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SUPPRESSION OF FUSARIUM WILT OF TOMATO BY SEED, SEEDLING AND SOIL TREATMENTS WITH CERTAIN FUNGAL AND BACTERIAL BIOCONTROL ORGANISMS.

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ABSTRACT

Three different cultivars of tomato were treated with biocontrol agents. these biological agents were, *Pseudomonas fluorescens, Trichoderma harzianum* , commercial powder (Rhizo-N) containing *Bacillus subtilus* , commercial liquid (Plant-Guard) containing *T. harzianum*, Rizolex T-60 , combinations between Rhizo-N , Plant-Guard and *P. fluorescens, T. harzianum*. The treated seeds were evaluated for seed germination and vigour index. The treated seedling and soil were evaluated for reduction the percentage of Fusarium wilt. It was found that seedling in treatment was more effective in reducing Fusarium wilt than soil treatment. Also, *P. flourescens* was more effective in increasing seed germination, vigour index and reduction of Fusarium wilt, followed by combination between *P. fluorescens*, *T. harzianum*, *T. harzianum*, Rhizo-N , Plant-Guard and the combination between Rhizo-N , Plant-Guard. Rizolex T-60 treatments was the test effective.

Keywords: Tomato cultivars, *Fusarium oxysporum f. sp. Lycopersici*, biological agents, vigour index, seed germination

INTRODUCTION

Fusarium wilt of tomato (Lycopersicon *esculentum* Mill.), caused by *Fusarium oxysporum* Schlechtend.: Fr. f. *sp. Lycopersici* (Sacc.) W. C. Snyder & H. N. Hansen, is one of the most damaging diseases either in the nurseries or in the open fields. The disease infected seedling or mature plants. Seedling appear stunted with periodic wilting the older leaves droop and curve downward, and some might turn yellow. The vascular tissue becomes dark brown, the bases of affected stems enlarge, and the plants frequently wilt and die (Jones *et al.*, 1991). The disease is widely spread particularly through early summer and autumn tomato plantations in upper Egypt Governorates and newly reclaimed sandy regions in Noubareya, Ismailia and El- Salheya (Awad, 1990). *F. oxysporum f. sp. Lycopersici* is a soilborne pathogen, which cansurvive as dormant chlamydospores in soil for extended periods of time (Katan, 1971). The dissemination of *F. oxysporium f. sp. Lycopersici* is via seed, tomato stakes, soil and ingected transplantes or infested soil clinging to transplants (Jones *et al.*, 1991). Fusarium wilt disease controlled in tomato by use resistant cultivars (Awad, 2000), by preplant soil fumigation with methyl bromide (Ristaino and Thomas, 1997), and by biological control. Biological control has potential for the management of Fusarium wilt. A variety of soil microorganisms have demonstrated activity in the control of Fusarium wilt pathogen. Larkin and Fravel,1998 used *Trichoderma spp., Gliocladium virens, Pseudomonas fluorescens* and *Burkhdderia cepacia* in controlling Fusarium wilt of tomato. De Cal *et al.*, 2000 used *Penicillium oxalicum* against tomato Fusarium wilt. Fuchs *et al.*,

(1997) used nonpathogenic *Fusarium oxysporum* to induce resistance to Fusarium wilt in tomato. The use of combinations of multiple antagonist organisms also may provide improved disease control over the use of single organisms. Several researchers have observed improved disease control using various combinations of multiple compatible biocontrol organisms (Duffy *et al.*, 1996 and Pierson and Weller, 1994). The objectives of this research were to evaluate the effect of biocontrol agents, including commercial formulations, with known activity against soilborne fungal pathogens for their efficacy in controlling Fusarium wilt of tomato. In addition, to compare the effective of different methods of applying these biocontrol agents for controling the disease .

MATERIALS AND METHODS

Isolation and identification of the causal organism

The causal pathogen of tomato wilt disease (Fusarium *oxysporum f. sp. Lycopersici*) was isolated from the vascular bundles of tomato plants which were collected from Alexandria and El-Bahira governments and showed typical external wilt symptoms and internal brown xylem discoloration. Purification of the isolated fungus was carried out following single spore technique. The purified fungal culture was identified, according to cultural and morphological diagnostic criteria according to Booth, (1971).

Source and preparation of biocontrol agents

The antagonistic strains of *Pseudomonas fluorescens* and *Trichoderma harzianum* were isolated from the native soil. *P. fluorescens* was inoculated on Kings-B- medium and incubated at 27C°. After 48 hr. of incubation, bacterial suspensions were prepared by flooding the plates with 0.01 M. Mg So_4 and scraping off the bacteria. Bacterial suspensions were adjusted to (10^7 cfu) / ml. As described by Abdalla *et al.*,(1999). *T. harzianum* was grown on potato dextrose agar (PDA) petri plates and incubated at 25C° for 7 days then flooded with sterile distilled water and conidia were gently freed from the culture surface with abrush. The suspension was filtered through cheesecloth and the density of conidia was adjusted to 10^7 conidia / ml with the aid of hemocytometer. Egyptian commercial powder named Rhizo-N produced by El-Nasr company containing *Bacillus subtilis* at the rate of 30×10^6 cell / g. (5 g / liter distilled water were used) . Also, Plant-Guard produced by the company earlier, containing *Trichoderma harzianum* at the rate of 30×10^6 cell / ml (5 ml / liter distilled water were used). Combinations of Rhizo- N, Plant- Guard and *P. fluorescens, T. harzianum* were also applied.

Screening trials

(a) Seed treatments

Tomato seeds of three cultivars, namely Strain-B, Pearson and Roma were treated with the tested antagonist and grown in seedling plug trays (plug size 3.4 by 3.4 by 5 cm , 48 plugs / tray). The treatments were,

(1) seeds coated with *P. fluorescens* cornstarch (autoclaved cornstarch was mixed with *P. flourescens* suspensions 10% w/v), for 30 seconds and dried in sterile air as described by El- Meleigi, (1989), (2) Seeds coated with *T. harzianum* (5 ml of conidial suspension mixed with 1000 seeds and dried), (3) seeds coated with Rhizo-N at the rate of 5g/kg seeds, (4) seeds coated with Plant-Guard at the rate of 5ml/kg seeds, (5) seeds coated with mixed of Rhizo-N and Plant-Guard at the rate of 2.5g, 2.5 ml respectively /kg seeds, (6) seeds coated with mixed of *P.fluorescens* and *T. harzianum* 2.5 ml, 2.5 ml respectively /1000 seeds, (7) seeds coated with Rizolex T-60 at the rate of 3g /kg seeds. Five tomato seeds were planted in each cell of the plug tray and maintained in the greenhouse at 25ºC After 3 weeks vigour index was calculated.

(b) Seedling treatments

The three tomato cultivars were sown in seedbeds which contained autoclaved sandy-loam soil. Tomato seedlings were removed carefully after 21 days, roots were washed gently in running tap water to remove soil particles. Seedling's roots of each of the three tomato tested cultivars were dipped in the suspensions of the biological agent which previously described, for 24 hr, before transplanting in 15 cm diameter pots (5 plants / pot) containing sandy loam soil infested with *F. oxysporum f. sp. Lycopersici* grown on potato dextrose. Culture were grown for 10 days on a rotary shaker at 150 rpm at 25C °, blended and propagule counts determined on a hemacytometer, Inoculum was added to the soil at rate of 10^4 cfu/g soil.

(c) Soil treatments

Each biocontrol agent suspension was mixed with soil, previously infested with *F. oxysporum f. sp. Lycopersici*. The bioagent were prepared and inoculated at the rate of 50 ml / pot (each pot = 15 cm), and the seedlings of the three tomato tested cultivars removed carefully after 21 days were transplanted into these pots (5 plants / pot).

In b and c experiments control treatments were (i) plants inoculated with the wilt pathogen and not treated with biocontrol agents, (ii) plants not inoculated with the wilt pathogen and treated with biological agents, and (iii) plants not inoculated with the wilt pathogen and not treated with bioagents. Five replicates were used in each treatment and after 3 weeks disease was monitored and assayed as the total percentage of seedlings showing symptoms of Fusarium wilt (yellowing, dropping of leaves, vascular discoloration, etc.) Stem sections of wilted seedlings were surface–disinfested in 0.5 % sodium hypochlorite and plated on PDA medium to confirm the presence of the wilt pathogen. The experiments were carried out twice. Analysis of variance was conducted using the general linear model procedures of the SAS program and Duncan's multiple range test was used the (Duncan, 1955).

Germination test

Treated seeds (400) were also placed between paper rolls in four replicates of 100 seed each for germination. The rolls were kept at 25C° in seed germinator. The first count of normal seedlings was taken on the fourth day and the second count on the seventh day.

Vigour index (VI)

Normal seedlings were evaluated for vigour index. The root and shoot lengths of the normal seedlings were measured and vigour index was calculated using the formula of Abdula Baki, and Anderson 1973.

VI = (mean root length + mean shoot length) × percentage of germination.

RESULTS

Fffect of bioagents on seed germination

The increase in seed germination in all cultivars of tomato treated with biocontrol agents and Rizolex- 60 were compared with the values of the untreated seeds and they are presented in (Table 1). Treatment of tomato seeds with *P. fluorescens* increased the germination by 42.8%, whereas *T. harzianum* increased the germination by 23.1 %.

While the combination of *P. fluorescens* and *T. harzianum* increased the germination by 35.2 %. However, the commercial powder (Rhizo- N) Plant-Guard increased the germination by 27.5 % and 27.4 % respectively. While, the combination of Rhizo- N and Plant- Guard increased the germination by 23.7 %. On the other hand, Rizolex T- 60 increased the germination by 13.1 %.

Table 1: Effect of the tested bioagents on seed germination of the tested tomato cultivars.

Treatments	Germination (%) Cultivars			Mean	Increase (%)
	Pearson	Roma	Strain - B		
Control	67.7 e	64.7 e	58.3 g	63.3	0.0
Pseudomonas fluorescens	95.7 a	91.7 a	85.0 a	90.8	42.8
Trichoderma harzianum	81.0 c	82.3 bc	71.7 e	78.3	23.1
Commercial powder(Rhizo – N)	84.0 bc 85.7 b	85.3 b	74.0 d	81.1	27.5
Commercial liquid(Plant – Guard)	85.7 b	79.0 c	78.3 c	81.0	27.4
Combination of (*P. fluorescens and T. harzianum*)	93.0 a	84.3 b	80.7 b	86.0	35.2
Combination of (Rhizo – N and Plant – Guard)	82.7 bc	78.0 c	75.3 d	78.7	23.7
Rizolex T – 60	77.3 d	73.0 d	65.3 f	71.9	13.1

Data are average of 4 replicates

Means followed by the same letter (s), in each column, are not significantly different at p= *0.05*.

Effect of biocontrol agents on seed vigour.

Data Table 2 show that *P. fluorescens* increase the vigour by 107.5 % and the combination of *P. fluorescens* and *T. harzianum* increased the seed vigour by 98.3 %. *T. harzianum* and Plant – Guard increased the vigour

by 70.2 % and 48.4 , respectively. However, Rhizo-N increased the vigour by 59.6 %, while the combination of Rhizo-N and Plant-Guard increased the vigour by 39.6 %. As a result Rizolex T-60 treatment increased the vigour by 27.9 % (Table 2).

Table 2: Effect of different biocontrol agents on vigour index (VI) of tomato tested cultivars.

Treatments	Germination (%) Cultivars			Mean	Increase (%)
	Pearso	Roma	Strain - B		
Control	338.6 f	297.6 f	244.9 e	293.7	0.0
Pseudomonas fluorescens	682.1 a	576.1 a	570.2 a	609.5	107.5
Trichoderma harzianum	518.4 c	508.2 c	473.2 b	499.9	70.2
Commercial powder(Rhizo – N)	487.2 d	511.8 c	407.0 c	468.7	59.6
Commercial liquid (Plant – Guard)	481.5 d	418.7 d	407.2 c	435.8	48.4
Combination of *(P. fluorescens and T. harzianum)*	651.0 b	531.1 b	564.8 a	582.3	98.3
Combination of (Rhizo – N and Plant – Guard)	474.9 d	411.0 d	343.9 d	409.9	39.6
Rizolex T – 60	425.2 e	365.0 e	336.5 d	375.6	27.9

Data are average of 5 replicates
Means followed by the same letter (s), in each column, are not significantly different at p= 0.05.

Effect of biocontrol agents as seedling treatments on tomato wilt.

The percentages of Fusarium wilt in tomato cultivars were calculated after dipping tomato seedling of the tested cultivars in the suspensions of the biological agents and the results were presented in (Table 3). *P. fluorescens* reduced the Fusarium wilt by 74.3 % and the combination of *P. fluorescens* and *T. harzianum* reduced the disease by 71.5 %. *T. harzianum* and Plant-Guard reduced disease by 59.3% and 51.3 %, respectively. However, Rhizo-N reduced wilt by 54.4 %, while combination of Rhizo-N and Plant-Guard reduced the disease by 51.4 %. Rizolex T-60 reduced wilt by 46.7 %.

Table 3: Effect of different biocontrol agents as seedling treatment on tomato wilt of the tested cultivars.

Treatments	Fusarium wilt (%) Cultivars			Mean	Reductio (%)
	Pearson	Roma	Strain -B		
Control (with the pathogen)	84.0 a	80.7 a	87.3 a	84.0	0.0
Pseudomonas fluorescens	20.6 f	17.6 g	26.7 f	21.6	74.3
Trichoderma harzianum	34.1 d	31.6 e	37.0 e	34.2	59.3
Commercial powder (Rhizo – N)	38.3 cd	35.3 d	41.3 d	38.3	54.4
Commercial liquid (Plant – Guard)	40.2 c	38.2 c	44.7 c	41.0	51.2
Combination of *(P.fluorescens and T. harzianum)*	23.7 e	20.7 f	27.3 f	23.9	71.5
Combination of (Rhizo – N and Plant – Guard)	40.4 c	38.0 c	44.1 c	40.8	51.4
Rizolex T – 60	44.7 b	42.7 b	47.0 b	44.8	46.7
Control (without the pathogen)	0.0 g	0.0 f	0.0 g	0.0	100

Data are average of 5 replicates
Means followed by the same letter (s), in each column, are not significantly different at p= 0.05.

Effect of biocontrol agents as soil treatment:

Soil was treated with bioagents and Fusarium wilt of tomato tested cultivars was presented in (Table 4). *P. fluorescens* reduced the Fusarim wilt by 62.5 % and the combination of *P. fluorescens* and *T. harzianum* reduced the disease by 55.8 %. *T. harzianum* and Plant-Guard reduced the wilt by 46.5 % and 27.3, respectively. Rhizo-N reduced the disease by 50.5 %, while the combination of Rhizo-N and Plant-Guard reduced the disease by 35.1 %. (Rizolex T-60) reduced wilt by 42.4 %.

Table 4: Effect of different biocontrol agents as soil treatment on wilt of tomato tested cultivars.

Treatments	Fusarium wilt (%)			Mean	Reduction (%)
	Cultivars				
	Pearson	Roma	Strain -B		
Control (with the pathogen)	84.0 a	80.7 a	87.3 a	84.0	0.0
Pseudomonas fluorescens	31.3 f	28.7 f	34.6 f	31.5	62.5
Trichoderma harzianum	43.7 d	41.3 d	49.7 d	44.9	46.5
Commercial powder (Rhizo – N)	41.7 d	40.7 d	42.3 e	41.6	50.5
Commercial liquid (Plant – Guard)	60.3 b	58.3 b	64.7 b	61.1	27.3
Combination of(*P. fluorescens and T. harzianum*)	36.7 e	34.0 e	40.7 e	37.1	55.8
Combination of (Rhizo – N and Plant – Guard)	54.7 c	50.0 c	58.7 c	54.5	35.1
Rizolex T – 60	45.3 d	48.7 c	51.3 d	48.4	42.4
Control (without the pathogen)	0.0g	0.0 g	0.0 g	0.0	100

Data are average of 5 replicates

Means followed by the same letter (s), in each column, are not significantly different at p= 0.05.

DISCUSSION

Seed germination and seed vigour tests showed that all the antagonists tried significantly increased the seed germination and seed vigour. Rizolex T – 60 treated seeds showed higher seed germination and seed vigour than the untreated ones, *P. fluorescens* was the best biological agent applied to improve tomato seed germination. These results are in agreement with those of Leeman *et al.*, (1995), who reported that *P. fluorescens* increase the germination in treated seeds of radish in commercial greenhouses. Also Voisard *et al.*, (1989) suggested that some *Pseudomonas* have the ability to synthesis hydrogen cyanide which is known to inhibit the expression of pathogenic fungi. However, Dubeikovsky *et al.*, (1993) reported that *P. fluorescens* is known to produce the plant growth regulators like gibberellines, cytokinins and indole acetic acid.The obtained results show that *T. harzianum* has shown increased seed germination and seed vigour. In this respect Gopinath *et al.*, (1992) recorded that *T. harzianum* increased seed germination and seedling vigour of lettuce seeds. Also, Haran *et al.*, (1996) reported that *T. harzianum* is know to produce chemical compounds which is known to inhibit pathogenic fungi such as chytinolytic enzymes, glucanase and proteases.Also, *Bacillus subtilis* had a good inhibitory effect on Fusarium wilt of tomato. Similar results were obtained by other researchers using *B.*

subtilis for inhibiting the growth of various fungi and bacteria (Shekhawat, *et al.*1993 and Korsten *et al.* 1997). On the other hand Loeffler *et al.*, (1986) reported that *B. subtilis* produce different antibiotic as subtilin, bacillin, bacillomycin, subtenolin, mycosubtilin and iturin which inhibit growth of pathogenic fungi and bacteria.

Data show that seedling and soil treatment suppress tomato Fusarium wilt using biocontrol agents and seedling treatment was more effective in this respect. In this study, combination of biocontrol agants including *P. fluorescens* and *T. harzianum*, Rhizo-N and Plant-Guard did not improve the level of wilt incidence compared to the application of *P. fluorescens* alone. Similar results was observed by Larkin and Fravel, (1998), when used non-pathogenic *Fusarium* spp. And *Gliocladium virens* as a biological agent, these combinations reduced wilt of tomato but did not provide significantly better control than the non-pathogenic Fusarium antagonists alone. In recent years much attention has been given to non-chemical systems to protect the plants against seed-borne or soil-borne pathogens. The present study has shown that the applied biological agents such as *P. fluorescens, T. harzianum* and *B. subtilis* can be used as alternative methods to chemical fungicides.

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خفض الإصابة بمرض الذبول الفيوزاريومى في الطماطم بتطبيق معاملة البذور أو البادرات أو التربة بأستخدام بعض الكائنات ذات التأثير الحيوى٠

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جرى أختبار كفاءة بعض الكائنات ذات التأثير الحيوى على ثلاثة أصناف من الطماطم وهي البكتيرة سودوموناس فلوريسينس والفطر تريكوديرما هارزيانيم والمسحوق التجاري رايزو – ان المحتوى على بكتيريا باسيلس سابتلس والسائل التجاري بلانت جارد المحتوى على الفطر تريكوديرما هارزيانيم ومخلوط كل من البكتيرة سودوموناس فلوريسينس والفطر تريكوديرما هارزيانيم وأيضا خلط كل من المسحوق التجاري رايزو – ان والسائل التجاري بلانت جارد والمبيد الفطري ريزولكس ت – ٦٠ وذلك لاختبار فاعليتها في التأثير على نسبة الإنبات وحيوية وقوة البادرات vigour index عند أستخدامها فى معاملة البذور وأيضا لاختبار فاعليتها في خفض نسبة الإصابة بالذبول الفيوزاريومى بطريقتين الأولى هي معاملة بادرات الطماطم بهذه الكائنات ذات التأثير الحيوى والطريقة الثانية هي معاملة التربة .وقد وجد ان الطريقة الأولى كانت أكفأ من الطريقة الثانية في خفض نسبة الإصابة بالذبول الفيوزاريومى كما وجد ان أستخدام البكتيرة سودوموناس فلوريسينس كان اكثر تأثيرا في زيادة نسبة الإنبات لبذور الطماطم وانتاج بادرات قوية وخفض نسبة الإصابة بالذبول الفيوزاريومي يلي ذلك معاملات الخلط بين كل من البكتيرة سودوموناس فلوريسينس والفطر تريكوديرما هارزيانيم يليه الفطر تريكوديرما هارزيانيم ثم المسحوق التجاري رايزو– ان ثم السائل التجاري بلانت جارد ثم الخلط بين كل من رايزو– ان والبلانت جارد ويأتى فى المرتبة الأخيرة المبيد الفطري ريزولكس ت –٦٠ وذلك مقارنة بالكونترول٠