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## Short Communication

# Soil mycoflora in tomato fields

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**Density and species richness of fungal communities in soils of *Fusarium* infested and non-infested tomato-growing localities were studied by comparison of rhizoplanes, rhizospheres, and root-free soils. The rhizosphere soils harbored the highest counts of fungi, followed by root-free soil and rhizoplanes in both localities. Species richness was high in the rhizosphere and root-free soil but distinctly low in the rhizoplane. The population density of the rhizosphere and the rhizoplane showed a significant difference between infested and non-infested localities.**

Key Words——*Fusarium*; soil mycoflora; tomato field.

Since the proposition of the term "rhizosphere" as a compartment of the root environment (Hiltner, 1904), voluminous studies have been carried out dealing with different aspects of rhizosphere microorganisms (Rovira, 1965a; El-Abyad et al., 1982; Richards, 1987; Harris, 1988; Lynch and Wood, 1988; Lynch, 1990; Barber, 1995; Tate, 1995). The quantitative and qualitative composition of these organisms are greatly affected by root exudates as well as soil type (Rovira, 1965 b; Parkinson, 1967; Youssef et al., 1975; Richards, 1987; Harris, 1988). It is well known that these exudates vary with plant age (Rovira, 1956; Vancura and Hovadik, 1965). On the other hand, rhizosphere microorganisms have some effects on plant growth (Rovira, 1965a; Youssef and Mankarios, 1974; Lynch and Wood, 1988; Hoflich et al., 1994). Though some studies have referred to considerable variations in the rhizospheres of resistant and susceptible varieties (Subba-Rao, 1977), none of the previous studies have dealt with the rhizospheric microorganisms of healthy and diseased roots of the same variety.

Tomato wilt induced by *Fusarium oxysporum* f.sp. *lycopersici* is a very serious disease of wide distribution in Egyptian soils. The main objective of the present investigation is to contrast the mycoflora of the root environment of healthy and diseased tomato plants in non-infested and infested soils, respectively.

Soil properties in the Governorate of Ismailia vary considerably from one locality to another. Soils of the region consist of arid and semi-arid desert soils, some of which are cultivated, with textures ranging from sandy and sandy loam in reclaimed localities to sandy clay in old cultivated sites. The pH of such soils is slightly alkaline, fluctuating between 7 and 8, and the salinity ranges from 0.5 to 7 mmhos.

A total of 150 random soil and root samples were collected from both naturally infested and non-infested tomato growing localities for screening the mycoflora of rhizospheres, rhizoplanes and root-free soils. Samples thereafter were transferred to the laboratory in sterile-tight polyethylene bags and stored at 5°C until microbiological analyses were performed.

Dilution plating (as described by Johnson et al., 1959) and serial washing techniques (Harley and Waid, 1955) were adopted as isolation procedures. Czapek's yeast extract agar medium (CYA) amended with a combination of rose bengal (67 mg/L) and chloramphenicol (50 mg/L) was used for isolation. For each sample six replica plates were prepared and incubated at 28°C for 10 d, then developing colonies were identified and counted as colony forming units (cfu) per gram (dry soil or dry root). Pure cultures of isolated fungi were grown on standard media for proper identification: Ascomycetes on oatmeal agar (OA); mucoraceous fungi on malt extract agar (MEA) and potato dextrose agar (PDA); Hyphomycetes on PDA and potato carrot agar (PCA); *Aspergillus* and *Penicillium* on MEA and CYA. For species identification the following references have been consulted: *Aspergillus* (Raper and Fennell, 1965); *Penicillium* (Raper and Thom, 1949; Pitt, 1979); *Chaetomium* (Arx et al., 1986); *Fusarium* (Booth, 1971); dematiaceous Hyphomycetes (Ellis, 1971, 1976); general taxonomy (Domsch et al., 1980).

The genera *Aspergillus* and *Penicillium* were the richest amongst all the genera of class Hyphomycetes with 12 species each (Table 1). These were followed by *Fusarium* (5 spp.). Other genera of Hyphomycetes were represented by 3 or fewer species. Ascomycetes followed with 18 species, contributing 19% of total fungi isolated. *Chaetomium* came first among all genera of this class, being represented by 5 species. Other genera of Ascomycetes were represented by 2 or 1 species. Zygomycetes were represented by 8 species, accounting for only 8% of total fungi isolated. The other two class-

Table 1. Percentage frequency of species isolated from the three microhabitats in *Fusarium*-infested and non-infested tomato fields.

| No. | Organisms | Root-free soil | | Rhizosphere | | Rhizoplane | |
|---|---|---|---|---|---|---|---|
| | | H[a] | I[a] | H | I | H | I |
| 1 | *Absidia corymbifera* | 8 | 16 | 4 | 8 | — | — |
| 2 | *A. glauca* | 8 | 8 | — | — | — | — |
| 3 | *Acremonium strictum* | 12 | 20 | — | — | — | — |
| 4 | *A. terricola* | 4 | 4 | 24 | 24 | — | — |
| 5 | *Actinomucor elegans* | 4 | 4 | — | 12 | — | — |
| 6 | *Alternaria alternata* | 24 | 36 | 52 | 40 | 24 | 16 |
| 7 | *Arachniotus dankaliensis* | 12 | 12 | 8 | 12 | — | — |
| 8 | *Ascotricha chartarum* | 8 | — | — | — | — | — |
| 9 | *Aspergillus aegyptiacus* | 24 | 24 | — | — | — | — |
| 10 | *A. carneus* | — | — | — | 8 | — | — |
| 11 | *A. clavatus* | — | — | — | 4 | — | — |
| 12 | *A. flavus* | 60 | 56 | 56 | 90 | 20 | 24 |
| 13 | *A. niger* | 92 | 92 | 88 | 84 | 52 | 36 |
| 14 | *A. ochraceus* | 52 | 56 | 44 | 32 | 4 | 4 |
| 15 | *A. sydowii* | 40 | 32 | 36 | 36 | — | — |
| 16 | *A. terreus* | 96 | 88 | 84 | 76 | 20 | 12 |
| 17 | *A. terricola* | — | — | 4 | — | — | — |
| 18 | *A. ustus* | 8 | 8 | 4 | 8 | — | — |
| 19 | *A. versicolor* | 40 | 48 | 20 | 16 | — | — |
| 20 | *A. wentii* | 32 | 32 | 12 | 16 | — | — |
| 21 | *Bartalina robillardoides* | 4 | 4 | — | — | — | — |
| 22 | *Botryotrichum piluliferum* | 44 | 40 | 20 | 16 | 12 | 4 |
| 23 | *Botrytis cinerea* | — | 8 | — | — | — | — |
| 24 | *Byssochlamys nivea* | 24 | 20 | 8 | 8 | 8 | — |
| 25 | *Cephaliophora irregularis* | — | 4 | 4 | — | — | — |
| 26 | *Chaetomium bostrychodes* | 8 | 4 | 20 | 8 | — | — |
| 27 | *C. globosum* | — | — | 16 | 12 | — | — |
| 28 | *C. gracile* | 16 | 8 | 20 | 12 | 4 | — |
| 29 | *C. madrasense* | — | — | 20 | 20 | — | — |
| 30 | *C. nigricolor* | 8 | 8 | 12 | 8 | — | — |
| 31 | *Chrysosporium tropicum* | — | — | 16 | 4 | — | — |
| 32 | *C. xerophilum* | 8 | 4 | — | — | — | — |
| 33 | *Circinella muscae* | 4 | 4 | — | — | — | — |
| 34 | *Cladosporium cladosporioides* | 22 | 20 | 12 | 24 | 4 | 4 |
| 35 | *C. herbarum* | 16 | 24 | — | — | — | — |
| 36 | *Curvularia oryzae* | 4 | 4 | 16 | 4 | — | — |
| 37 | *C. tuberculata* | 24 | 12 | 16 | 12 | — | — |
| 38 | *Drechslera holmii* | — | — | 8 | 8 | — | — |
| 39 | *D. rostrata* | 8 | 8 | — | — | 4 | 4 |
| 40 | *D. spicifera* | 12 | 8 | — | — | — | — |
| 41 | *Emericella nidulans* | 88 | 80 | 88 | 56 | 16 | 12 |
| 42 | *Emericellopsis salmosynnemata* | — | 4 | — | — | — | — |
| 43 | *Epicoccum purpurascens* | 4 | 4 | 4 | 4 | — | — |
| 44 | *Eurotium chevalieri* | — | — | 24 | 12 | — | — |
| 45 | *E. rubrum* | 32 | 24 | — | — | — | — |
| 46 | *Fusarium concolor* | — | — | 4 | — | 24 | 24 |
| 47 | *F. dimerum* | 24 | 8 | 8 | — | — | — |
| 48 | *F. equiseti* | — | 8 | — | — | — | — |
| 49 | *F. oxysporum*[b] | 64 | 72 | 56 | 80 | 80 | 96 |
| 50 | *F. solani* | 52 | 48 | 36 | 28 | 60 | 52 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 51 | *Geotrichum candidum* | 4 | 4 | 8 | 8 | — | — |
| 52 | *Gliocladium roseum* | — | — | — | — | 8 | 4 |
| 53 | *Gliocladium* sp. | 20 | 12 | — | — | — | — |
| 54 | *Graphium* sp. | 8 | — | 8 | 4 | — | — |
| 55 | *Humicola fuscoatra* | 20 | 24 | 16 | 24 | — | — |
| 56 | *H. grisea* | 8 | 8 | — | — | — | — |
| 57 | *Macrophomina* sp. | — | — | — | — | 8 | 8 |
| 58 | *Melanospora* sp. | — | — | — | — | — | 4 |
| 59 | *Microascus cinereus* | — | — | 14 | 12 | — | — |
| 60 | *M. trigonosporus* | 20 | 22 | — | — | — | — |
| 61 | *Mucor circinelloides* | 16 | 24 | 12 | 28 | 20 | 40 |
| 62 | *M. racemosus* | 8 | 4 | 4 | 16 | — | — |
| 63 | *Myrothecium roridum* | 28 | 12 | — | — | — | — |
| 64 | *M. verrucaria* | 28 | 28 | 20 | 20 | — | — |
| 65 | *Neocosmospora vasinfecta* | — | — | 4 | — | — | — |
| 66 | *Nigrospora oryzae* | — | — | 4 | — | — | — |
| 67 | *Paecilomyces lilacinus* | 28 | 32 | 28 | 16 | — | — |
| 68 | *P. variotii* | 12 | 12 | 12 | 4 | — | — |
| 69 | *Papulaspora* sp. | — | — | — | — | — | 4 |
| 70 | *Penicillium brevicompactum* | 4 | 12 | — | 4 | 4 | 8 |
| 71 | *P. canescens* | 40 | 32 | 20 | 24 | — | — |
| 72 | *P. chrysogenum* | 8 | 8 | 16 | 8 | — | — |
| 73 | *P. citrinum* | 12 | 8 | — | — | 12 | 16 |
| 74 | *P. cyclopium* | 8 | 8 | 8 | 4 | — | — |
| 75 | *P. funiculosum* | 8 | 12 | 24 | 20 | — | — |
| 76 | *P. oxalicum* | 16 | 12 | 12 | 8 | — | — |
| 77 | *P. purpurogenum* | 32 | 28 | — | — | — | — |
| 78 | *P. roquefortii* | — | 4 | — | — | — | — |
| 79 | *P. rubrum* | 20 | 28 | 36 | 8 | — | — |
| 80 | *P. rugulosum* | 12 | 8 | 8 | 4 | — | — |
| 81 | *P. variabile* | — | — | 12 | 12 | 4 | 4 |
| 82 | *Phoma leveillei* | 4 | 4 | — | — | 8 | 4 |
| 83 | *Rhizoctonia solani* | — | 4 | — | 12 | 40 | 20 |
| 84 | *Rhizopus stolonifer* | 52 | 48 | 44 | 56 | 56 | 52 |
| 85 | *Scopulariopsis brevicaulis* | 52 | 52 | 24 | 28 | — | — |
| 86 | *S. candida* | — | — | 8 | 4 | — | — |
| 87 | *S. hanii* | 28 | 24 | 16 | 28 | — | — |
| 88 | *Sporormiella minimoides* | 8 | — | — | — | — | — |
| 89 | *Stachybotrys chartarum* | 24 | 20 | 12 | 4 | — | — |
| 90 | *Syncephalastrum racemosum* | — | — | 8 | 8 | — | — |
| 91 | *Talaromyces flavus* | 8 | 4 | — | — | — | — |
| 92 | *Tilletiopsis* sp. | — | — | 12 | 8 | — | — |
| 93 | *Trichoderma harzianum* | 32 | 24 | 28 | 24 | 20 | 12 |
| 94 | *T. koningii* | 20 | 28 | 16 | 8 | — | — |
| 95 | *Trichosporon beigelii* | 4 | 4 | 12 | 4 | — | — |
| 96 | *Ulocladium oudemansii* | 16 | 4 | 8 | 8 | — | — |
| 97 | *Verticillium* sp. | 12 | 4 | 8 | 4 | — | — |
| Total number of species | | 71 | 74 | 64 | 63 | 24 | 24 |
| | | 77 | | 69 | | 26 | |

a) H: Non-infested; I: Infested.
b) Including pathogenic and non-pathogenic isolates.

es, Agonomycetes and Coelomycetes, were poorly represented.

Species frequency (%) was calculated as the number of cases of isolation out of 25 samples (Table 1) for each microhabitat. Four classes of species frequency were recognized: a high occurrence class, consisting of species showing frequencies of more than 50%, e.g., *Aspergillus flavus*, *A. niger*, *Emericella nidulans*, and *F. oxysporum*; a moderate occurrence class, including species showing frequencies ranging between 49 and 25%; a low occurrence class, containing species showing frequencies ranging between 24% and 12%; and a rare occurrence class, including species showing frequencies of less than 12%, e.g., *Absidia glauca*, *Aspergillus ustus*, *Drechslera rostrata*, and *Penicillium brevicompactum*. It was observed that fungi of high occurrence were almost the same in both root-free soil and rhizospheres. Some species (13 spp.) were common in all three microhabitats, of which *F. oxysporum*, *F. solani* and *Rhizopus stolonifer* were of high occurrence rank. Other species were restricted to a specific microhabitat. Egyptian soils tend to be slightly alkaline and the annual average temperature is relatively high. Both factors are quite favorable for *Fusarium* (Jones et al., 1982; Agrios, 1988) and this may account for its high frequency.

Collectively, root-free soils revealed 78 species, rhizospheres 68 species, and rhizoplanes 26 species. The limited number of species recovered from the rhizoplane indicates its selective effect on the occurrence of certain fungi on the root surface. The genera *Fusarium*, *Aspergillus*, *Mucor*, *Trichoderma*, *Penicillium*, and *Gliocladium* have been reported before as rhizoplane fungi (Subba-Rao, 1977). When the mean spectrum of species hosted by each one of the three microhabitats is considered (Table 2), it was evident that root-free soil accommodates the widest spectrum of species (ca. 16 spp.), while the rhizoplane showed the narrowest spectrum (5 spp.). Statistical analysis showed no difference between non-infested and infested status of the three microhabitats. But significant differences were found between root-free soil *versus* both rhizosphere and rhizoplane as well as between rhizosphere versus rhizoplane.

Comparison of mean cfu's for the three different microhabitats (Table 2) revealed marked difference in colony counts. Rhizosphere soils showed the highest counts while rhizoplane samples had the lowest counts. Root-free soil showed intermediate counts. It was noticed that the R:S ratio (mean count of fungi in the rhizosphere to that of root-free soil) was 7.0 for the non-infested habitat and 8.5 for the infested one. The increasing number of fungal populations in the rhizosphere is attributed to the carbon compounds released from living roots into the surrounding soils (Subba-Rao, 1977; White, 1989). The concentration of these exudates increases and the number of fungal propagules becomes greater closer to the root surface. The slight increase in fungal populations of the *Fusarium*-infested compared with non-infested localities might be attributed to the contribution from the death of plant roots, which provide an additional food source for the growth of saprophytic fungi (White, 1989).

Comparison of data of the three microenvironments in both non-infested and infested localities (Table 2) revealed that there was no difference in species composition of fungal flora between non-infested and infested localities. At the same time, the total number of species recorded from each locality was approximately the same. There was no correlation between number of species recorded and population density in either locality. While the species number and richness tend almost to be alike in non-infested and infested localities, the count of fungi varied between them slightly (significant at $P=0.01$). The absence of a significant difference in quantitative and qualitative composition of fungal populations from root-free soil in both non-infested and infested habitats

Table 2. Comparison between the three microhabitats of non-infested and infested soils.

| Parameter | Non-infested | Infested | Mean | *t*. Value |
|---|---|---|---|---|
| Species richness[a] | | | | |
| Root-free soil | 16.6 | 15.9 | 16.3 | 0.52 |
| Rhizosphere | 13.4 | 12.8 | 13.1 | 0.52 |
| Rizoplane | 5.3 | 4.8 | 5.1 | 0.8 |
| Total number of species | | | | |
| Root-free soil | 71 | 74 | – | – |
| Rhizosphere | 64 | 63 | – | – |
| Rizoplane | 24 | 24 | – | – |
| Population density[b] | | | | |
| Root-free soil | $6.3\times10^2$ | $7.3\times10^2$ | $6.8\times10^2$ | 0.92 |
| Rhizosphere | $4.5\times10^3$ | $6.2\times10^3$ | $5.3\times10^3$ | 51.4[c] |
| Rizoplane | $1.1\times10^2$ | $1.3\times10^2$ | $1.2\times10^2$ | 2.1[c] |

a) Expressed as number of species recovered from each habitat. Mean of twenty five samples.
b) Expressed as total cfu/g. Mean of twenty five samples.
c) Significant at $P=0.01$.

has previously been reported (Abdul Wahid, 1990). The difference between non-infested and infested habitats in both the rhizosphere and the rhizoplane might be due to the introduction of extra nutritional elements in the soils resulting from the lysis of the diseased roots and sloughed off cells and tissues (White, 1989).

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