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INTERACTION BETWEEN TOMATO WILT PATHOGEN, ROOT-KNOT NEMATODE AND A MYCORRHIZAL FUNGUS

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ABSTRACT

The studied Fusarium oxysporum Iycopersici isolates differed in their virulence to tomato plants with 60 % maximum vessels discolouration. The mycorrhizal fungus Fusarium equiseti was nonpathogenic to tomato. Mycorrhiza significantly decreased the population of (Meloidogyne javanica) and root galls formation in the presence or absence of the wilt fungus. plant roots.

Nematode and wilt pathogen significantly decreased tomato fresh and dry weights; than control; when applied individually or in combination to the soil. In the meantime; plant weight was highly increased in response to the mycorrhizal application.

Nematode Population on the roots and galls were exhibited by mycorrhiza.

Reducing sugars were increased in mycorrhizal fungus treated plants and decreased in nematode infected ones. Total protein was significantly decreased than control. Fusarium wilt fungus had a slight negative effect on total protein, while nematode inoculated plants resulted much higher protein than control. Phenolic compounds were decreased in response to the inoculation with nematode and wilt fungus. Mycorrhizal fungus treated plants showed great increase of phenols than control ones.

INTRODUCTION

Tomato root- knot nematode (*Meloidogyne javanica*) can be successfuly controlled by nematicides, but the later had bad effects on soil fauna and flora

(Abo-Elghar and El-Shinnawi, 1978).

Salem *et al.* (1984) found that *Fusarium equiseti* and *Pythium butleri* increased the phosphorus content of broad - bean seedlings and reduced the pathological stress on the seedlings through decreasing the galls formed by *M. Javanica.* Cooper and Grandison (1986) cleared that tomato mycorrhizal plants were more resistant than nonmycorrhizal ones to root nematode (*M. hapla*).

Concerning plant growth response, Carter (1978) examined the interaction between *M. inocognita* and *Fusarium oxysporum lycopersici* on three tomato cultivars with different genetic resistance to fusarium wilt. He found that the dry weight of stem was reduced and the combination of both pathogens caused synergistic decrease in stem dry weight of some varieties than others.

Samborski *et al.* (1958) observed that less of resistance involves the break - down of proteins to amino acids essential to pathogen development. They added that resistant cultivars normally lack these amino acids in sufficient quantities to maintain the parasite. Lewis and McClure (1975) found that quantities of certain free amino acids of cotton root- knot susceptible cultivar (M8) were greater than the resistant one (Clevawill).

Matta and Dimond (1969) who demonstrated the increase of soluble in susceptible tomato plants followed the infection with *F. oxysporum lycopersici.* Landecker (1982) mentioned that mycorrhizal plants secrete high amounts of phenols as a resistant mechanism to fungal invasion. Mahmoud (1985) found that the susceptible soybean cultivar Gorsoy Contained high levels of total, poly, mono and diphenols as compared with the resistant cultivar Forrest to *Fusarium* wilt. Cooper

and Grandison (1986 found that tomato mycorrhisal plants had larger root system as compared to the nonmycorrhizal ones.

The aim of the present investigation was to study the interaction between M. Javanica, F. oxysporum lycopersici and F. equiseti on tomato in relation to nematode population, plant fresh and dry weights and changes in reducing sugars, total protein and phenolic compounds.

MATERIAL ADN METHODS

1 - Isolation and identification of the fungi :

Wilted tomato plants were collected from different fields of Shebin El-Kom in the summer of 1986. Plant roots were washed thoroughly, surface sterilized with 0.1 % mercuric chloride for two minutes, rinsed several times in sterilized water and dried between two sterilized filter papers. Small pieces of each sample were plated on plain agar and incubated at 30 oC for 2 days. Single spore cultures were obtained using streak method. The obtained isolates were identified on the bases of spore characteristics (Gilman, 1957) and (Carter, 1978).

An identified mycorrhizal fungus fusarium equistia isolate was obtained from Agricultural Botany Depat., Faculty of Agric., Menoufia University (Salem *et al.*, 1984).

2- Nematode isolation, identification, reservation and estimation :

Inoculum of *Meloidogyne javanic* (Treub) was derived from the infected roots of black night shade (*Solanum nigrum* L.); grown in the Farm of Faculty of Agriculture, Menoufia Unversity. Pure stock cultures were maintained on *Solanum*

nigrum plants grown in lysimeters filled with sterile sand. Cultures were moistend daily with tap water and once a week with nutrient solution (Hewitt, 1951). Obtained root - knot nematode was identified according to Taylor *et al.* (1955).

To extract and enumerate *M. Javanica* stages; plants were uprooted and the roots were carefully washed using running tap water to remove soil particles. Roots of each replicate were weighed and stained with lactophenol - acid fuchsin for 24 hours. Stained roots were rinsed in water and macerated to release various nematode stages. A dissecting microscope was used for counting each individual stage and later was estimated per gram roots per treatment.

3- pot experiments :

Pots (15 cm in diameter) were sterilized by dipping in 5% formalin for 3 minutes. Soil sterilization was conducted using one liter of 5% formalin cubic foot soil and covered with plastic sheet for 7 days. The cover was then removed and the soil was left in open air until complete evaporation of formalin. Fungal inocula were separately grown on sand barly medium for 15 days. Each inoculum was applied to the soil at the rate of 3 % of soil weight, potted and irrigated daily after 7 days. Control pots recived sterilized soil mixed with the same rate of sand barley medium.

Wilt disease isolates and the mycorrhizal one were individually tested for their pathogenicity by trnsplanting 45 days old Nile Prichard tomato cultivar into the potted soil. Roots of used seedlings were surface sterilized and rinsed in sterilized water just before transplanting. Percentage of vessels discolouration was estimated at plant stem bases (cross sections) after two months of soil infestation.

Five pots were used as replicates for a fusarium isolate.

Nematode inoculation was conducted by applying 500 second stage larva of *M. Javanica* per pot at the time of seeding. Two experiments were carried out : the first was accomplished on April, 1987 and included the following treatments :

(1) Blank (check), (2) *F. oxysporum* lycopersici (F), (3) *F. equiseti* (M), (4) *M. Javanica* (N), (5) F+M, (7) M+N and (8) F+M+N. Nile irichard tomato seeds were surface sterilized and sown at the rate of 20 seeds per pot. One month later plants were thined to 5/ pot. Ten pots assembled each treatment and data were recorded two months after sowing as nematode population and galls formation. The second experiment was carried out at December, 1987 under greenhouse conditions. Ten surface sterilized seeds per pot and five pots per treatment were included. Four treatments were included i.e., nematode inculation, nematode + mycorrhizal fungus, nematode + fusarium wilt pathogen and all the three organisms together. Plant samples were collected every week to enumerate different nematode stages until the 6th week of seeding.

data recorded :

1- Fresh and dry weight of tomato plants:

samples of the first experiment were picked up 45 days after sowing (2 plants / replicate). The average fresh weight was determined and the plants were then dried at 70 °C for 3 days and the dry weight was estimated.

2 - Nematode population in tomato roots :

Different stages of *M. Javanica* were enumerated in root macerated tissues.

Besides; formed galls were counted both at the end of the first experiment and every week of the second one.

3 - Chemical constituents of tomato plants:

One month old tomato plants of the first experiment were used for determination of some chemical compounds. Reducing sugars were colourimetrically determined according to Dubois *et al.* (1956). Total nitrogen was estimated in yeast biomass using the semimicrokhjldahl method according to Ling (1963). Nitrogen percentage was multiplied by 6.25 to obtain crude protein percentage.

Total phenols estimation was conducted by treating a known volume of the ethanolic extract with 0.25 ml Hcl in test tube, boiling in water bath for 10 minutes and then cooled. One ml of phenol reagent (folins) and 1 ml of 20% sodium carbonate were added. The mixture was completed by distilled water to be 10 ml and its colour density was measured at 620 nm.

RESULTS AND DISCUSSION

1 - Identification and virulence of tested fungi :

Obtained isolates of tomato wilted plants resulted *Fusarium oxysporum* fungus as the causal organism according to Gilman (1957). All isolates were pathogenic; with different virulence. The most virulent isolate (caused 60% vessels discolouration) was choosed for further studies.

Mycorrhizal fungus (*Fusarium equiseti*) was nonpathogenic to tomato plants as reported by Landecker *et al.* (1982) and Cooper and Grandison (1986).

2 - Nematode population in response to mycorrhiza and *F. oxysporum* :

Results present in Table (1) show clear that the mycorrhizal fungus significantly decreased nematode population and galls as compared with controls. Fusarium wilt pathogen also reduced nematode population insignificantly. Application of both fungi to the soil significantly decreased the total number of nematode and galls formation.

These results are in harmony with those obtained by Salem *et al.* (1984) who suggested that the VAM fungi may limit nematode activity. Gordon *et al.* (1986) and Grandison and cooper (1986 too) reported that mycorrhizal inoculation into the soil reduced nematode population and adult development in plant roots. Cooper and Grandison (1986) observed that tomato mycorrhizal plants were more resistant to root-knot nematode than nonmycorrhizal ones.

3 - plant growth response:

Results in Table (2) indicate that soil infestation with either *M. Javanica* or *F. oxusporum* and both of them significantly decreased fresh and dry weight of tomato plants than control. Plants were severly affected with the combined inoculation (nematode + wilt disease fungus) as recorded also by Ibrahim *et al.* (1982).

In the meantime, the alone application of the mycorrhizal fungus to the soil resulted the highest fresh and dry weights. Landecker *et al.* (1982) mentioned that mycorrhizal fungi when established in the root secrete 3 variety of growth regulators, including auxins, cytokinins and gibberllins. These compounds are

Table (1) : Influence of the mycorrhizal fungus and wilt disease pathogen on Meloidogyne javanica population into tomato roots two months after seeding.

Treatment	Average number of nematode population per 1 g roots					Reduction of total nemat-Ode (%)
	Larvae	Females	Total	Egg mas.	Galls	
Nematode (N)	0	25.67	25.67	6.00	24.33	–
Mycorrh. (M) + (N)	1.33	3.67	5.00	1.00	4.00	80.52
F. Oxysporum + (N)	1.67	16.00	17.67	2.33	14.33	31.66
M+N+*F. Oxysporum*	1.67	4.67	6.34	1.00	5.00	75.30

L.S.D. at 5% between treatments : 10.63

Table (2) : Fresh and dry weights of 45 old tomato plants as influenced with nematode, mycorrhiza and fusarium wilt pathogen.

Treatment	Average fresh and dry weights (g)		
	Fresh weight	Dry weight	Dry matter %
Nematode (N)	0.86	0.20	23.26
Mycorrhiza (M)	2.88	0.64	22.22
Wilt pathogen (F)	1.46	0.32	21.91
(M) + (N)	2.26	0.40	17.69
(F) + (N)	0.74	0.10	13.52
(M) + (F)	1.58	0.33	20.89
(M) + (N) + (F)	2.00	0.38	19.00
Check	2.12	0.40	18.87
L.S.D. (P : 0.05)	0.45	–	0.08

homlogous to those formed normally by the host plant and which regulate cell division, growth and other physiological processes such as the mobilization and control of nutrient translocation. They further mentioned that secretion of growth regulators by the fungus may be beneficial to the plant. Landexker *et al.* (1982) and salem *et al.* (1984) reported that the mycorrhizal fungi increased the phosphorous content of plant seedlings. This may result in improving tomato plant growth which was recorded also by Gordon *et al.* (1986) and Grandison and Cooper (1986).

Application of mycorrhizal fungus + nematode showed increase in plant fresh weight. While the application of the three organisms; into the soil together; insignificantly decreased the fresh and dry weights. This may come back to the role of mycorrhizal fungus in protecting tomato from nematode and wilt pathogen invasions as reported also by Salem *et al.* (1984), Grandison and Cooper (1986), Gordon *et al.* (1986) and Cooper and Grandison (1986).

4 - Chemical constituents of tomato plants :

Results presented in Table (3) indicate that application of the VAM fungus with or sithout Fusarium wilt pathogen increased reducing sugar than control by 4.01 and 8.12 % respectively. This may be due to the secretion of hydrolytic enzymes by the fungi which hydrolyze large chains of cabohydrate as reported by Dcese and Stahman (1962), Madkour and Aly (1980) and Landexker *et al.* (1982).

Other treatments decreased plant content of reducing sugars with maximum reduction (36.71 %) in nematode + fusarium wilt fungus inoculation. This may be attributed to nematode feeding and /or fusarium pathogen consumption on plant sugars. It could be noticed that nematode often cause great sugar reduction. Soil infestation with *F. oxysporum* alone had negligable effect on reducing sugars content.

Tables (4) : Chemical constituents of one month old tomato plants as affected by nematode, VAM fungus and fusarium wilt pathogen.

Treatments	Reducing sugars mg / 100g	dr.wt.Alt.%	Total protein (%)	Phenolic compounds mg / 100g	dr.wt.Alt.%
Nematode (N)	7.12	- 28.59	2.00	17.95	-- 20.43
Mycorrhiza (M)	10.78	+ 8.12	0.49	61.54	+ 172.78
Wilt pathogen (F)	9.76	- 2.11	1.40	19.17	- 14.94
(M) + (N)	7.94	- 20.36	1.54	66.66	+ 195.48
(F) + (N)	6.31	- 36.71	1.79	33.33	+ 47.74
(M) + (F)	10.37	+ 4.01	1.55	63.07	+ 179.57
(M) + (N) + F)	9.36	- 6.12	2.21	26.66	+ 18.17
Check	9.97	-	1.53	22.56	-

Total protein content of tomato plants was sharply decreased in response to the alone application of the VAM fungus. While the single application of wilt disease pathogen slightly decreased total protein than control. This could be due to nutrition requirements of Fusarium spp. to amino acids as recorded by Samborski *et al.* (1958) and Landecker *et al.* (1982).

Nematode inoculated plants showed higher protein percentages than control. This was also reported by Lewis and McClure (1975). The highest protein percentage (2.21) was observed in tomato plants raised on the inoculated soil with the three agents.

Phenolic compounds were decreased in response to either nematode or wilt fungus inoculations. Great phenol increases could be noticed in the mycorrhizal plants (different treatments). Matta and Dimond (1969), Carter (1978) and Mahmoud

(1985) attributed changes in phenolic compounds to plant susceptiblity and rate of infection. But Landecker *et al.* (1982) mentioned that plants produce a high - molecular- weight phenolic compound in order to inhibit mycorrhizal fungal growth.

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"العلاقة المتداخلة لنيماتودا تعقد الجذور وفطر الذبول الفيوزاريومى والميكرهيزا فى الطماطم"

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" الملخص العربى "

تعتبر نيماتورا تفقد الجذور من الطفيليات الهامه المنتشره فى الاراضى المصريه خاصة حديثة الاستصلاح . وقد أجريت هذه الدراسه بغرض مقاومتها حيويا حيث تسبب مقاومتها كيميائيا خللا ضارا بكائنات التربة النباتية والحيوانية ، ويمكن تلخيص أهم النتائج فيما يلى

١ - فطر الميكروهيز المستخدم (فيوزاريوم اكويستى) غير ممرض لنباتات الطماطم ، بينما تفاوتت عزلات فطر الذبول (فيوزاريوم أوكسيبورم) قدرتها المرضية .

٢ - لوحظ نقص معنوى لأعداد نيماتودا تعقد الجذور (ملويدوجين جافانيكا) وكذلك العقد المتكونه على جذور الطماطم كإستجابة لوجود فطر الميكروهيزا فى التربه سواء فى وجود أو غياب فطر الذبول .

٣ - أدت العدوى الصناعية للتربه بفطر الذبول أو النيماتودا أو كلاهما معا إلى الغض المعنوى فى الوزن الفص والجاف لنباتات الطماطم بينما إزدات أوزان النباتات كاستجابة لوجود فطر الاليكروهيزا بالتربة .

٤ - قلت أعداد اليرقات ، الاناث ، كتل البيض داخل جذور الطماطم كما قلت عدد العقد المتكونة على الجذور لوجود فطر الميكروهيزا بالتربة عند تقديرها أسبوعيا بالمقارنه بغير المعاملة بفطر الميكروهيزا .

٥ - تزايد معدل السكريات المختزله فى النباتات المعامله بالميكروهيزا وتناقص فى