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CORRELATION BETWEEN SOIL CHEMICAL CHARACTERISTICS AND SOIL-BORNE MYCOFLORA IN CUCUMBER TUNNELS

HALIMA QUDSIA, ARSHAD JAVAID*, RASHID MAHMOOD AND NAUREEN AKHTAR

Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.
**Corresponding author's email: arshadjpk@yahoo.com, arshad.iags@pu.edu.pk*

Abstract

Twelve soil samples were collected from fields of cucumber (*Cucumis sativus* L.) tunnels from various localities of Lahore and Shekhupura districts, Pakistan. Soil samples were analyzed for various characteristics viz. pH, EC_e, organic matter, nitrogen (N), phosphorus (P) and potassium (K). Soil mycoflora was isolated using dilution plate method. Soil pH, EC_e, organic matter, N, P and K were in the range of 7.42–8.13, 107–2520 (µS cm^{-1}), 0.98–1.40%, 0.039–0.070%, 7–357 mg kg^{-1} and 88–946 mg kg^{-1} in different soil samples, respectively. A total of 18 fungal species belonging to 10 genera viz. *Aspergillus, Alternaria, Cladosporium, Drechslera, Emericella, Fusarium, Mortierella, Mucor, Penicillium* and *Sclerotium* were isolated from various soil samples. Saprophytic fungi were more prevalent than pathogenic ones. Number of colonies of saprophytic fungi ranged from 360–2754 g^{-1} soil in different samples. In contrast, number of pathogenic fungal colonies were limited to 1–234 g^{-1} soil. Number of colonies of pathogenic fungi were positively and significantly correlated with soil organic matter and nitrogen contents. This study concludes that high nitrogen and organic matter in cucumber tunnels favour population of pathogenic fungi.

Key words: Correlation, Cucumber tunnels, Mycoflora, Soil nutrients.

Introduction

Cucumber (*Cucumis sativus* L.) belongs to the family Cucurbitaceae (gourd family). It is cultivated worldwide for its delicious green fruit; consumed as raw, in salads and pickles, and also cooked as vegetable in some parts of the world. In Pakistan, annual production of cucumber is 15949 tons and is cultivated on 1251 hectares area annually. To ensure its availability all the yearlong, its cultivation has been frequently shifted from open fields to greenhouses and plastic tunnels (Anon., 2006). Tunnel cultivation of cucumber differs in various aspects from that in open fields. For instance, to compensate the high cost of tunnel installation and maintenance, farmers prefer to cultivate high yielding hybrid varieties. As these varieties are very responsive to added nutrients, therefore, fertilizer doses applied in tunnel are much more than those in open fields. Secondly, in the closed humid environment of plastic tunnels, crop is frequently sprayed with pesticides to suppress fungal and bacterial pathogens. Thus during the whole year, cucumber tunnel floor soil remains under mono-cropping system, pool up with heavy pesticide and fertilizer additions. These extremes may give the soil a unique set of physico-chemical characteristics different from that of open cucumber fields.

Soil-borne fungi are important biotic component of the soil. They not only cause a number of plant and animal diseases but also have some beneficial aspects like phosphate solublization, organic matter decomposition etc. Therefore, growth of crop plants is greatly influenced by the type and size of fungal populations in the soil. It is now a proven fact that the fungal genera which reside in soil are sensitive to one or the other soil physico-chemical characteristics (Kanwal *et al.,* 2017). For instance, *Phytophthora* spp. and *Pythium* spp. are suppressed in soils having high organic matter or by the addition of composts (Hoitink *et al*., 1996; Boehm *et al*., 1997). On the other hand, an increase in soil nitrogen content results in an increase of disease incidence due to *Pseudomonas syringae* on beans and cucumber (Rotenberg *et al*., 2005), *Gaeumanomyces graminis* var *tritici* and *Rizoctonia solani* on wheat (Pankhurst *et al*., 2002), and *Fusarium* spp. on *Asparagus* (Hamel *et al*., 2005).

The explanation of the effect of altered tunnel soil characteristics on soil-borne fungi is needed to design soil and crop management systems. The present study was planned to correlate the chemical characteristics of the cucumber tunnel floor soil, collected from different areas of Punjab, Pakistan, with fungal diversity in the soil.

Materials and Methods

Soil sampling: Twelve soil samples were collected from the rhizosphere of different cucumber tunnels in Lahore and Sheikhupura districts, Punjab, Pakistan. These samples were taken from up to 15 cm depth of the rhizosphere. Each soil sample was about 500 g. After taking a fresh soil sample for isolation of fungal flora, rest of the soil of each sample was air dried, crushed, sieved and stored in clean plastic bottles for chemical analysis and isolation of soil mycoflora.

Soil chemical analysis: Electrical conductivity of soil extract (EC_e) and pH were determined using EC and pH meters, respectively. Soil organic matter was determined by acidified potassium dichromate ($K_2Cr_2O_7$) method following Moodie *et al.* (1959). Ten milliliter $K_2Cr_2O_7$ was added to a flask containing 1 g soil sample followed by addition of 10 mL sulphuric acid (H_2SO_4) and left for 30 min. Thereafter, 200 mL water was added in the flask. Same procedure was repeated in another flask without addition of soil sample referred as blank. Materials in both the flasks were titrated against ferrous sulphate ($FeSO_4$) solution. Soil organic matter was determined by applying the following formula:

$$\text{Organic matter (\%)} = \frac{FeSO_4 \text{ used for blank} - FeSO_4 \text{ used for sample} \times 0.698}{\text{Weight of soil}}$$

Extractable phosphorus was determined with Olsen's method by developing the colour with antimony potassium tartrate and measuring the absorbance on spectrophotometer at 880 nm (Watanabe & Olsen, 1965). Potassium was extracted with ammonium acetate and determined on flame photometer. Soil nitrogen was determined by Kjeldahl's method (Jackson, 1962).

Isolation of soil mycoflora: Serial dilution method was used for isolation. One gram of soil was suspended in 10 mL distilled water. One milliliter of this suspension was then added in 9 mL distilled water in another test tube to prepare a dilution of 10^{-1}. The process was repeated several times until a dilution of 10^{-4} was prepared. One hundred micro liters of dilution of each sample was spread on malt extract agar (MEA) medium in 9-cm diameter Petri plates. Plates were incubated at 25 $\pm$ 2 °C for seven days. Thereafter, different fungal colonies were purified separately on MEA. The purified cultures of soil fungi were identified on the basis of morphological and cultural characters (Domsch *et al.*, 1980; Barnett & Hunter, 1998; Bennett, 2010). Data regarding number of colonies of different saprophytic and pathogenic fungal per gram soil were recorded.

Statistical analysis: Data regarding number of total colonies of saprophytic and pathogenic fungi were analyzed by one way of analysis of variance followed by mean separation by LSD method at $p \leq 0.05$. Correlation of number of fungal colonies with different soil characteristics was calculated using MS Excel software.

Results and Discussion

Soil chemical characteristics: Analysis revealed that soil of all the experimental tunnels was slightly alkaline within a narrow pH range of 7.42–8.13. However, other chemical properties of soil of different cucumber tunnels were quite different from each other that showed the over or under use of soil fertilizers or pesticides. For example, soil electrical conductivity (EC_e), an important factor for plant health, showed a wide range in different soil samples. Although crop plants vary in salt tolerance but as a general rule EC_e up to 4 dS m^{-1} (4000 µS cm^{-1}) is considered normal for plant growth (Ghafoor *et al.*, 2004). In the current study, EC_e in different soil samples ranged from 0.1 to 2.5 dS m^{-1} which indicates that no tunnel was installed on saline soils (Table 1). EC_e affects the membrane permeability of plant for available nutrients in soil (Alpaslan & Gunes, 2001). Chartzoulakis (1992) during a study on cucumbers growing in green house found that germination, growth and yield of cucumber are reduced with increasing EC_e.

Organic matter and soil nitrogen content in different soil samples were found within medium to fertile range (Rashid, 2001) i.e., 0.98–1.40% and 0.039–0.070%, respectively (Table 1). In general, cultivated soil of Pakistan is deficient in both of these important factors (Azam *et al.*, 2001). The recommended amount of organic matter in agricultural soils is about 1.30% while highly productive agricultural soil in Australia contain upto 30% organic matter (Kirkbey & Mengel, 1987). Soil phosphorus and potassium in different samples ranged from 7-357 mg kg^{-1} and 88-946 mg kg^{-1}, respectively (Table 1). Phosphorous is important for living cells as it is the part of important biomolecules and potassium is involved in a number of metabolic activities of cells. Agricultural soil of Pakistan is approximately 68-88% and 5-52% deficient in phosphorous and potassium, respectively (Zia *et al.*, 1998). Current analysis of soil samples indicated that most of the studied fields has amount of potassium and phosphorous in adequate range (Rashid, 2001).

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

Samples	pH	EC_e (dS m^{-1})	Organic matter (%)	Nitrogen (%)	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})
1	7.53	2.0	1.05	0.070	177	402
2	7.86	0.79	0.98	0.039	357	118
3	7.78	0.84	1.12	0.050	7	108
4	7.66	0.11	0.98	0.048	42	126
5	7.81	0.79	0.98	0.049	105	88
6	8.13	1.19	1.12	0.060	56	349
7	7.90	2.52	0.98	0.050	26	946
8	8.00	0.66	0.98	0.049	10	114
9	7.75	0.48	1.12	0.050	29	214
10	7.85	1.57	1.40	0.070	24	228
11	7.42	1.46	1.40	0.070	103	652
12	8.10	1.00	1.40	0.069	21	190

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

Field No.	No. of fungal colonies per gram of soil																	
	AN	AF	AP	AT	ASF	AA	CC	CH	DR	EN	FO	MC	MS 1	MS 2	PI	PE	PR	SC
1	144	54	0	18	0	0	0	0	0	54	0	36	36	0	18	0	0	0
2	252	126	0	18	0	0	18	0	0	144	18	234	0	54	54	0	0	0
3	324	108	0	198	0	18	18	36	0	18	0	216	0	36	126	54	18	0
4	342	108	0	216	0	0	36	0	0	306	0	72	180	0	126	36	36	0
5	1062	414	0	0	0	0	36	0	0	234	0	144	144	0	126	216	0	0
6	738	522	0	18	0	54	0	72	0	36	0	558	72	0	486	324	0	0
7	486	630	0	0	0	18	0	18	0	288	18	378	36	0	288	72	0	0
8	36	90	0	1890	18	0	0	0	36	90	0	378	108	0	54	0	18	0
9	126	126	0	522	18	0	0	0	54	126	0	36	144	0	0	0	36	0
10	288	36	0	90	0	0	0	0	72	0	18	144	18	0	0	0	36	0
11	72	144	18	504	0	0	0	36	126	288	0	342	108	0	54	72	18	0
12	90	18	36	72	90	0	0	0	126	72	0	90	0	0	0	36	0	108

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

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

	pH	EC	Organic matter	Nitrogen	Phosphorus	Potassium
Total No. of colonies	0.35	-0.06	-0.37	-0.32	-0.29	0.20
No. of colonies of saprophytic fungi	0.33	-0.06	-0.42	-0.36	-0.26	0.18
No. of colonies of pathogenic fungi	0.30	0.05	0.81**	0.56*	-0.30	0.1

*, **, Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

Soil mycoflora: A total of 18 fungal species belonging to 9 genera namely *Aspergillus, Alternaria, Cladosporium, Drechslera, Fusarium, Mortierella, Mucor, Penicillium* and *Sclerotium* were isolated from various soil samples. Among these, species of *Aspergillus* were most common. These included *Aspergillus niger* van Tieghem, *A. fumigatus* Fresenius, *A. penecilloides* Speg., *A. flavus* Link, *A. terreus* Thom and *Emericella nidulans* G. Winter with 36–1062, 18–630, 0–36, 0–1890, 0–90 and 0-306 colonies g^{-1} soil in different fields, respectively. Likewise, there were three species of *Penicillium* namely *Penicillium italicum* Wehmer, *P. expansum* Link and *P. restrictum* Gilman & Abbott with 0–486, 0–324 and 0–36 colonies g^{-1} soil in various surveyed fields, respectively. Other isolated fungal species were *Alternaria alternata* (Fr.) Keissl., *Cladosporium cladosporoides* (Fresen) De Vries, *C. herbarum* (Pers.) Link, *Drechslera* sp., *Fusarium oxysporum* (Schlecht) Synder & Hansen, *Mortierella chlamydospora* (Chesters) Plaats-Nit., *Mucor* sp.1, *Mucor* sp. 2, and *Sclerotium rolfsii* Sacc. with 0–54, 0–36, 0–72, 0–126, 0–18, 36–558, 0–144, 0-54 and 0–108 colonies g^{-1} soil, respectively (Table 2). Different species of *Aspergillus* especially *A. niger* and *A. flavus*; *Penicillium*, *Fusarium* etc are reported as postharvest pathogens of cucumber (Amin *et al.,* 2009). *S. rofsii* and *F. oxysporum* are the most common root rot causing soil-borne pathogen of cucumber (Abd-El-Kareem, 2009).

Total number of fungal colonies per gram of soil ranged from 360–2880 in samples taken from different fields. Out of 12 fields, in 8 fields number of fungal colonies per gram soil was above 1100. Saprophytic fungi were more common than pathogenic ones. There were 360–2754 colonies of saprophytic fungi as compared to 1–234 colonies of pathogenic fungal species g^{-1} soil (Fig. 1A–C).

Correlation studies: Correlation of total number of fungal colonies as well as number of saprophytic and pathogenic fungal species with soil pH, EC, phosphorus and potassium was insignificant. However it has been established by many researchers that level of potassium in soil is negatively correlated with the prevailing pathogenic fungal flora (Amtmann *et al.*, 2008). Results of the present study suggested that correlation of number of pathogenic fungi with soil nitrogen and organic matter was positive and significant (Table 3), which indicates that high soil nitrogen and organic matter favour growth and reproduction of pathogenic fungi. Several studies demonstrated that application of more nitrogen fertilizers than recommended dose results in increase in disease severity of powdery mildews (Jensen & Munk, 1997; Hoffland *et al.*, 2000), leaf rust by *Puccinia recondita* (Mascagni *et al.*, 1997), rice blast by *Magnaporthe grisea*, wheat leaf spot by *Septoria tritici* and blotch, by *Stagonospora nodorum* (Howard *et al.*, 1994; Simon *et al.*, 2003).

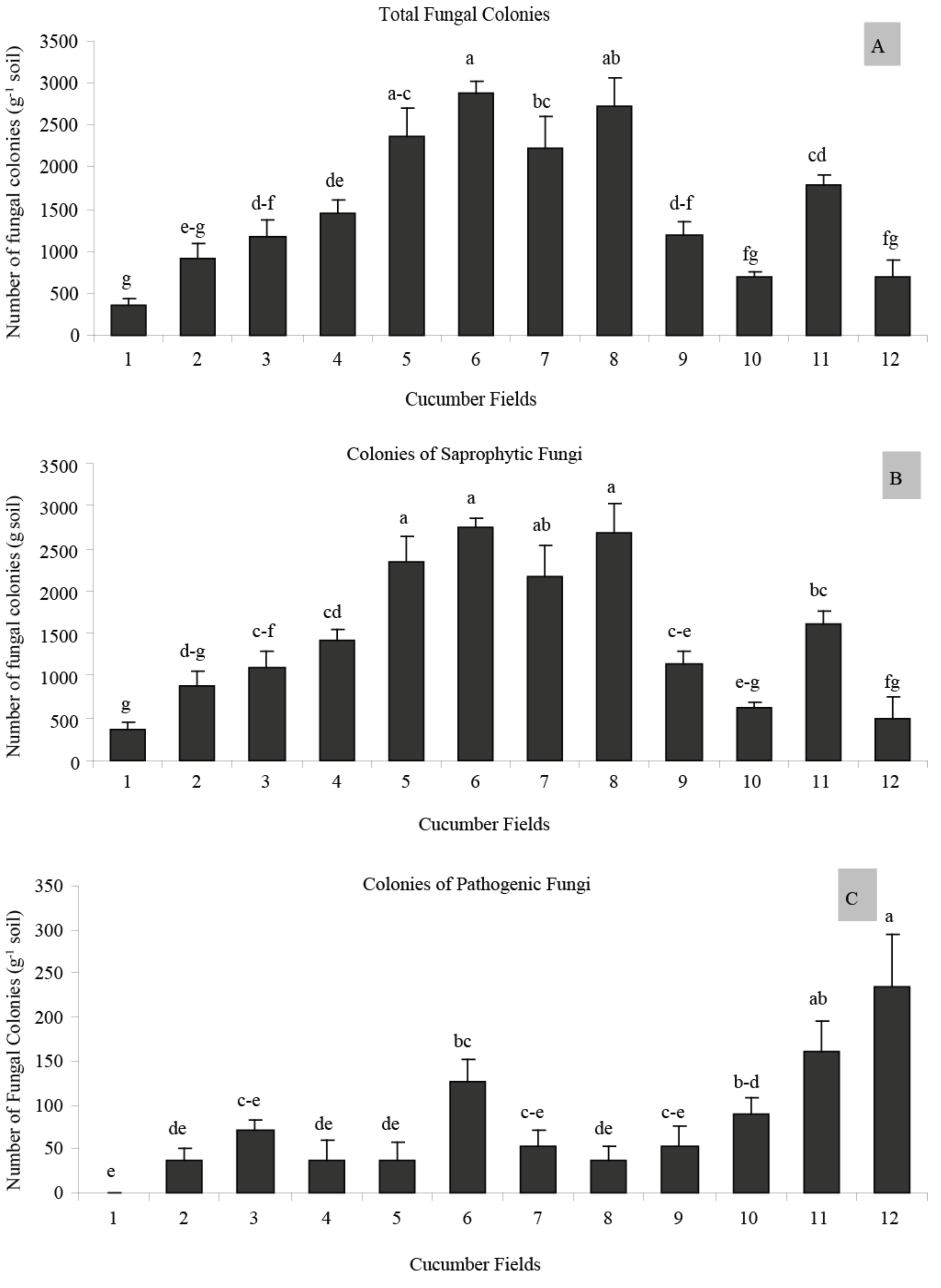

Fig. 1. Total number of fungal colonies, and number of saprophytic and pathogenic fungal colonies in soils of different cucumber tunnels. Vertical bars show standard error of means of five replicates. Bars with different letters show significant difference ($p \leq 0.05$) as determined by LSD method.

Conclusion

It is concluded from the findings of the present study that high nitrogen and organic matter in cucumber tunnels favour population of pathogenic fungi.

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