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BIOLOGICAL TOMATO SEED TREATMENT AND APPLICATION OF SOME FERTILIZERS TO CONTROL FUSARIUM ROOT- ROT

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ABSTRACT

Both Trichoderma viride and Bacillus subtilis inhibited the linear growth of Fusarium solani (Mart) Sny. And Hans, the causal agent of tomato root-rot disease. T. viride showed greater inhibitory effect compared with B. subtilis.

Greenhouse and field experiments indicated that seed coating with either T. viride or B. subtilis were effective biocontrol treatments against Fusarium root-rot of tomato. On the other hand, these treatments were found to be responsible for the increasing both fresh and dry weights of tomato shoots and roots. Furthermore, the population density of the isolated bacteria, fungi and Fusarium spp., were lower in the plants developed from treated seeds.

Different fertilizers, i.e. ammonium nitrate, calcium super phosphate, potassium sulphate and the combination among them in various rates in greenhouse experiments, significantly decreased the percentage of post-emergence damping-off caused by F. solani. However, the lowest percentages occurred in $N_0 P_1 K_2$, $N_1 P_1 K_2$ *and* $N_1 P_2 K_1$ *treatments.*

On the other, most fertilizer treatments, significantly increased the number of healthy survival plants and tomato yield in the field. The highest percentages of healthy plants and yield were recorded with the application of $N_2 P_2 K_2$ *(100 Kg ammonium nitrate + 200 Kg calcium super phosphate + 100 Kg potassium sulphate).*

Key words: Tomato *(Lycopersicon esculentum, Mill.)*, biological, fertilizer amounts, Fusarium root-rot, disease severity index (*DSI).*

INTRODUCTION

Tomato (*Lycopersicon esculentum, Mill).* Is considered the main glasshouse crop in most European countries. While, in Egypt, it is one of the most important vegetables crops. Tomato fruits have a high nourishing value, as it contains a considerable amounts of nutrients, vitamins and mineral salts, (Moursy and El-Morabaa, 1960). The estimated total production produced from commercial tomatoes grown in approximately 599615 feddans was 10.278.539 tons in 2009. The Egyptian government is pressing hard to increase tomato production to face the increase our exportation.

Several diseases attack tomato plants causing serious losses in either nurseries or field conditions. For instance, *Fusarium spp., Rhizoctonia sp., Pythium spp., Phytophthora spp.* And *Selerotium rolfsii,* attack the root and stem base of tomato plants, causing root and stem rots (Moursy and El-Morabaa, 1960; Grinstein *et al.*, 1979; El-Mougy 1995;Ghanem, 1998 and Kokalis-Burelle and Gransnamanickam, 2002). Fusarium root-rot has increased considerably in Egypt. It causes high damage in tomato cultivations especially in old lands of Egypt (El-Fahham,1993).

Biological control has recently become one of the most important methods for controlling many fungal diseases particularly tomato root-rot (El-Eraky *et al., 1993;* El-Fahham, 1993; Roberti *et al.;* 1993; Hamed, 1996 and Montealegre *et al.,* 2002*).*

Many investigators strongly have reported the importance of using macro elements fertilizers in reducing tomato root-rot disease (Dubey, 1958; Moursy and El-Morabaa, 1960; Duffy and Defago, 1999 and Cheuk *et al.*, 2003). Using fertilizer applications; Kokalis-Burelle and Gransnamanickam (2002) found that supplementing soil nitrogen increased root-rot disease on tomato. In addition, Garrett (1941) recommended that a generous supply of plant nutrition probably may increase plant resistance chiefly by promoting more rapid production of new roots to replace those destroyed by the disease. Also, Blair (1943) noticed that the addition of nitrogen to the soil had not increased the growth of *R. solani.* Dubey (1958),Papavizas and Davey (1962) found that the high nitrogen application decreased the disease incidence. On the other hand, Moursy and El-Morabaa (1960) showed that potassium and phosphorus fertilizers increased the root growth of plants. This research aimed to study the effect of biological tomato seed treatment and application of some fertilizers for arriving to the best methods to control tomato root-rot disease for safe and pollution free tomato production.

MATERIALS AND METHODS

Certain antagonists, i.e. *Trichoderma viride,* and *Bacillus subtilis,* were used in laboratory, greenhouse and field experiments to evaluate their effectiveness against Fusarium root-rot of tomato (cv. Castle Rock). Antagonistic microorganisms used in this study were isolated from rhizosphere region of tomato plants. The isolates of *Fusarium solani* were obtained from tomato plants cultivated in Fayoum governorate. On the other hand, Vitavax T (Vitavax 37.5% +37.5% Thiram) was used to compare its efficiency with the tested microorganisms.

In vitro, studies were carried out to select the most effective antagonistic microorganisms that could be applied in both greenhouse and open field. The antagonistic organisms were separately tested against *Fusarium solani* on potato dextrose agar medium (PDA) using the inhibition zone technique. Cultures were examined after one week of incubation. Experiments were carried out in four replicates. The antagonistic efficiency criterion was estimated by the presence or absence of an inhibition zone, as well as its length.

In the greenhouse trials, seeds were soaked in the spore suspension of *Trichoderma viride* or bacterial suspension of *Bacillus subtilis,* then sown in soil artificially infested with *Fusarium solani* (loamy clay soil pH 7.6 and moisture holding capacity 35%) using 25 cm diameter pots, kept 20± 4^0 C, all treatments were carried out in completely randomized design with four replicates.

Inoculum applied to seeds was prepared by growing *T. viride on* PDA *for 2 weeks at* 25^0 *C and B. subtilis* on PDA + peptone 5% for I week at 25^0 C. The antagonistic microorganisms were apllied to seed at the rate of 2 Petri plates (9 cm in diameter)/10 g seeds. Vitavax T was used at the rate of 2 g/ kg of seeds. Seeds screened for the control treatments were similarly treated, but without coating with the fungicides. In field trials, seeds were soaked with the tested microorganisms as mentioned before and planted in the field (loamy clay soil, pH 7.6 and moisture holding capacity 35%) in which the pathogen of tomato root-rot was naturally established.

Field experiments were designed in a complete randomized block design in the farm of Fayoum Research Station in two successive seasons; 2008 and 2009.

Disease severity index (DSI) of root-rot was recorded based on a scale from 0 (non visible damage) to 4 (complete destruction), 12 weeks after planting according to Lewis and Papavizas (1977). Also, fresh and dry weights of both tomato shoots and roots were estimated at the same time. Analysis of variance was performed and means were compared by using the least significant differences at 5% level.

The population density of bacteria, *Fusarium spp.* And other fungi was estimated per gram of soil. These estimations were carried out on 3 replicates at the three stages of plant growth, I.e. seedling, vegetative and flowering stages (30, 60 and 90 days after planting, respectively) in the rhizosphere of tomato plants.

The fertilizer experiments included 12 treatments (Table 1), each treatment was replicated four times and occupied $^{1}/_{50}$ feddan (each replicate contained three rows with 25 hills). Four seeds of tomato plants (Castle Rock cv.) were planted in each hill on the second half of October of each year. Half of the fertilizer amounts of each treatment was added before the first irrigation, and the other half was added at the time of flowering.

Table 1. Amounts of fertilizers added in each pot (in gm) and in the open field (in kg/feddan).

Fertilizer amounts	Fertilizers					
	Ammonium nitrate (N)		Calcium superphosphate (P)		Potassium sulphate (K)	
	Kg/feddan	g/pot	Kg/feddan	g/pot	Kg/feddan	g/pot
$N_1 P_1 K_1$	50	0.42	100	0.32	50	0.42
$N_1 P_2 K_1$	50	0.42	200	0.66	50	0.42
$N_1 P_1 K_2$	50	0.42	100	0.32	100	0.84
$N_1 P_2 K_1$	50	0.42	200	0.66	50	0.42
$N_2 P_1 K_1$	100	0.84	100	0.32	50	0.42
$N_2 P_2 K_1$	100	0.84	200	0.66	50	0.42
$N_2 P_2 K_2$	100	0.84	200	0.66	100	0.84
$N_0 P_1 K_2$	0	0	100	0.32	100	0.84
$N_0 P_2 K_1$	0	0	200	0.66	50	0.42
$N_0 P_1 K_1$	0	0	100	0.32	50	0.42
$N_0 P_2 K_2$	0	0	200	0.66	100	0.84
Control ($N_0 P_0 K_0$)	**0**	**0**	**0**	**0**	**0**	**0**

Plants were counted twice, *i.e.* after complete emergence, and before harvest to calculate the percentage of emergence and healthy survival plants. Fruit yield of tomato plants was also determined.

RESULTS AND DISCUSSION

1. In vitro studies:

Data shown in Table (2) indicated that, there was antibiotic effect among the tested microorganisms and the causal pathogen. The greatest inhibitory effect was observed in the case of *T. viride* (5.2 cm inhibition zone) comparing with *B. subtilis* (3.1 cm inhibition zone). *Trichoderma viride* inhibited and overgrew the

Table 2. **Mean inhibition zones among the two antagonists *Trichoderma viride* and *Bacillus subtilis,* and the *Fusarium solani.***

Treatments	Mean inhibition zones (cm)
1. *T. viride*	5.2
2. *B. subtilis*	3.1
3. Control (*F. solani* alone)	0.0
L.S.D. at 5%	1.4

growth of *F. solani*, whereas *B. subtilis* only inhibited the growth of the tested pathogens.

The obtained results may have an important approach, respect to the usage of antagonistic microorganisms and their effectiveness against *F. solani*, to control a root-rot disease of tomato. Similar results were reported by Elad *et al*. (1982), Filippi *et al*. (1987), El-Fahham, (1993) and Roberti *et al*.(1993).

The above authors suggested that the biological behaviour may be due to the three mechanisms; secretion of antibiotics by the antagonist, mycoparasitism and lyses of mycelium of the parasite. They also emphasized that these mechanisms were probably the most important ones for controlling the causal pathogens. Also, there was an *in vitro* evidence of a positive relationship between the decrease in the severity of the disease and the magnitude of the inhibition zone between the antagonist and the causal fungus finally controlling this pathogen.

2. Greenhouse studies:

The two antagonistic microorganisms; *T. viride* and *B. subtilis* were active in the rhizosphere of tomato plants and inhibited Fusarium root-rot of tomato (Table 3). Application of the two antagonistic microorganisms, as well as the use of Vitavax T. fungicide; resulted in a sharp decrease in root-rot decrease severity, expressed as the lower disease severity index (DSI), compared with the control. Since the disease severity index were; 1.7, 1.2 and 1.2 in the infested soil, when the tomato seeds were previously treated with the two antagonistic microorganisms; *T. viride* and *B. subtilis* and the fungicide; Vitavax T, respectively. While, this disease index was found to be significantly higher (3.7) in the tomato plants resulted from the control untreated seeds.

Data in Table (3) were confirmed by those of El-Eraky et al. (1993) and Abd El-Moiety and Abo-Zeid (1988). They reported that Vitavax, Benlate and Brassicol completely inhibited the growth of *R*. solani. The present results are in parallel with the findings of the mentioned investigators, since they reached to the conclusion that using of *T. harzianum* and *Bacillus subtilis* as seed and/or

Table 3. Effect of certain antagonistic microorganisms compared with Vitavax T on disease severity index (DSI) of Fusarium root-rot of tomato, under greenhouse conditions.

Treatments	Disease severity index (DSI)	
	Non-infested soil	Infested soil
T. viride	0.2	1.7
B. subtilis	0.0	1.2
Vitavax/Thiram	0.0	1.2
Control	0.5	3.7
L.S.D.5%	**0.05**	**0.9**

soil treatments for the control of root-rot caused by *Fusarium sp.*, under field conditions performed the best results. Similar results were also obtained by Hamed (1996) and Montealegre *et al*. (2002) in their previous studies.

3. Field studies:

The effect of the two antagonistic microorganisms; *T. viride* and *B. subtilis,* compared with Vitavax T, on Fusarium root-rot disease severity, was studied under field conditions in the two seasons; 2008 and 2009. The obtained results showed that, the disease severity index (DSI) decreased significantly in seeds treated with the tested microorganisms; *T. viride* and *B. subtilis.* It was 1.0 and 1.7 in 2008 and 1.2 and 1.0 in 2009, for the two antagonistic microorganisms, respectively. Vitavax T was also effective in reducing the disease severity of Fusarium root-rot to a level of o.7 in the two seasons of the study (Table 4).

Plant growth, as indicated by the average of fresh and dry weights of shoots and roots per plant, increased significantly by coating seeds with the two tested antagonistic microorganisms. Data in Table (4) suggested that some microorganism products stimulated plant growth. In the same time, the two antagonistic microorganisms reduced population density of plant pathogens. Therefore, the reduction in disease severity may be due to the low level of Fusarium population in the rhizosphere and the surrounding soil of tomato roots (Table 5).

On the other hand, the population density of rhizospheric micro flora was low at the first growth stages of plants developed from treated seeds, but they increased progressively with plant growth. (Table 5). Papavizas *et al.* (1968) stated that, from the voluminous accumulated rhizospheric data which support the presence of antagonism in soil, it is reasonable to believe that antagonism is an important factor affecting the population density and survival of the pathogens in the rhizosphere.

Table 4. Effect of certain antagonistic microorganisms compared with Vitavax T, on Fusarium root-rot disease severity index (DSI) and on average fresh and dry weights of both shoots and roots of tomato plants under field conditions in 2008 and 2009.

Treatments	Season 2008					Season 2009				
	DSI	Shoots		Roots		DSI	Shoots		Roots	
		F. W.*	D.W**	F. W*	D.W**		F. W*	D.W**	F. W*	D.W**
T. viride	1.0	183.7	17.5	22.2	3.7	1.2	264.2	30.7	30.2	7.2
B. subtilis	1.7	158.5	15.5	18.0	2.0	1.0	34.0	51.7	39.2	8.0
Vitavax T	0.7	229.7	22.2	27.0	5.0	0.7	377.2	66.5	43.7	10.7
Control	2.7	78.5	11.2	16.7	1.8	3.2	213.5	29.2	25.2	5.2
L,S.D at 5%	1.0	5.1	5.8	6.9	1.2	0.8	6.8	6.9	10.7	2.5

F.W. = Fresh weight / g., D.W. = Dry weight g.
DSI = Disease severity index.

Some bacteria increased plant growth, by the production of gibberellin or auxin of soluble phosphate, while others have been shown to alter the rhizosphere microbial flora (Filippi *et al*., 1987).

Therefore, the prime purpose of biological control of tomato root-rot disease was not only to raise the plant growth and yield, but also to reduce density of soil-borne pathogens, so taking all factors into consideration and the difficulties involved.

Effect of different levels of fertilizers on diseases incidence:

Different levels of fertilizers were used as shown in Table (1). The effect of different levels of nitrogen, phosphorus and potassium fertilizers on disease incidence, was studied under the greenhouse and field conditions.

a. Greenhouse experiment:

All fertilization treatments significantly decreased the percentage of post emergence damping-off caused by *F. solani*. However, the lowest percentage of post-emergence damping off was obtained by N_0 P_1 K_2 followed by $N_1P_1K_2$ and N_1 P_2 K_1 treatments (Table 6). It was; 18.5%, 18.9% and 20.7% for the three treatments, respectively.

Table 6. Effect of different levels of fertilizers on tomato root-rot disease incidence caused by *F. solani*, under greenhouse conditions.

Treatment	Disease incidence percentages of	
	Post-emergence damping-off	Survival plants
N_1 P_1 K_1	32.5	24.0
N_1 P_2 K_1	20.7	28.0
N_1 P_1 K_2	18.9	30.5
N_1 P_2 K_2	63.7	21.5
N_2 P_1 K_1	42.5	24.5
N_2 P_2 K_1	31.4	30.2
N_2 P_2 K_2	38.0	20.5
N_2 P_1 K_2	29.6	26.5
N_0 P_1 K_1	28.3	27.5
N_0 P_1 K_2	18.5	28.5
N_0 P_2 K_2	27.0	24.5
Control (N_0 P_0 K_0)	70.0	18.0
L.S.D at 5%	**23.0**	**12.4**

b. Field experiments:

Results of the two field experiments are presented in Table (7), it is clear that all fertilizer treatments significantly increased the healthy survival plants as well as the yield, over the control, except treatments of N_2 P_1 K_2 in 2008 with regard to the percentage of surviving plants and N_0 P_1 K_2 regarding the yield only.

The highest percentage of healthy survival plants and yield were obtained by application of N_2 P_2 K_2. Similar results were also gained during 2009.

These results are in harmony with those obtained by many investigators such as Garrett (1941) who recommended that a generous supply of plant nutrition probably increased plant resistance chiefly by promoting more rapid production of new roots to replace those destroyed by the disease Blair (1943) noticed that the addition of nitrogen to the soil did not increase the growth of *Rhizoctonia solani.* While, Moursy and El-Morabaa (1960) showed that potassium and phosphorus fertilizers increased the root growth of plants. Also, Dubey (1958) and Papavizas and Davey (1962) observed that high nitrogen application decreased the root-rot disease incidence. In conclusion, the obtained results demonstrated the importance of fertilizers in reducing root-rot disease of tomato.

Table 7. Effect of some fertilizer treatments on the root-rot disease incidence and yield of tomato, under field conditions during 2008 and 2009 seasons.

Treatments	2008		2009	
	% Survival Plants(%)	Dry yield (Kg/pot)	Survival plants (%)	Dry yield (Kg/pot)
N_1 P_1 K_1	37.6	2.200	76.1	2.162
N_1 P_2 K_1	38.1	2.362	78.6	2.312
N_1 P_1 K_2	38.7	2.400	81.4	2.437
N_1 P_2 K_2	38.6	2.525	85.5	2.437
N_2 P_1 K_1	32.2	1.812	72.2	2.072
N_2 P_2 K_1	37.4	2.312	82.6	2.425
N_2 P_1 K_2	30.3	2.050	77.4	2.150
N_2 P_2 K_2	42.0	3.042	87.6	3.925
N_0 P_1 K_2	32.7	0.875	83.5	2.062
N_0 P_1 K_1	37.0	2.025	84.9	2.425
N_0 P_2 K_1	37.3	2.025	85.9	2.462
Control N P K	31.5	1.475	70.8	2.062
LSD at 5%	**2.2**	**0.475**	**10.0**	**0.462**

Also, these results are in harmony with the works of Duffy and Defago (1999) who concluded that disease severity of tomato root-rot was reduced by ammonium nitrate. On the other side, Cheuk et al. (2003) found significant reductions in tomato root-rot disease and improved crop yield with the addition of compost.

Conclusively, from these results recommended that tomato is one of the most important vegetable plants in Egypt and in order to reduce the harmful influence of organisms that cause root-rot of tomatoes must use anti-vital transactions and also use a combination of fertilizer in line with the rapid growth of the roots of tomato in order to give the highest productivity in the vegetative growth and fruit yield.

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معاملة بذور الطماطم بيولوجيا لمكافحة عفن الجذور الفيوزارمي وتأثير بعض معاملات التسميد على ظهور المرض

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نباتات الطماطم أحد أهم نباتات الخضر في مصر سواء من حيث الاستهلاك المحلي أو التصدير وأثبتت التجارب المعملية التي أجريت بهذا البحث قدرة الكائنات الحية الدقيقة المستخدمة (فطر ترايكوديرما فيردى وبكتريا باسيليس سانلس) على تثبيط النمو الميسيليومي للفطر فيوزاريوم سولاني أحد مسببات أمراض أعفان الجذور في الطماطم ،هذا وقد تفوق فطر التضاد ترايكوديرما فيردي على البكتيريا باسيليس سانلس في ذلك المجال.

وقد أوضحت التجارب التي أجريت بالصوبة والحقل أن تغطية بذور نباتات الطماطم بالكائنات الحية الدقيقة المستخدمة في البحث كان لها تأثير حيوي مضاد للفطر المسبب للمرض مما يقلل الضرر الناشئ عنه. كما أثبتت الدراسة أيضا أن هذه المعاملات الحيوية سببت زيادة في الوزن الطازج والوزن الجاف للجذور والمجموع الخضري للنباتات على حد سواء .

كما أوضحت النتائج أيضا انخفاض تعداد الكائنات الممرضة الأخرى من فطر وبكتريا وأنواع أخرى من فطر الفيوزاريوم في منطقة المحيط الجذري لنباتات الطماطم المعاملة بالكائنين المضادين المستخدمين في البحث (الفطر ترايكوديرما فيردي وبكتيريا باسيليس سانلس).

وعلى الجانب الآخر فقد أوضحت النتائج أن التسميد بنترات الأمونيوم وسوبر فوسفات الكالسيوم وكبريتات البوتاسيوم بمستويات مختلفة قد قلل بوضوح نسب موت بادرات الطماطم بعد الإنبات المتسبب عن الفطر فيوزاريوم سولاني ، وكانت أفضل المستويات السمادية في خفض نسب الإصابة هي $N_1 P_2 K_1$ and $N_1 P_1 K_2$ ، $N_0 P_1 K_2$.

وفي التجارب التي أجريت بالحقل ، أدت معظم المستويات السمادية إلى زيادة ملحوظة في النسب المئوية لعدد النباتات الحية وأيضا زيادة كمية المحصول المنتج وكانت أفضل معاملة هي المعاملة $N_2 P_2 K_2$ (١٠٠ كجم نترات أمونيوم و ٢٠٠ كجم سوبر فوسفات و ١٠٠ كجم كبريتات بوتاسيوم للفدان).

<u>التوصية</u> :نباتات الطماطم أحد أهم نباتات الخضر في مصر و لكي نقلل من التأثير الضار للكائنات المسببة لعفن جذور الطماطم يجب استخدام معاملات حيوية مضادة وأيضا استخدام تركيبة سمادية تتماشى مع سرعة نمو جذور الطماطم لكي تعطي أعلى إنتاجية في النمو الخضري والثمري.