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Features of broad bean-wilt-suppressive soil in Ismailia Governorate

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Summary – The wilt suppressive soil "Fm2" showed the highest percentage of healthy plant (81%) comparing to the other soils which showed more than 60% healthy plant. When the ten field soil samples (including the suppressive soil "Fm2") were artificially inoculated with *Fusarium oxysporum* f. sp. *fabae*, they exhibited high receptivity value (over 50% diseased plants) while "Fm2" soil (the suppressive soil) showed the almost least value (less than 10% diseased plants). The fungal flora of the soil samples was studied where 64 spp. belonging to 37 genera were recovered with the highest total and species counts was found in "Fm2" soil. Chemical analysis revealed that chloride and calcium ions were higher in "Fm2" soil. Chloride ions were higher than calcium ions in all samples.

Zusammenfassung – Der Welk-Hindernis-Boden «Fm2» zeigt den höchsten Prozentsatz gesunder Pflanzen (81%) im Vergleich zu den andern Böden, die mehr als 60% gesunde Pflanzen aufweisen. In der vorliegenden Untersuchung wurden 10 Bodenproben (darunter der Welk-Hindernis-Boden «Fm2») künstlich mit *Fusarium oxysporum* f. sp. *fabae* infiziert. «Fm2»-Boden (der Welk-Hindernis-Boden) zeigte immer den geringsten Prozentsatz an (weniger als 10% kranke Pflanzen), während die anderen Böden den höchsten Prozentsatz an kranken Pflanzen (mehr als 50%) aufwiesen.
Die Pilzflora der studierten Bodenproben enthielt 64 Arten von 37 Gattungen mit der höchsten Frequenz und Artenzahl im «Fm2»-Boden.
Die chemischen Analysen ergeben, dass Chlorid- und Calciumionen im «Fm2»-Boden hoch waren. Chloridionen waren zahlreicher als Calciumionen in allen untersuchten Proben.

Introduction

Fusarium wilt of *Vicia faba* L. caused by *Fusarium oxysporum* f. sp. *fabae* Ya *et* Fang (F.o. *fabae*) was first reported to occur in Egypt in 1962 (Abd-El-Rehim, 1962). Soils that are naturally suppressive to *Fusarium* wilt of numerous crop plants are known to occur in many regions of the world (Toussoun, 1975; Schneider, 1982; Cook and Baker, 1983; Alabouvette, 1986, and Abdul Wahid et al., 1998). These soils have certain characters that discriminate them from other soils. These characters include texture, chemical composition and microbial content of the soil (Hornby, 1983; Lumsden et al., 1987, and Oyarzun et al., 1994). These soils reduce the incidence of root diseases caused by soil-borne fungi, even though a strong pathogen and susceptible host are present. Fusarium suppressive soils are classified into two types: the classical type which suppresses only pathogenic *Fusarium* spp., and the forest – soil which suppresses all *Fusarium* spp. isolates (Hornby, 1983).

The intent of this investigation was to determine the features of broad bean-wilt-suppressive soil in Ismailia Governorate.

Materials and methods

Infestation level and potentiality

Infestation level and potentiality of the ten soils were examined using high-quality seeds of *Vicia faba* L. cultivar Giza two (G2) and the pathogen *Fusarium oxysporum* f. sp. *fabae* Ya et Fang. Firstly, the seeds were grown in pots containing normal soils to determine the natural infestation level. Secondly, the soils were artificially inoculated with the pathogen to determine the level of interaction of these soils with the pathogen which in turn means the diseasepotential of the soils.

Biotic and abiotic characters of the soil

Fungal population of the ten soils was estimated using dilution plate methods (Johnson et al., 1959, and Kiewnick et al., 1997). Growing colonies were identified and counted as colony forming units (cfu) per gram dry soil.

Soil texture was determined by sieve techniques (Black, 1965) and typified according to the soil texture triangle (Hodgson, 1974). Chemical composition and electrical conductivity (EC as mS/cm) were measured as described by Page *et al.* (1982).

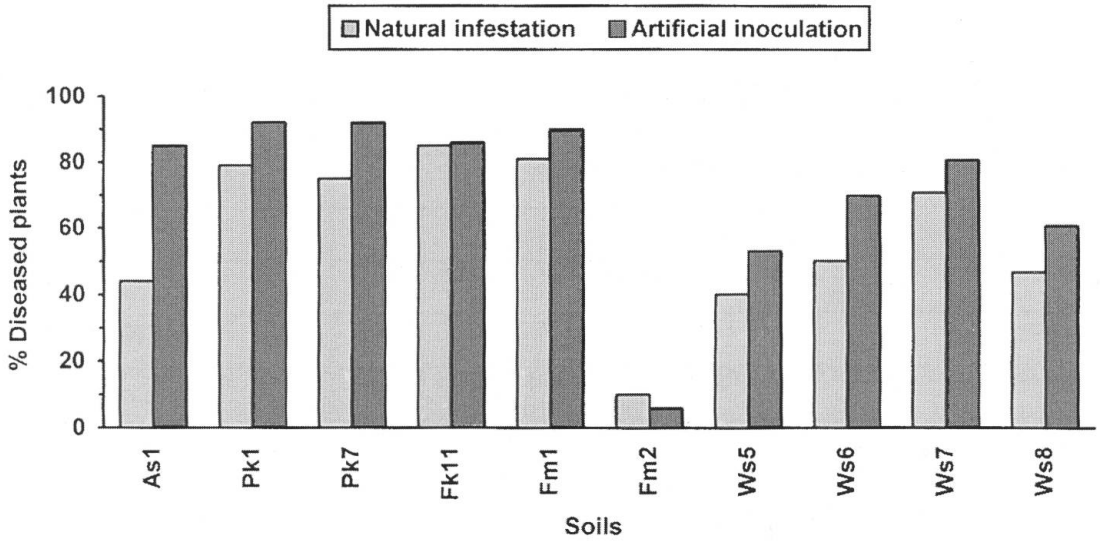

Fig. 1.: Percentage of diseased broad bean plants in the naturally infested and artificially inoculated soils.

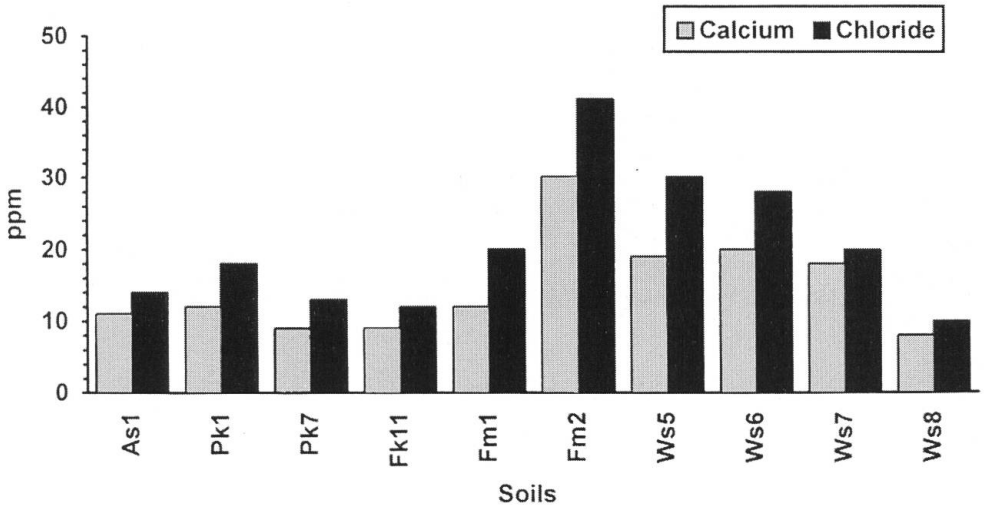

Fig. 2.: Calcium and Chloride ion concentration in the different soil samples.

isolated Fungi	As1	Pk1	Pk7	Fk11	Fm1	"Fm2"	Ws5	Ws6	Ws7	Ws8
Absidia corymbifera	-----	-----	-----	-----	-----	1,66	-----	-----	-----	-----
Acremonium strictum	13,33	3,33	5,00	6,66	-----	18,33	8,33	10,00	-----	-----
Alternaria alternata	4,16	-----	1,66	10,00	11,66	-----	-----	-----	5,00	5,00
A .tenuissima	-----	-----	-----	3,33	-----	13,33	-----	-----	-----	-----
Aspergillus flavus	24,00	60,00	60,00	8,33	3,33	5,00	5,00	2,16	-----	6,66
A . niger	1,66	3,33	5,00	1,66	5,00	26,66	2,00	-----	15,00	23,33
A . terreus	35,00	13,33	11,66	16,00	21,66	8,33	31,66	5,00	13,33	38,33
A . versicolor	-----	-----	-----	11,66	6,66	11,66	8,33	-----	-----	-----
Botryotrichum piluliferum	-----	-----	5,00	-----	1,66	6,66	-----	3,33	-----	-----
Chaetomium globosum	1,66	-----	1,30	3,33	-----	8,33	-----	-----	-----	-----
Cladosporium herbarum	-----	1,66	5,00	10,00	6,66	5,00	1,66	-----	1,66	-----
Drechslera spicifera	-----	-----	3,33	-----	-----	25,00	-----	-----	3,33	-----
Emercella nidulans	13,33	6,66	15,00	43,33	46,40	51,66	16,66	5,00	16,66	15,00
Fusarium dimerum	6,66	-----	6,40	-----	50,00	10,00	5,00	5,00	5,00	-----
F . equiseti	-----	-----	3,33	-----	8,33	11,66	-----	5,00	-----	-----
F . oxysporum	16,66	16,66	18,33	8,33	6,66	46,60	20,00	8,33	18,33	16,66
F . solani	5,00	8,33	10,00	3,33	1,66	5,00	15,00	-----	18,33	-----
Myrothecium verrucaria	-----	-----	16,33	11,66	-----	26,66	-----	-----	-----	-----
Penicillium canescens	1,66	3,33	4,50	-----	6,66	11,66	-----	-----	5,00	-----
P . cyclopium	-----	-----	8,33	-----	25,00	36,66	-----	3,33	-----	-----
Rhizopus stolonifer	6,66	5,00	-----	-----	10,00	16,66	8,33	11,66	5,00	8,33
Trichoderma harzianum	-----	-----	-----	-----	-----	6,66	-----	-----	-----	-----
T . koningii	-----	-----	-----	-----	-----	8,33	-----	-----	-----	-----

Table 1: Counts of the most frequent and dominant fungi isolated from the ten soils.*

* count is in cfu/g soil and represents the mean of six replica (count$\times 10^2$)

Statistical analysis

The data were analized by one-way-analysis of variance (after arcsine-transformation for proportions). Means were separated by Duncan's multiple range test ($P = 0.05$) using SAS (1988) computer program.

Results

Infestation level

The percentage of diseased plants (DP) varied greatly among the ten soils. The "Fm2" soil showed a limited infestation potentiality (DP = 19%), while the highest infestation level was recorded in soils "Pk1", "Pk7", "Fk11" and "Fm1"(DP >80%). When these ten soils were artificially inoculated with the pathogen, the infestation potential of all soils increased except for the soil "Fm2" where the infestation level decreased (DP <10%) (Fig. 1). Statistical analysis revealed significant differences between this soil and all other soils.

Soil characterizations

Clay represented a minor part of the soil composition (0.8%–0.4%), while silt was the intermediate compartment (34.5%–24.5%). Sand was the major component of the ten soil samples examined (72%–65%). All soils were alkaline, pH ranged between 8.08 to 8.78. Electric conductivity was fluctuating from 0.44 mS/cm to 2.15 mS/cm. Chemical analysis revealed that there were no distinct features amongst the ten soils except for the chloride and calcium

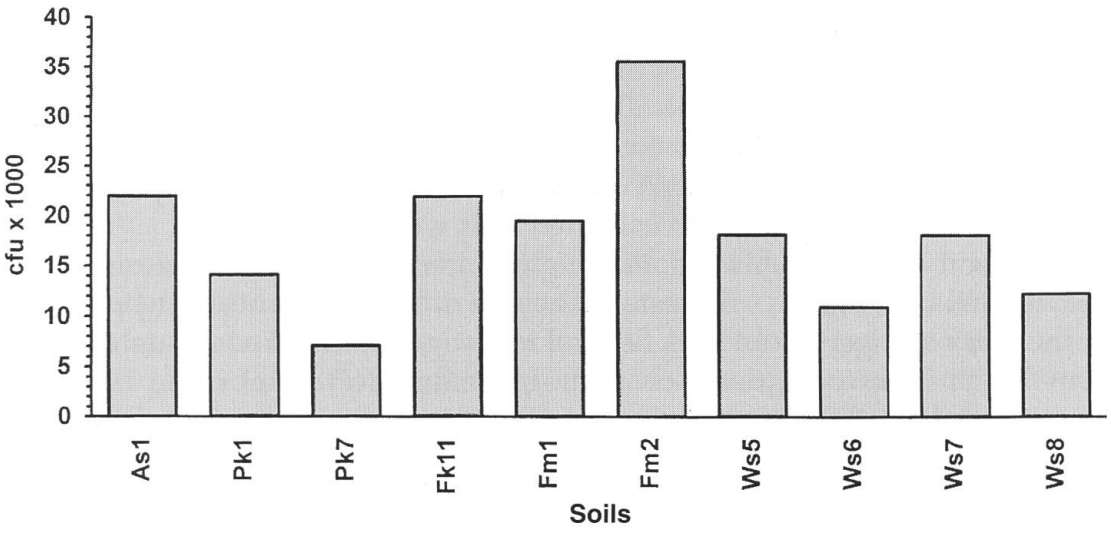

Fig. 3.: Total counts of fungi (cfu) recorded in the different soil samples.

ions. Both ions were much higher in the "Fm2" soil comparing to all other remained soil samples (Fig. 2).

It was possible to isolate about 64 spp. belonging to 37 genera in 5 classes. Table 1 represents the most frequent isolated species. Several species were more abundant in "Fm2" soil than other soils. The "Fm2" soil accommodated the highest total count of colony forming units (Fig. 3) reported during this investigation (3,55 × 104 cfu/g), which was highly significant ($P < 0.001$).

Discussion

According to their response to artificial inoculation the ten soils could be categorized into suppressive (SS) and conducive (CS) soils. The "Fm2" soil could be classified as the most suppressive (S) soil (>80% healthy plants = HP), whereas other soils could be ranked as either moderate suppressive (MS) soil (50–80%HP) or conducive (C) soils (<50% HP). This arbitrary suppressive index could be used for surveying and classifying soils with respect to their infestation potential.

To explore which factor(s) may be responsible for suppressiveness among the tested soils, it was necessary to study the biotic and abiotic features of the soil samples. Although many publications reported that clay or clay-loam soils have a potency to suppress wilt disease (Stotzky, 1973, and Tu et al., 1975), our results proved that the soil "Fm2", which is sandy loam, successfully suppressed *Fusarium* wilt of broad bean. This is coincident with the findings of Baker (1980) that sandy loam soil could reduce the *Fusarium* wilt of carnations by 60 %. Smith and Snyder (1971) and Scher and Baker (1980) reported the existence of suppressiveness to *Fusarium* wilt in sandy loam soil.

The role of pH in disease suppressiveness was proved by many scientists (Hornby, 1983, and Lumsden et al., 1987). Some attributed this phenomenon to the alkaline medium of the soil (Louvet et al., 1976, and Scher and Baker, 1980), still others suggested that acidic pH is the motive factor (Garrett, 1970). The alkalinity of "Fm2" soil cannot account for its capability to suppress *Fusarium* wilt of Vicia faba as all investigated soils were alkaline.

The soil "Fm2" exhibited the highest concentration of chloride and calcium ions among the soils tested. The role of both elements in suppression of the wilt pathogen is obvious. Several investigators clarified the deleterious action of these two elements on pathogenic fungi (Fletcher *et al.*, 1982 and Lumsden et al., 1987). Other elements such as magnesium, potassium and phosphorus have no effect on chlamydospore germination in suppressive soils (Chuang, 1988).

Results obtained in our investigation confirmed the findings of others that chemical substances may be contributing to suppressiveness of soils to Fusa-

rium wilt, at least partially. It is still evident that suppressiveness exhibited by the "Fm2" soil may be a function of soil microbiota as well. Biological origin of suppressiveness cannot be neglected, it was proved by many workers (Cook and Baker, 1983; Hornby, 1983; Alabouvette et al., 1985, and Oyarzun et al., 1994). The "Fm2" soil displayed the highest count of fungi (3,55×104 cfu/g) as well as the greatest fungal diversity. This coincides with the results of Kiewnick et al. (1997). Mycobiota of this soil may act as a whole community to reduce the ability of the pathogen *F.o. fabae* to establish as a saprophyte in the soil, which in turn decline the probability of infection. The saprophytic activity of a pathogen is affected by the community of its microbial associates (Odum, 1971; Marois and Mitchell, 1981). This could be through the competition for space and nutrient (Alabouvette, 1986) as well as the deplation of oxygen and creation of deleterious metabolites (Hornby, 1983). The diversity of microorganisms in a suppressive soil has a regulation mechanism tends to establish a microbial equilibrium that affects the survival of the pathogen (Louvet et al., 1976 and Alabouvette et al., 1979).

Lumsden et al. (1987) found that *Fusarium, Trichoderma, Pseudomonas*, and *Actinomycetes* were consistently present in higher number in suppressive soil. Their findings confirm our results, where *Fusarium spp., Trichoderma koningii, T. harzianum, Penicillium cyclopium* were isolated in high quantity from "Fm2" soil comparing with other soils. These fungi were isolated frequently from suppressive soils elsewhere (Hornby, 1978; Lui and Baker, 1980 and Bara et al., 1982).

Innumerable researchers have referred to the role of non-pathogenic *Fusarium oxysporum* strains in the suppressiveness (Toussoun, 1975; Hornby, 1983; Schneider, 1982 and 1984; Cook and Baker, 1983; Alabouvette, 1986, and Larkin et al., 1996). Although non-pathogenic *Fusarium* strains are closely related to the pathogenic strains and have nearly the same ecological niches, they grow well in suppressive soils and chlamydospores are produced more rapidly than what happen in conducive soils. On the other hand germination of pathogenic *Fusarium* is lower and germ tubes are strikingly shorter and appear to be stunted (Smith and Snyder, 1972; Toussoun, 1975, and Larkin et al., 1993).

Our results implied that some other biological factor, in addition to the fungi, might be engaged in the suppressiveness of "Fm2" soil. Although we did not evaluate the biomass of bacteria in this investigation, many other scientists proved their noticeable role in *Fusarium* suppression (Tu and Cheng, 1981, and Alabouvette et al., 1985). The suppressiveness of a soil may not be due to an individual antagonist but a group of antagonists integrally acting together and belonging to diverse taxonomical groups.

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