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FIELD APPLICATION OF CERTAIN BIOLOGICAL AND CHEMICAL APPROACHES- ON CONTROLLING TOMATO WILT DISEASE.

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ABSTRACT

Different isolates of *Fusarium oxysporum* f.sp. *lycopersici* were isolated from tomato plants infected with wilt collected from tomato fields located at different locations of Ismaillia, Giza and Minia. These isolates differed significantly in their virulence to induce wilt disease of tomato. The present virulent isolates were found to cause wilt disease only to tomato. These isolates were then considered specialized which named *F. oxysporum* f.sp. *lycopersici*. In laboratory testes, the growth of the mentioned pathogen was highly inhibited by 75.5% - 70.3% as a result of the antagonistic effect of *Streptomyces spp.* and *pseudomonas fluorescens*. The growth of different isolates of the tested fungus were completely inhibited at concentrations 100 and 400 ppm of Topsin M and Tecto / Mancozeb, respectively. Application of biological and fungicidal treatments reduced wilt incidence of tomato grown under field conditions. Combination between Topsin M and soil drench with *Streptomyces spp.* or *Pseudomonas fluorescens*. Induced more significant reduction in disease incidence and yield increase than seed coating with bioagents and / or each treatment alone. Data of this study indicated that the combined biological and chemical treatments could be considered as promising technique for application to control certain soil borne fungi causing wilt disease. Perspective application of integration technique may held to decrease the usage of fungicides and reduce environmental pollution.

Keyword : Biological control, *Fusarium oxysporum* f.sp. *lycopersici*, *Streptomyces spp.*, chemical control, Topsin M, Tecto / Mancozeb, *Pseudomonas fluorescens* and tomato wilt.

INTRODUCTION

Tomato (*Lycoperscon esculentum* Mill) is one of the most important vegetable crops in Egypt. Tomato fruits are used for fresh meals and food industries. Tomato attacked by certain soilborne fungi causing rots and wilt disease. Wilt disease appears during the growing season at different stages of plant growth from seedling to mature stages and may happen earlier to cause pre – emergence wilt. *Fusarium oxysporum* f. *sp. lycopersici* is reported to be the main pathogen causing wilt disease of tomato. (Fassihiani, (1985) , Awad, (1990) and Zaki et al(1998).

Tomato fusarium wilt considered important in view of its wide prevalence in Egypt especially in sandy soil. The application of biological control using antagonistic microorganisms proved to be successful for controlling the mentioned disease. It is still not easy and costly in application, however it can serve as one of the good control measure under greenhouse conditions.

The inhibitory effect of bacteria *pseudomonas fluorescens* and *streptomyces sp.* on different soilborne plant pathogens are reported by

Dufour (1994) Larkin and and Fravel (1998). The inhibitory effect on the growth of F. oxysporum f. sp. *lycopersici* isolates by using Tecto / Mancozeb and Topsin – M was studied by Abdel – Kader, 1983 Ziedan (1993) and Wahid *et al*, (1995). Sivan (1987) mentioned that the application of biological control using antagonistic microorganisms proved to be successful for controlling various plant diseases in many countries. Cipriano *et al*, 1989 indicated that some pseudomonas strains inhibited mycelial growth of the 55 isolates examined 19 inhibited F. *oxysporum* F. sp. *lycopersici* growth in vitro. El – Abyad *et al* (1993) recorded that three *Streptomyces spp.* were used to evaluate the potential of microbrial antagonism for the control of some tomato diseases including fusarium wilt. In vitro studies showed that an 80 % conc. of culture filtrate of *strepotmyces* significantly inhibited spore germination, mycelial growth and sporulation of *F. oxysporum* f. sp. *lycopersici*. In vivo studies the seed coating treatment was the most effective in controlling the pathogen at 42 and 63 days after sowing. The results also revealed that seed-coating with antagonistic *Streptomyces spp.* significantly improved tomato growth. Dufour (1994) tested isolates of *Acremoniella atra, Cylindrocarpon sp., Gliocladium spp., streptomyces sp.* and *Trichoderma spp.* for their antagonism towards *F. oxysporum* f. sp. *lycopersici* in vitro studies, pot tests and field experiments although evidence of antagonism was observed, results of the 3 types of test did not agree, and individual isolates were inconsistent in their activity. Tu and Zheng (1994) found that four well characterized biological control agents, *Gliocladium roseum, G. Virens, Bacillus subtilis* and *pseudomonas fluorescens*, were found to control *F.oxysporum* f. sp. *lycopersici* in greenhouse tomatoes. Chin (2001) recorded that the phenazine – 1- carboxamide producing bacterium *pseudomonas chlororaphis* pcl 139, controls tomato foot and root rot caused by *F. oxysporum* f. *sp. lycopersici*, and Wahid *et al* (1995) reported that *F. oxysporum* growth was completely inhibited within a range of 50 – 100 ppm of Topsin – M and 100 – 800 of tecto mancozeb.

Larkin and Fravel (1998) tested numerous fungi and bacteria, for their efficacy in controlling wilt of tomato caused by *F. oxysporum* . They found that isolates of *Gliocladium virens*, *T. Hamatum*, *P. fluorescens* and *Burkholderia Cepacia* significantly reduced Fusarium wilt compared with disease controls (30 to 65 % reduction), but were not as consistently effective as the non pathogenic fusarium isolates.

MATERIALS AND METHODS

Samples of tomato (*Lycopersicom esculentum* mill) showing wilt symptoms were collected from different tomato fileds located at Ismailia,Giza and Minia were subjected to isolation trials for the causal organisms. The purified fungi isolated were identified according to cultural and microscopically characters described by Nelson and Marasan (1983). Pathogenicty ability of these isolates to induce wilt of tomato plants was tested under greenhouse conditions.

Tomato seeds (CV. Super Marmande) were sown in pots (25 cm diameter) containing steamed loamy soil artificially infested individually at the rate of 5 % W : W with the inoculum of each isolate tested which was previously grown for two weeks on sand barley medium (1 : 1, W : W and 40 % water).

Four pots each containing five seeds were used as replicate for each isolate tested as well as control. Disease symptoms were noticed and recorded after 30 and 45 days from sowing date as percentage of pre – and post emergence wilt incidence. Isolation from infected tomato seeds at pre – emergence stage as well as infected tomato plants at post – emergence stage was carried out. The fungi obtained were compared with that used in soil infestation. The inhibitory effect of bioagents and fungicides on growth of F. *oxysporum* f. sp. *lycopersici* isolates was evaluated in vitro.

Two bacterial isolates i.e. *Streptomyces spp.* And *Pseudomonas fluorescens* obtained from Bacterial Disease dept. Agriculture Research center were used in this study.

These bacterial isolates were isolated from Egyptian soil and proved to be highly antagonistic against wide spectrum of soil borne fungi. Dual culture technique (Ferreira *et al*, 1991) was followed for determination the antagonistic effect of the above mentioned bacteria against the present wilt pathogens. The fungicidal effect on fungal growth was tested as the following description. Five concentrations of each Topsin M and Tecto / Mancozeb i.e. 25, 50, 100, 200 and 400 ppm based on the active ingredient were prepared in PDA medium paused in Petri – dishes. Control treatment was fungicide – free medium.

Petri – dishes were inoculated at the center with 5 mm disc of 10 days old fungal culture. Four replicates were used for each treatment as well as control. All plate were incubated at 25 ^{0}C for 7 days then examined. Percentage of growth reduction was calculated in relative to that grown in control treatment as shown in the following formula.

$$\text{Growth reduction \%} = \frac{\text{growth in control} - \text{growth in treatment}}{\text{growth in control}} \times 100$$

Biological and chemical approaches for controlling tomato wilt disease were applied under field conditions, naturally heavily infested with wilt pathogen at El. Badrashen, Giza, Governorate. Application of *Streptomyces spp.* and *Pseudomonas fluorescens* as seed coating or soil drenching : either individually or in combination with Topsin M were evaluated in this experiment (table 1)

The bacterial inoculums were prepared according to sallam *et al* (1978). A fields experiment, consisted of plots (3.5 × 6 m) each comprised of 6 rows and 12 holes / row the bacterial inoculums at the rate of 500 ml / row (3×10^{6} c f u) were incorporated with the top 20 cm of the soil surface at planting row saites three days before sowing date, while tomato transplants were immersed for one hour in the same previous bacterial inocula at planting time. Topsin M was applied as foliar spray at the recommended dose

(2g / 1) after 14 days of sowing data with revelant to the specific treatment. In order to drench the plant roots region with fungicide, Topsin M was applied following the method proposed by Abdel – Kader (1999).

Table (1): Evaluated control treatments of tomato wilt disease under field condition at El – Badrasheen, Giza Governorate , 2002

Serial No.	Biological treatment		Chemical treatment
	Seed coating	Soil drench	Soil drench
1	*Streptomyces spp.*	--	--
2	*P. fluorescens*	--	--
3	*Streptomyces spp.*	--	Topsin M
4	*P. fluorescens*	--	Topsin M
5	--	--	--
6	--	*Streptomyces spp.*	--
7	--	*P. fluorescens*	--
8	--	*Streptomyces spp.*	Topsin M
9	--	*P. fluorescens*	Topsin M
10	--	--	Topsin M
	--	--	--
			--

Shoot system of tomato plants and the soil surface around them were sprayed with the prepared suspension of the fungicide tested. Four replicates for each treatment as well as control were conducted in complete randomized block design. Tomato transplants cv. Super Marmande were used in all treatments at the rate of one transplant/ holt.

Average percent of pre – and post. emergence with incidence as well as yield obtained were calculated. All date were statistically analyzed according to Steel and Torrie (1980).

RESULTS

Isolation from the collected tomato samples showing wilt symptoms results in four isolates of *Fusarium oxysporum* f. sp. *lycopersici*. pathogenicity test for these isolates revealed that they are able to induce wilt disease on tomato plants (table 2). Data also show that *F. oxysporum* No.2 (Ismailialsolate) was the most aggressive one to induce wilt infection on tomato plants in which its pathogenicity differed significantly in comparison with the other isolates tested. Fusarium isolate No.3 (Giza isolate) was the weakest in this concern.

Table (2): Pathogencity test of different *F. oxysporum f. sp. lycopersici* isolates to induce wilt disease of tomato plants in greenhouse.

F. oxysporum f. sp. *lycopersici* isolates		Wilt disease (%)	
Location	Serial No.	Pre-emergence	Post-emergence
El- Menia	1	12.7	37.8
Ismailia	2	25.9	53.6
Giza	3	10.7	24.3
L.S.D. at 5 %		5.4	7.5

The fungi isolated from both infected germinated tomato seeds and plants were found to be identical with the isolates of *F. Oxysporum* which were used in soil infestation.

The effect of the antagonists *Streptomyces* spp. and *pseudomonas fluorescens* as well as the fungicides Topsin M and Tecto / Mancozeb on the growth of *F. oxysporum* f. sp. *Lycopersici* isolates are presented in table (3).

Table (3): Growth reduction (%) of *F. oxysporum f. sp. lycopersici* isolates affected by bioagents and fungicids.

Isolate of *F. oxysporum f. sp. Lycopersici*	Antagonistic tester		Fungicidal Concentrations ppm									
	Strepomy-ces spp.	*P.fluores-cens*	Tecto / Mancozel					Topsin M				
			25	50	100	200	400	25	50	100	200	400
No 1	75.5	70.3	12.1	25.2	63.1	85.2	100	67.1	85.2	100	100	100
No 2	76.5	70.3	14.2	41.5	61.3	72.1	100	69.1	86.7	100	100	100
No.3	75.5	70.3	16.1	51.3	70.1	88.1	100	70.7	92.2	100	100	100
L.S D. at 5 % Antagontists (A) : N.S			Concentration (C) : 8.2									
Fungal isolates (F.) : N.S			Fungal isolates (I) : 4.2									
Between (A) × (F) : N.S			Funficides (F) : 12.5									
			Between (C) × (I) × (F) : 17.7									

The growth diameter of fusarial tested in control treatment was 90 mm each.

Data obtained showed that both bacterial isolate have high inhibitory effect on the wilt pathogen growth causing almost the reduction, without significant differences, in growth of all isolates tested which recorded as 75.5 % and 70.3 % for *Streptomyces spp.* and *P. fluorescens* respectively. On the other hand, the inhibitory effect on the growth of *F. oxysporum* f. sp. *lycopersici* isolates was increased by increasing both of Tecto / Mancozeb or Topsin M Concentrations in media. The growth of fungal isolates was complately inhibited by Topsin M at concentration of 100 ppm, while they tolerated the same concentration of tecto / Maconzeb and their growth was inhibited by increasing Tecto / Mancozeb up to 400 PPM.

Data in table (4) indicated that the yield of tomato fruits, in all treatments was significantly higher than in control. The highly effective treatments which induce the best records of tomato yield were Topsin M combined with either *Streptomyces spp.* or *P. fluorescens* applied as soil drench followed by soil application with bioagentes only. Moderate increase in yield records was observed when the bioagents were applied as seed coating combined with *spraying* of Topsin M followed by seed coating with bioagents only.

Spraying with Topsin M only at the recommended dose (2 g / 1) gave the lowest yield record.

Table (4): percentage of tomato wilt incidence as affected by bioagents and or fungicidal treatments under field conditions.

Treatment	Wilt incidence %		Healthy survival plants %	Yield (fruits)	
	Pre-emergence	Post emergence		Kg/ plot ($21m^2$)	Increase %
Seed coating					
1- *Strepotmyces spp.*	14.7	23.8	61.5	14 5	38.5
2- *P. fluorescens*	15.8	24 7	59.5	13 1	25 4
3- *Streptomyces* + Topsin	13.9	21 5	64.6	16 5	54 3
4- *P. fluorescens* + Topsin	16 3	22.3	61.4	15 3	47 2
Soil drench					
5- *Strepotmyces spp.*	8 9	20.7	70 4	18 5	75 3
6- *P. fluorescens*	11.7	23.5	64.8	17.3	64 3
7- *Streptomyces* + Topsin	9.1	18.5	72 4	20 1	90 3
8- *P. fluorescens* + Topsin	12.1	19.3	68 6	19 3	85 7
9- Topsin M	19.5	29.2	51.3	13 2	13 2
10- Control	19.3	38.3	42.4	10.6	-
L.S.D. at 5 %	2.3	3.2	4.6	-	-

*** Topsin M used as foliar application.**

DISSCUTION

Data in table (2) revealed that the isolate of *F. Oxysporum* No.2 (Ismailia isolate) was the most aggressive one to induce wilt infection on tomato plants. The results obtained are in a harmony with those reported by Salgado *et al* (1995) and Buruchara *et al* (1999).

Data in table (3) show that both bacterial isolate have inhibitory effect on the wilt pathogen growth causing almost the same reduction, without significant differences.

Semilar results concerning the inhibitory effect of bacteria on different soil borne plant pathogens are reported by *Sallam et al*, (1978) and Ragab *et al*, (1999). on the other hand, the inhibitory effect on the growth of *F. oxysporum* f. sp. *lycopersici* was increased by increasing both of Tecto / Mancozeb and Topsin M Concentrations in media.

This was in harmony with those of Abdel- Kader, 1983 and Wahid *et al*, (1995).

Data in table (4) show that the accumulated yield as tomato fruits in all treatments was significantly higher than in control.

The highly effective treatments, which induce the best records of tomato yield, were Topsin combined with either *Streptomyces spp.* or *P. fluorescens* applied as soil drench.

The mentioned data were reported by Dufour (1994), Larkin and Fravel, (1998) and Abdel - Kader (1999)

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التطبيق الحقلى لبعض طرق المكافحة البيولوجيةوالكيمائية لمـــرض الذبـــول فـــى الطماطم

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أمكن الحصول على عزلات مختلفة من فطر فيوزاريم أكسيسبورم من نباتات طمـــاطم ظـــهرت عليها أعراض الذبول وقد تم جمعها من حقول طماطم فـــى محافظـــات مختلفـــة هـــى محافظـــة الجـــيزة ، الإسماعيلية ، المنيا وقد اختلفت هذه العزلات فى قدرتها على أحداث مرض الذبول لنباتات الطمـــاطم وكـــان أقواها عزلة الإسماعيلية بينما كانت أضعفها عزلة الجيزة.

وقد أظهرت التجارب المعملية حدوث تثبيط فى نمو العزلات المختبرة مـــن الفطـــر فيوزاريـــوم أوكسيسورم فـ سبياســـيلس لايكوبرســـيكاى قـــدر بنســـبة ٧٥,٥ % ، ٧٠,٣ % كنتيجـــة لتـــأثير البكتريـــا ستربتوميسس وسيدومانس فلورونس على التوالى ومن ناحية أخرى أدى اســـتخدام تركـــيزات ١٠٠ و ٤٠٠ جزء فى المليون من مبيد التوبسين وتكتو / مانكوزيب على التوالى إلى حدوث تثبيـــط كـــامل لنمـــو هـــذه العزلات.

أظهرت تجربة الحقل أن زراعة نباتات الطماطم فى تربة معاملة بالمبيد الحيوى ستربتومســـيس أو سيدوموناس فلوروسنس ثم رش النباتات بمبيد التوبسين أدى إلى نقص شديد المعنوية فـــى حـــدوث مـــرض الذبول يليه معاملة البذور بالبكتريا ثم المبيد وكانت هذه المعاملات أكثر معنوية من استخدام البكتريا أو المبيـــد بصورة منفردة فى عمليات المقاومة وقد أظهرت كل المعاملات السابقة نقص معنوى فى حـــدوث الإصابـــة بالذبول بالإضافة إلى زيادة معنوية فى المحصول الناتج من الثمار وذلك بالمقارنة بالكنترول.

تشير النتائج المتحصل عليها فى هذه الدراسة إلى أن استخدام مبيد التوبيسن رشا على المجمـــوع الخضرى كتأثير مساعد للمعاملة بالمبيد الحيوى ستريتومسيس أو سيدوموناس فلوروسنس يمكـــن اعتبارهـــا طريقة تطبيقية فى مكافحة أمراض الذبول المتسببة عن فطريات التربة الممرضة.