

## SUPPRESSION OF FUSARIUM WILT OF TOMATO BY CHITOSAN INVOLVING BOTH ANTIFUNGAL ACTIVITY AND ROOT PROTECTION.

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**Abstract:** Pathogenicity tests of five *Fusarium oxysporum* f. sp. *lycopersici* (FOL) isolates on five tomato cultivars revealed that the foliar yellowing and vascular browning were produced by all isolates, however, the isolates differed in their degree of virulence. Isolate No. 1 was the most aggressive one in inducing the disease in tomato plants. Tomato cultivars reacted differently to the infection by *Fusarium* isolates. Pritchard cv. was the most susceptible, while, Super Marmande cv. gave the highest level of resistance. In *vitro*,

using chitosan as antifungal agent with concentration of 1000 ppm significantly inhibited the mycelial growth of all tested *Fusarium* isolates. In greenhouse experiments, the treatment of tomato seeds or transplants roots with chitosan at concentration of 500 and 1000 ppm reduced both foliar yellowing and vascular browning incidence of tomato plants growing in soil infested with FOL (Isolate No. 1). The treatment of transplants roots by chitosan was more effective than the treatment of seeds.

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**Key words:** fusarium, tomato wilt, chitosan.

### Introduction

*Fusarium oxysporum* f. sp. *lycopersici* (FOL) is one of the most destructive pathogens of tomato. It is a wide spread soil borne pathogen that may be responsible for severe tomato yield losses (Jenkins and Averre, 1983 and Jarvis, 1989). *Fusarium* crown and root rot of tomato caused by FOL killed about 70-83 % of tomato young plants causing root rot and basal stem decay and severe death (Kuckareck *et al.*, 2000).

Various strategies for controlling *Fusarium* wilt of tomato introduced over the years; fungicide treatments,

biological control, using resistant cultivars and cultural practices, but serious damage still occur because the difficult application of biological agents or resistant break of resistant cultivars or side effects of fungicides. Thus, there is a growing need to develop alternative approaches for controlling soil borne diseases; one approach that is being actively pursued involves the use of bioactive substances (Benhamou *et al.*, 1994). Among the most promising bioactive oligosaccharides is chitosan ( $\beta$ -1, 4-linked D- glucosamine polymer), a mostly deacetylated derivative of

chitin occurring in the cell wall of several fungi.

Chitosan is a component of cell wall of phytopathogenic fungi and released during plant fungus interactions, which stimulate the plant defense mechanisms or as an antifungal activator (Walker-simmons *et al.* 1983, and Kauss *et al.*, 1989). It is a very common polymer found in nature and has been reported in shells of crustaceans insects and fungi (Hadwiger, 1999 and Takechi *et al.*, 2000).

Recently, several works have demonstrated that chitosan appears to play a dual function by interfering directly with fungal growth (Oh, *et al.*, 1998 and Asran, 2005).

Also, chitosan treatment suppresses crown and root rot of tomato caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* (Benhamou and Theriault 1992). They also mentioned that the elicitor chitosan applied by root coating was found to sensitize the plants to respond faster to root colonization and, thus, to halt pathogen invasion in the tissues. In growth chamber experiments, chitosan applied as seed coating significantly controlled root rot and seedling blight severity of maize caused by *Fusarium graminearum* (Asran, 2005).

The aim of this research was to determine the reaction of certain tomato cultivars to different FOL

isolates; effects of chitosan at different concentrations on FOL mycelial growth and the application of chitosan as seed coating or root coating on the incidence of tomato Fusarium wilt.

## Materials and Methods

### Reaction of some tomato cultivars to Fusarium wilt

Five isolates of *Fusarium oxysporum* f. sp. *lycopersici* were isolated from natural diseased tomato showing wilt symptoms from different localities in Assiut governorate and identified by Assiut University Mycological Center (AUMC). For studying the reaction between the isolates and some commercial cultivars, five tomato cultivars were tested (Strain B, Supper Marmand, Marmand, Pritchard and Peto 95) during autumn 2005. Seeds of each cultivar were surface sterilized in 2% sodium hypochlorite solution for 2 min., rinsed in sterile distilled water, then air dried and sowed in tray contains sterilized peat: sand: clay 1:1:1. The nursery was irrigated when needed. Barley grain medium was inoculated by each isolate (10 days old) and incubated at 25 °C for 2 weeks. The inoculum was added to sterilized clay-sand soil (2: 1) at rate of 3 % and thoroughly mixed. Sterilized pots (25 cm. in diameter) were used. After one week, tomato transplants (45 days old) were transferred to the infested pots. Four replicates were used for each isolate and uninfested

pots were used as control. Plants were irrigated when necessary and fertilized with NPK. Disease severity was estimated after 30 days from transplanting, as a foliar yellowing percent and vascular browning percent using the rating scale in which infected plants were classified according to a numerical grades ranging from 0 to 4 in which, 0 = healthy.

- 1 = > 25 of plant leaflets are yellow and of vascular root bundles are dark brown.
- 2 = < 25 – 50 of plant leaflets are yellow and of vascular root bundles are dark brown.
- 3 = < 50 – 75 of plant leaflets are yellow and of vascular root bundles are dark brown.
- 4 = < 75 – 100 of plant leaflets are yellow and of vascular root bundles are dark brown.

For calculating the foliar yellowing and vascular browning indices of each plant, the following formulae were used: [(sum of yellowing values /4 x number of leaflets) x100%] and [(sum of vascular browning values /4 x number of internodes) x 100%] (Fakhouri and Buchenaure, 2003).

#### **Effect of chitosan on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici***

To determine the optimal concentrations of chitosan for

treating tomato seeds and transplant roots, purified chitosan solution was prepared according to Asran (2005). Chitosan from crab shell was dissolved in 0.25 N HCl and adjusted to pH 5.6 with 2N NaOH by stirring for 8h. at 45 °C. Undissolved particles were removed by centrifugation (10,000xg 15 min). Chitosan was precipitated with 2N NaOH and washed three times in deionized water to remove salts. The purified chitosan was then air dried and stored at room temperature until required. Chitosan was added to Potato Dextrose Agar (PDA) at concentrations of 0, 500, 750, 1000 ppm. The chitosan solution and PDA were autoclaved separately and combined after autoclaving. A 5 mm diameter plug from the advancing margins of fungal colonies of each isolates seeded centrally onto 5 plates of each chitosan concentration. Fungal growth was recorded as radical growth when the mycelia of the control plates reached to the edge of plate. The experiment was carried out twice.

#### **Seed treatment with chitosan**

Seeds of tomato cv. Pritchard (highly susceptible cultivar) were surface sterilized as mentioned before and immersed into each chitosan concentration 500 and 1000 ppm for 15 min., the wetted seeds were air dried. Seeds treated with sterile water were used as control. The seeds were nursery planted and transplanted in sterilized pots

containing infested soil with isolate No.1 (the highly pathogenic isolate) of *Fusarium oxysporum* f. sp. *lycopersici* in rate of 3% as mentioned above.

### Transplant roots treatment with chitosan

Surface sterilized seeds of tomato (cv. Pritchard) were sown in trays containing sterilized soil (peat: sand: clay 1:1:1) and irrigated by distilled water until two leaf stage (45 days). The roots of these seedlings were soaked in 500 and 1000 ppm of chitosan for 5 min. Seedlings were transplanted into sterilized pots containing infested soil as described previously. Water treatment was used as a control.

After 30 days from transplanting, disease severity was estimated as a

disease index of foliar yellowing and vascular root bundle browning as described above.

### Statistical analysis

All data were subjected to statistical analysis and means were compared with L.S.D. test (Gomez and Gomez, 1984).

### Results

#### Pathogenicity tests

Pathogenicity tests for the 5 isolates of *Fusarium oxysporum* f. sp. *lycopersici* on five tomato cultivars revealed that all fungal isolates were able to cause foliar yellowing (Table 1) and vascular browning root bundles (Table 2) in tomato plants. Isolates No. 1 and No. 2 caused the highest disease severity followed by isolate No. 3.

**Table (1):** Foliar yellowing percent as a reaction of certain tomato cultivars to FOL isolates.

Isolate No.	Cultivars					Mean
	Strain B	Supper Marmand	Marmand	Pritchard	Peto 95	
1	71.88*	34.40	50.00	81.30	46.90	56.88
2	68.80	31.30	53.10	68.80	50.00	54.38
3	50.00	18.80	43.80	59.40	40.60	42.50
4	28.10	6.30	12.50	28.10	12.50	17.50
5	18.80	9.40	9.40	31.30	9.40	15.63
Mean	47.50	20.00	33.75	53.75	31.88	

\* Foliar yellowing %

L.S.D. 0.05

Isolates ( I ) 12.63

Cultivars ( C ) 12.63

Interaction ( IC ) 21.56

**Table (2):** Vascular browning percent as a reaction between certain tomato cultivars and FOL isolates.

Isolate No.	Cultivars					Mean
	Strain B	Supper Marmand	Marmand	Pritchard	Peto 95	
1	65.63*	28.13	43.75	75.00	40.00	50.50
2	59.38	28.13	50.00	65.63	46.88	50.00
3	46.88	15.63	34.38	46.88	37.50	36.25
4	18.75	0.00	9.38	18.75	12.50	11.87
5	6.25	0.00	0.00	12.50	6.25	5.00
Mean	39.37	14.37	27.50	43.75	28.62	

\* Vascular browning %

L.S.D. 0.05

Isolates ( I ) 10.20

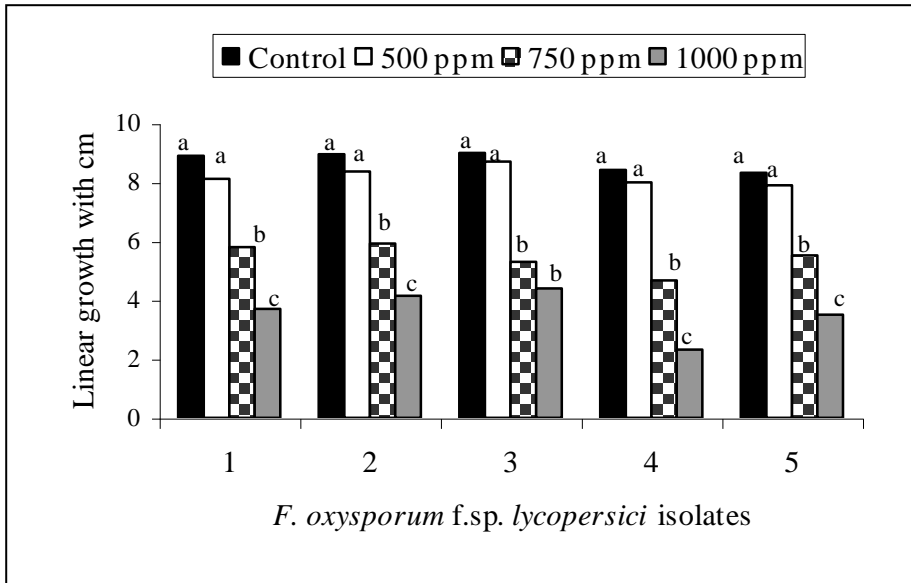
Cultivars ( C ) 10.82

Interaction (IC) 18.87

Meanwhile isolates No. 4 and No. 5 were the weakest isolates. The tested tomato cultivars reacted differently to FOL isolates from moderately susceptible to resistant. The cultivars Pritchard and Strain B were moderately susceptible cultivars. However Peto 95 and Marmand were moderately resistant while Supper Marmand was resistant cultivar. **Effect of chitosan concentration on mycelial growth of FOL isolates *in vitro*.**

Antifungal activity of chitosan was determined by investigating its direct inhibitory effect on mycelial

growth of FOL isolates. Data in figure 1 indicate that chitosan significantly inhibited the mycelial growth of FOL isolates with a marked effect at 750 and 1000 ppm. At the 1000 ppm concentration, chitosan inhibited the mycelial growth of FOL isolates by more than 70 % for isolate No. 4) and by more than 50 % for other isolates. Chitosan appeared to be more effective in inhibiting mycelial growth of isolate No. 4 than the other tested isolates.



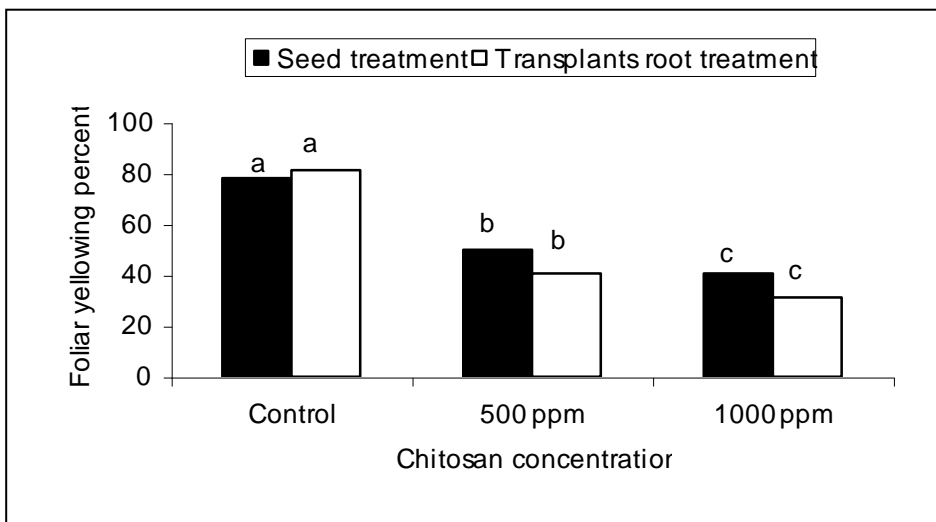
**Figure (1):** Effect of different concentrations of chitosan on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*. Different letters indicate significant differences among treatments according to least significant difference test ( $P = 0.05$ ).

### Effect of chitosan on protection against *Fusarium* wilt of tomato plants

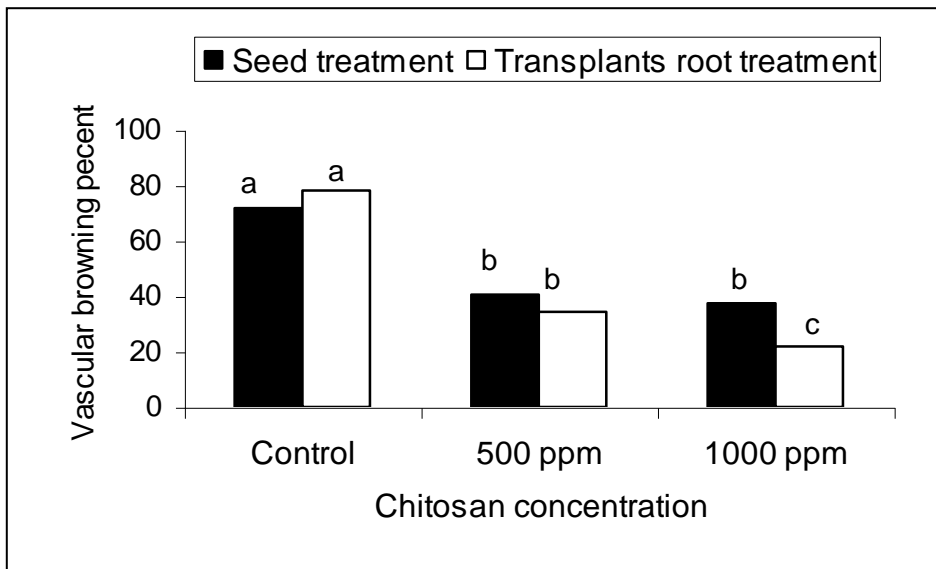
The chitosan concentrations 500 and 1000 ppm were tested to control *Fusarium* wilt of tomato plants caused by FOL isolate No. 1. The chitosan was used as seed and/or transplanting roots treatment. The disease was evaluated as foliar yellowing (Fig.2) and vascular browning root (Fig.3). Data in these figures indicate that treated tomato seeds or roots with any tested chitosan concentration were significantly suppressed disease severity in comparison with control treatment (0 ppm). Increasing chitosan concentration from 500 ppm to 1000 ppm caused decreasing

in the disease severity at the transplanting root treatment. In general, chitosan transplants root treatment was more effective than chitosan seed treatment.

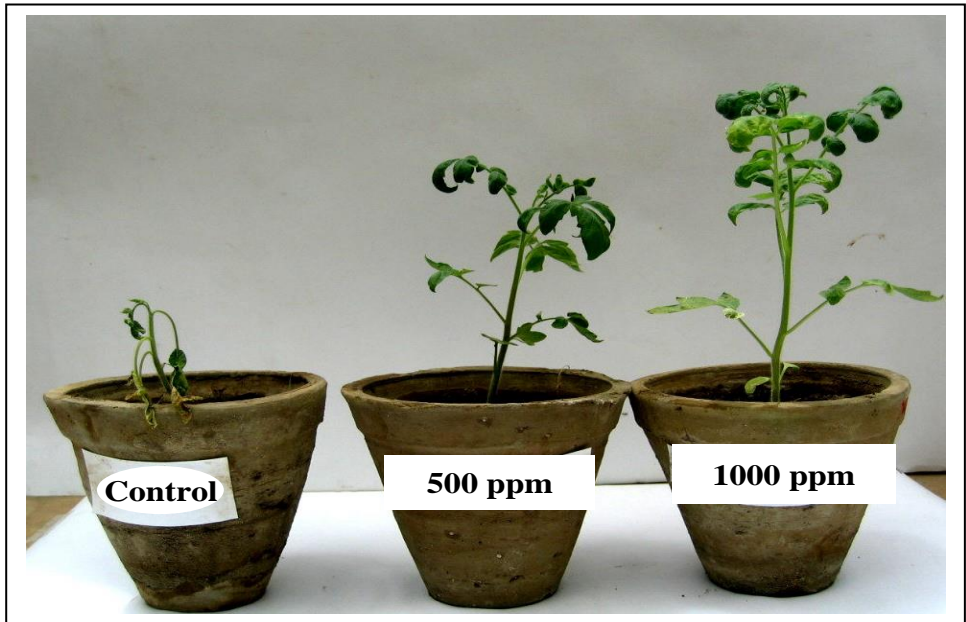
Tomato plants grown in the presence of different concentrations of chitosan and infested with FOL remained healthy and did not exhibit wilt symptoms. Plants grown in the presence of fungal inoculum alone (Control) started showing symptoms of wilting within 30 days after transplanting. By 30 days, control plants (chitosan untreated) appeared to be not only yellowing of leaves and wilting but also reached to an advanced stage of plant death (Fig. 5).



**Figure(2):** Effect of treating tomato plants cv. Pritchard with different concentrations of chitosan on foliar yellowing percent. Different letters indicate significant differences among treatments according to least significant difference test ( $P = 0.05$ ).



**Figure(3):** Effect of treating tomato plants cv. Pritchard with different concentrations of chitosan on vascular browning percent. Different letters indicate significant differences among treatments according to least significant difference test ( $P = 0.05$ ).



**Figure(4):** Effect of chitosan treatment on growth of tomato plants and protection against FOL wilt.

### Discussion

The *F. oxysporum* f.sp. *lycopersici* isolates used in this study were isolated from wilted tomato plants. These isolates had been previously characterized by morphological traits and identified, in Assiut University Mycological Center (AUMC), as *F. oxysporum* f.sp. *lycopersici* (FOL). It is not known that all fungal isolates can cause Fusarium wilt disease in certain tomato cultivars. The results of this investigation revealed that all tested isolates were able to cause yellowing and vascular root bundles discoloration in tomato plants. However, the isolates varied considerably in disease severity.

These results are in accordance with those other investigators (Jarvis, 1989, Benhamou, 1992, Rattink, 1993, Lafontaine and Benhamou, 1996, McGovern *et al.*, 1998 and Lubna Nawar, 2005) who stated that different isolates of *F. oxysporum* f.sp. *lycopersici* differed in their aggressiveness on tomato plants. The tested tomato cultivars reacted differently to FOL isolates from moderately susceptible to resistant. Pritchard and Strain B cvs. were moderately susceptible cultivars, while Supper Marmand cv. was resistant.

Antifungal activity of chitosan was determined by investigating its direct inhibitory effect on mycelial



growth of FOL isolates. Data represented herein indicate that chitosan significantly inhibited the mycelial growth of FOL isolates with a marked effect at 750 and 1000 ppm. Similar results were also reported by other investigators (El Ghaouth *et al.*, 1991, Wang, 1992, Oh *et al.*, 1998, Bautista *et al.*, 2003, Rabea *et al.*, 2003 and Lubna Nawar, 2005). The mode of action of chitosan as antifungal substance might be explained by its interaction with the fungal DNA and / or RNA as stated by Hadwiger and Loschke (1981). Additionally, Leuba and Stossel (1986) indicated that the antifungal activity of chitosan is related to its ability to interfere with the function of plasmamembrane of fungal cells.

Through the last 10 years, there has been an increasing interest in the use of chitosan as a protective agent in agriculture (Oh *et al.*, 1998) and several biological functions have been reported (El Ghaouth *et al.*, 1994).

Chitosan has been applied as a seed treatment or a transplant root treatment. Data indicate that treated tomato seeds or transplants roots with any tested chitosan concentration significantly decreased disease severity in comparison with control treatment. Increasing chitosan concentration to 1000 ppm caused decreasing in the disease severity at the different disease parameters. In general,

treating transplants roots with chitosan was more effective than chitosan seed treatment. The obtained results confirmed the previous studies which suggested that application of chitosan resulted in great reduction in severity of some root diseases caused by phytopathogenic fungi (Ragab *et al.*, 2001, Asran, 2005 and Lubna Nawar, 2005). The chitosan mode of action on suppression disease incidence might be due to their direct effect against the pathogen or its role in induction of disease resistance. Oh *et al.* (1998) reported that chitosan have dual functions on inhibition of mycelial growth of two pathogens (*Phytophthora infestans* and *Fusarium oxysporum* f. sp. *lycopersici*) and induction of expressions of several defense-related genes in the plant. These results suggest that both antifungal and plant defense-inducing activities are involved in suppression of disease by chitosan treatment.

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## وقف تطور مرض الذبول الفيوزارمى فى الطماطم باستخدام الشيتوزان ودوره فى تثبيط نشاط الفطر وحماية جذور النبات

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يعتبر فطر فيوزاريوم أوكسيسبوريم من أكثر الفطريات الممرضة لنبات الطماطم، وعند دراسة إختبار القدرة المرضية لعدد خمس عزلات من فطر فيوزاريوم أوكسيسبوريم طراز ليكوبيرسيى *Fusarium oxysporum* f. sp. *Lycopersici* ومدى قدرتها على إحداث الإصابة فى خمس أصناف من الطماطم ( إسترين ب، مارماند، سوبر مارماند، بريتشارد ، بيتو 95) فقد أظهرت النتائج أن العزلة رقم 1 من الفطر كانت أكثر العزلات قدرة على إحداث الإصابة. أما بالنسبة لقابلية أصناف الطماطم للإصابة فقد كان الصنف بريتشارد أكثر الأصناف قابلية للإصابة أما الصنف سوبر مارماند فقد أظهر درجة عالية من المقاومة.

معملياً ثبت فعلياً التأثير المثبط لمادة الشيتوزان بتركيزات 500 ، 750، 1000 جزء فى المليون على عزلات الفطر. وقد وصلت نسبة الإنخفاض فى النمو الميسليومى للفطر إلى أكثر من 70 % وذلك عند تركيز 1000 جزء فى المليون مع العزلة رقم 4 .

فى تجارب الصوبة تم استخدام الشيتوزان بتركيزات 500، 1000 جزء فى المليون فى معاملة البذور أو غمر جذور الشتلات صنف بريتشارد قد أظهرت النتائج أن الشيتوزان أدى إلى خفض نسبة الإصابة بالذبول الفيوزارمى سواء عند تقديرها كنسبة إصفرار الأوراق أو تلون الحزم الوعائية باللون البنى. وقد أظهر غمر جذور الشتلات فى الشيتوزان فاعلية أكثر فى مقاومة المرض مقارنة بمعاملة البذور.