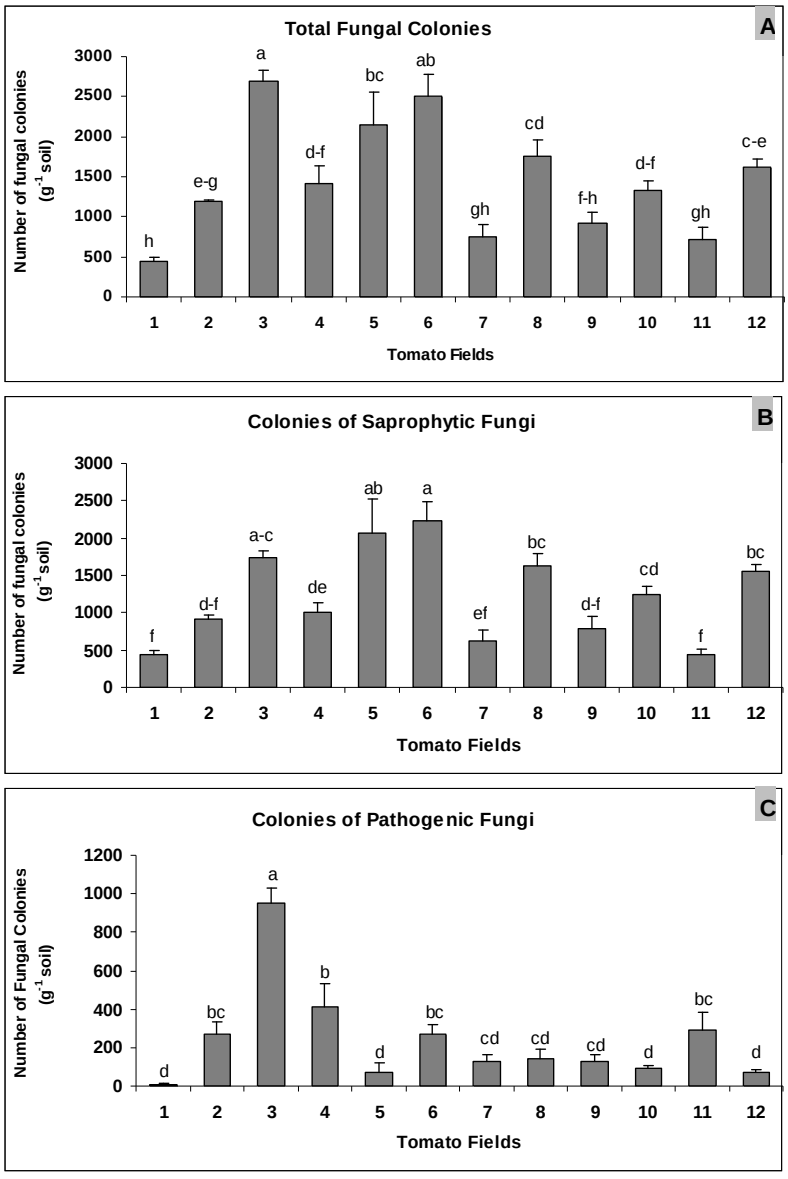

Fig. 1. Total number of fungal colonies and number of saprophytic and pathogenic fungal colonies in soils of different tomato tunnels. Vertical bars show standard error of means of five replicates. Bars with different letters show significant difference ($p \leq 0.05$) as determined by LSD method.

Cultivation of tomatoes in tunnels is becoming popular rapidly because it extends the growing season of this high-valued vegetables. However, soil management in these tunnels is highly challenging that affect the yield and quality of this vegetable. The range of pH of different soil samples was 7.7 - 8.3 which is slightly alkaline and might result in soil micro-nutrient sensitivity. It has been reported that pH of soil affects the availability of soil micronutrients to tomato plants. Recommended pH for efficient tomato tunnel soils is 6.5-7.0 (Reeve and Drost 2012). Soil pH influences the EC_e that directly affects the flow of nutrients through cell membranes. Ghafoor *et al.* (2004) suggested that despite of variable salt tolerance of different plants, EC_e up to 4 dS/m is highly favorable for plant growth. Results of the present study indicated that EC_e of different soil samples was in the range of 0.40 - 2.45 dS/m which is considered as optimum for cell membrane permeability for soil nutrients uptake (Alpaslan and Gunes 2001).

Table 3. Correlation between soil characteristics and number of fungal colonies of soil of tomato tunnels.

Fungal colonies	pH	EC	Organic matter	Nitrogen	Phosphorus	Potassium
Total No. of colonies	0.07	–0.42	0.33	0.37	–0.27	0.28
No. of colonies of saprophytic fungi	0.11	–0.46	0.12	0.17	–0.23	0.32
No. of colonies of pathogenic fungi	–0.06	–0.08	0.64*	0.65*	–0.22	0.01

*Significant at $p \leq 0.05$.

Soil organic matter and nitrogen content are the most significant fertility factors for growth and productivity of any crop. However, soil of Pakistan under cultivation, in general is deficient in soil organic matter as well as nitrogen content (Azam *et al.* 2001). For better plant growth, the recommended content of organic matter is 1.3% (Rashid 2001). However, up to 30% organic matter is reported in highly productive fields of Australia (Kirkbey and Mengel 1987). Amount of organic matter and nitrogen in soil samples were 0.42 - 2.02% and 0.02 - 0.10%, respectively, which indicated that such soils were within low to highly fertile range.

According to the criteria used by Punjab Soil Fertility Department, more than 21 mg/kg soil phosphorous and more than 180 mg/kg soil potassium should be present in fertile soil. Soil samples analyzed for the current study were found to have phosphorus 12 - 123 mg/kg and potassium 40 - 282 mg/kg. Although most of the agricultural soil of Pakistan is deficient in phosphorous and potassium (Zia *et al.* 1998), but analysis of soil samples in the present study indicated that most of the samples had adequate amount of potassium and phosphorous while some have medium level of these two important elements.

Alternaria alternata that was identified in different samples in the present study is known to cause leaf necrosis in a wide host range and has also been reported as leaf blight pathogen of tomato from Pakistan (Akhtar *et al.* 2004). *A. alternata* also causes black mold lesions on fruits of tomato and results in great economic losses (Morris *et al.* 2000). *Fusarium oxysporum* that was isolated from a number of soil samples during the present study is considered as the most devastating fungal pathogen that causes crown and root rot in tomato plants grown under greenhouse conditions (Rowe 1980). Genus *Cladosporium* is the largest genus of Dematiaceae and both of its isolated species were previously reported to cause rotting in tomato fruits (Rivas and Thomas 2005). Southern blight disease is also a notorious disease of tomato caused by *Sclerotium rolfsii* that infects the collar region by developing lesions on stem and causes wilting of the plant.

This sclerotial fungal pathogen also causes damping-off of seedlings, crown blight and rotting of tomato fruits (Mullen 2001, Kwon and Park 2002).

Results suggested significantly positive correlation among number of pathogenic fungi and amount of nitrogen and organic matter in soil. It has been previously established that higher the amount of nitrogen and organic matter in soil the more will be the number of pathogenic fungi. This hypothesis was further strengthened by the studies where unnecessary use of nitrogen fertilizers resulted in increased disease severity of many fungal diseases (Mascagni *et al.* 1997, Hoffland *et al.* 2000, Simon *et al.* 2003).

It may be inferred that a great variation in chemical and biological properties of soil of different tomato tunnels is present that should be regularly monitored to achieve the optimum growth condition for better yield of tomatoes grown in tunnels. In addition, population of the pathogenic fungi was positively correlated with organic matter and soil nitrogen. Therefore, addition of these two in the soil should be done with much care.

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