

EFFICIENCY OF BIOCIDES, FUNGICIDES AND PLANT EXTRACTS AGAINST *FUSARIUM SOLANI* ON TOMATO PLANTS

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ABSTRACT: *Tomato is one of the most important economic vegetable crops which are attacked by several serious diseases such as root-rot. The effect of biocides (Bio-Zeid, Bio-Arc, Plant guard and Clean-Root), fungicides (Rhizolex-T, Vitavax 200 and Maxiam) and plant extracts (Eucalyptus globules, Allium cepa, Datura spp, and Mentha viridis) on growth of Fusarium solani fungus in vitro and on root-rot disease of tomato caused by Fusarium Solani and their effect on biochemical changes were studied under greenhouse conditions. In vitro, all tested biocides were able to inhibit growth of F. solani. Seed treatment with biocides decreased percentage of disease incidence compared with control. Bio-Zeid was the most effective compared with other biocides. All fungicides were able to inhibit linear growth of F. solani compared to the control. Rhizolex-T and Vitavax 200 were the most effective fungicides treatments followed by Maxiam. All water extracts of camphor, onion, Datura and Mint were able to inhibit growth of F. solani compared to the control. Seed of tomato treatment with plant extract decreased disease incidence. Water extract of onion plants was the most effective compared with other plant extracts. Also, all treatments effected on enzymes activity, Bio-Zeid gave the highest increment in peroxidase followed by Bio-Arc and Rhizolex-T. Where, onion gave the highest increase in polyphenoloxidase activity followed by Bio-Zeid. Also it gave the highest increase in chitinase activity followed by Rhizolex-T compared to other treatments.*

Key words: *Biocides, fungicides, plant extract, Fusarium solani, tomato, Lycopersicon esculentum, root-rot disease.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is considered one of the most important economic vegetable crops in Egypt. Tomato plants are subjected to attack by several soil borne fungal pathogens, which cause serious diseases as root-rot and wilt (El-Mougy, 1995; Ghonim, 1999; Montealegre *et al.*, 2003; Morsy, 2005; Montealegre *et al.*, 2005; Srinon *et al.*, 2006 and Khalil *et al.*, 2009). *Fusarium solani* is a soil borne fungus pathogenic that attacks plants through roots at all stages of plant growth. Singh and Kamal (2012) recorded 10-90% loss in yield of tomato in temperate region due to this disease. The biological control may be the best alternative method of control against soil borne pathogens. Landa *et al.* (2004), Omar *et al.* (2006) and Morsy *et al.* (2009) tested the antagonistic *Bacillus megaterium* isolates against *F. oxysporum* the causal

organism of *Fusarium* crown and root-rot of tomato. Barhate *et al.* (2015) found that certain antagonists of *T. harzianum*, *T. hamatum* and *Bacillus subtilis* showed inhibitory effect on growth of the test fungus and were effective in reducing the growth of pathogen. Etenbarian (1992), Singh *et al.* (1993), and Narnavar and Kalekar (1997) reported that carbendazim was effective against tomato wilt and Musmada *et al.* (2009) reported that *in vitro* carbendazim (0.1%) completely inhibited the growth of the fungus. Natural substances, such as plant extracts have been investigated to study the effect on fungal activity. For example, there are studies evaluating the inhibitory activity of plant extract on fungi, Ushiki *et al.* (1996) tested suppression of soil borne plant diseases by medicinal plant extracts. Ejechi and Ilondi (1999) studied pepper extract against tomato rot fungi, Jasso de Rodriguez

et al. (2005) evaluated fungal activity of Aloe Vera pulpon mycelial growth of *Rhizoctonia solani* and *F. oxysporum*. They reported that this extract reduced colony growth rate of *R. solani* and *F. oxysporum* at concentration 15 µm. While *Thymus vulgaris* essential oil exhibit broad fungitoxic spectrum against eight fungal strains including *F. oxysporum* with concentration 0.7 µm/ml (Kumar et al., 2011 and Priyanka et al., 2014), The objective of the present investigation to study the effect of biocides, fungicides and plant extracts on the growth of *F. solani* fungus *in vitro*, on control of root-rot disease of tomato and their respective biochemical changes.

MATERIALS AND METHODS

1. In vitro:

An identified pathogenic isolate of *Fusarium solani* was selected from the fungal collection of the Plant Pathol. Res. Inst., ARC, and checked for pathogenicity.

1.1. Effect of different biocides on growth of *Fusarium solani* fungus:

Bio-Zeid (*Trichoderma album*), Clean root (*B. subtilis*), Plant guard (*T. harzianum*) and Bio-Arc (*B. megaterium*) were tested on Potato dextrose agar PDA medium against *F. solani* by dual culture inoculation technique. Mycelial discs (5-mm, diameter) were cut from the margin of 7 days old cultures of the pathogen in concern and antagonistic fungal agents as well, respectively and placed opposite to each other on PDA in petri plates (9 mm diameter). The discs were placed 3 cm away from each other. Inoculated plates were incubated at 27°C for seven days. The radial growth of *Fusarium solani* was determined to assess the antagonistic potential of *Trichoderma* spp. against the pathogen. The percent growth of test fungus was calculated by using formula given by Arora and Upadhyay (1978). For bacterial antagonists, *F. solani* culture was placed at the center of petri plate and bacterial isolates were streaked after 48 hours, streaks of bacterial

isolates were made equidistantly at the periphery of agar plates. Inoculated petri plates were incubated at 27°C for seven days. The growth and decrease in mycelial growth of the pathogen were calculated according to (Fokemma, 1973).

1.2. Effect of certain fungicides against *Fusarium solani* fungus:

Three fungicides (Rhizolex-T, Vitavax 200 and Maxiam) were used at different concentrations (10, 20, 50, 100 and 200 ppm). The desired concentrations of the fungicides were adjusted and added to autoclaved PDA medium, after reasonable cooling. Plates (9 cm in diam.) were inoculated at the center by discs (5 mm in diam.) of *F. solani* (7-old days). Three replicates were used for each concentration. Linear growth of fungus was measured when the pathogenic fungi completely covered control treatment by taking two perpendicular diameters (in mm) and averaged.

1.3. Plant extracts preparation:

Plant extracts were prepared by mixing 100 mg on each fresh plant leaves i.e. Camphore (*Eucalyptus globules*), Mint (*Mentha viridis*) and Datura (*Datura* spp.), onion bulbs (*Allium Sativum*) with 100 ml of water using electric blender for 5 minutes. The plant extracts filtered through double layers of cheese cloth. The extracts were centrifuged at 3000 rpm for 15 minutes and sterilized using Satman filter.

The plant extracts were added to PDA medium to give final concentrations of 10, 15, 20 and 25%. Poured plates were inoculated at the center by equal discs (5 mm in diam.) taken from 7 days old cultures plates of the pathogenic fungus (*F. solani*) and from 10 days old culture plates as well. Three replicates were used for each concentration. Plates were incubated at 28°C. Linear growth of fungus was measured when the pathogenic fungi completely covered the surface of the medium in a plate of the control treatment.

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2. Greenhouse experiments:

Greenhouse experiments were carried out to evaluate the effect of biocides, fungicides and plant extracts on decreasing the incidence of root-rot disease of tomato. Plastic pots (15 cm, diameter) containing weighted amounts of sterilized clay soil and infested with *Fusarium solani* grown on barley grain media of (5 g / kg) soil before sowing (Khalil *et al.*, 2009).

2.1. Management of root-rot disease of tomato using:

2.1.1. Biocides:

Ten tomato seeds (hybrid super GS) were treated with the Bio-Zeid, Clear-Root, plant guard and Bio-Arc at (rates giren) in Table (1), and treatment with 4% solution of carboxy methyl cellulose (CMC) as sticker. Rates of application are shown in Table(1). Three replicates were used for each treatment.

2.1.2. Fungicides:

This experiment was carried out in sterilized pots (15 cm diam.) containing sterilized clay soil. Soil was infested with *F. solani* as mentioned before. Ten tomato seeds were treated with Rhizolex-T (2 g / kg seed), Vitavax 200 (2 g / kg seed) and Maxiam (2 g / kg seed) by shaking them gently in glass container containing glue suspension (super film 70) as a sticker material / kg seed. The same

mentioned method was used without fungicides as control. Three replicates were used for each treatment.

2.1.3. Plant extracts:

The experiment was carried out in sterilized pots (15 cm diam.) containing sterilized clay soil infested with *F. solani* as mentioned before.

Surface sterilized tomato seeds were soaked for 2 hour in the plant extracts at concentration 50% using distilled water and treated with 4% solution of CMC as sticker, then air dried. The same method was used without plant extracts as control. Ten seeds were sown per pot, and three replicates were used for each treatment.

2.1.4. Disease assessment:

Percentage of pre- and post-emergence damping-off as well as healthy survival plants in each treatment were determined 15, 30 and 60 days after sowing, respectively using the following formulas according to Phillips and Hayman (1970):

$$\text{Pre - emergence (\%)} = \frac{\text{No. of non - germinated seeds}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Post - emergence (\%)} = \frac{\text{No. of dead seedlings}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Survival plants (\%)} = \frac{\text{No. of survival plants}}{\text{Total No. of sown seeds}} \times 100$$

Table (1). The biochemical tested compounds and used doses.

Biocide (Trade name)	Producer	Bio-agent (cfu / ml)	Dose Per kg/seed
Bio-Zeid	El-Nasr	<i>Trichoderma a lbum</i> , 3×10 ⁷ cfu/ml	2.5 g
Bio-ARC		<i>Bacillus megaterium</i> , 2.5×10 ⁷ cfu/ml	2.5 g
Plant guard		<i>Trichoderma harzianum</i> , 3×10 ⁷ cfu/ml	4.0 ml
Clean root		<i>Bacillus subtilis</i> , 30 × 10 ⁷ cfu/ml	5.0 ml

2.2. Biochemical changes:

Fifteen days after planting samples were taken from previously treated and untreated trials and extracted according to Goldschmidt *et al.* (1968). The extracts were used for assaying biochemical change in tomato plants following treatments. The activities of peroxidase enzyme (Allam and Hollis, 1972) and polyphenoloxidase enzyme (Snell and Snell, 1953) and chitinase enzyme (Tuzun *et al.*, 1989) were determined.

The six effective treatments were evaluated for their effects on the activities of peroxidase, polyphenoloxidase and chitinase enzymes in tomato plants.

2.2.1. Extraction of enzymes:

Five gram of tomato leaves were taken 6 weeks after sowing and ground in a mortar in presence of purified sand plus 4ml of 0.1 M sodium phosphate buffer (pH 7.1) (Tuzun *et al.*, 1989). The homogenate of each sample was filtered through four layers of cheesecloth then the filtrates were centrifuged at 3000 rpm for 20 min. at 6°C. The obtained supernatant fluids (crude enzyme extracts) were used for assaying activities of peroxidase, polyphenoloxidase (PPO) and chitinase enzymes at 425, 420 and 540 nm., respectively using spectrophotometer (Spectronic 20-D). Enzyme extract was replaced by distilled water in controlling cuvette. Changes in absorbency for all previous enzymes (Allam and Hollis, 1972) polyphenoloxidase enzyme (Matta and Diamond, 1963) and Chitinase

enzyme (Boller and Mauch, 1988) were determined.

3. Statistical analysis:

The obtained data were statistically treated by analysis of variance (ANOVA) (Gomez and Gomez, 1984).

RESULTS

1. *In vitro*:

1.1. Effect of different biocides on growth of *Fusarium solani* fungus:

Data presented in Table (2) indicated that all bio-agents tested were decreased significantly the linear growth of *Fusarium solani* on PDA compared to the control. Bio-Zeid was the most effective bio-agent recorded 83.33% followed by Bio-Arc 77.77%, Clean-Root 72.22% and Plant guard 63.33%.

1.2. Effect of fungicides certain on linear growth of the fungus:

Data in Table (3) show that *Fusarium solani* was completely inhibited by Rhizolex-T at all concentration ranging from 10-200 ppm. All fungicides decreased significantly the growth of the pathogen than the control. Rhizolex-T was the most effective ranked 100% reduction in all conc. followed by Vitavax 200 and Maxiam. It is observed an inversed correlation between fungicide concent and mycelial growth of the fungus. The higher concentration, the greater, the effect may be recognized.

Table (2). Effect of different biocides on linear growth of *F. solani*, *In vitro*.

Biocide	Rate / medium	Linear growth (cm)	Reduction (%)
Plant guard	4.0 ml	3.3	63.33
Clean-Root	5.0 ml	2.5.	72.22
Bio-Zeid	2.5 g	1.5.	83.33
Bio-Arc	2.5 g	2.0.	77.77
Control	-	9.0.	0.00
L.S.D. at 5%	-	2.15	-

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Table (3). Effect of certain fungicides at different concentration on the linear growth of *F. solani*, *In vitro*.

Fungicide	Concentration (ppm)	Linear growth (cm)	Reduction (%)
Rhizolex-T	10	0.0	100.00
	20	0.0	100.00
	50	0.0	100.00
	100	0.0	100.00
	200	0.0	100.00
Vitavax 200	10	3.5.	61.11
	20	2.0.	77.77
	50	0.0.	100.00
	100	0.0	100.00
	200	0.0	100.00
Maxiam	10	5.0	44.44
	20	3.0	66.66
	50	2.0	77.77
	100	0.0	100.00
	200	0.0	100.00
Control	-	9.0	0.00
L.S.D. at 5%	-	0.83	-

1.3. Effect of different plant extract on growth of *F. solani* fungus:

Data presented in Table (4) show that all extracts significantly decreased the linear growth of *F. solani* compared with the control. *Allium cepa* was the most effective recorded 100% reduction in all cons. except for 15 followed by *Eucalyptus globules*, gave 100% in 25 conc. *Datura* spp. and *Mentha viridis*. In general, effect of the plant extracts on the mycelial growth was increased by increasing concentrations of the plant extracts.

2. Greenhouse experiments:

2.1. Effect of biocides, fungicides and plant extracts on disease incidence:

Data in Table (5) show a significant difference between the tested biocides in

decreasing of pre- and post-emergence damping-off compared to check (control). All treatments significantly decreased the severity of root-rot symptoms caused by *Fusarium solani*. The tested fungicides varied significantly in decreasing pre- and post-emergence damping-off Rhizolex-T was the most effective fungicide recorded 0% disease severity followed by Vitavax 200 gave 5.55% and Maxiam 8.88%. There is a significant difference among plant extracts in decreasing pre- and post-emergence damping-of. *Allium cepa* was the most effective gave 5.55 disease severity followed by *Eucalyptus globules* (11.11%), *Datura* spp. (15.55%) and *Mentha viridis* (20.0%) compared to check. Moreover, all treatment significantly decreased the disease severity of root-rot symptoms caused by *Fusarium solani* compared to control.

Table (4). Effect of different concentrations of plant extracts on the linear growth of *F. solani*, *In vitro*.

Plant extracts	Concentration (%)	Linear growth (cm)	Reduction (%)
<i>Eucalyptus globules</i>	10	2.5	72.22
	15	1.5	83.33
	20	1.0	88.88
	25	0.0	100.00
<i>Datura spp.</i>	10	4.0	55.55
	15	2.5	72.22
	20	1.5	83.33
	25	0.0	100.00
<i>Allium cepa</i>	10	1.5	83.33
	15	0.0	100.00
	20	0.0	100.00
	25	0.0	100.00
<i>Mentha viridis</i>	10	3.5	61.11
	15	2.5	72.22
	20	1.5	83.33
	25	1.0	88.88
Control	-	9.0	0.00
L.S.D. at 5%	-	2.43	-

Table (5). Effect of biocides, fungicides and plant extracts on root-rot incidence of tomato under greenhouse conditions (2014).

Treatment	Concentration	Pre-emergence (%)	Post-emergence (%)	Survival (%)	Disease severity (%)
Plant guard	4.0 ml/L	10.0	10.00	80.00	18.88
Clean root	5.0 ml/L	6.67	10.00	83.33	16.66
Bio-Zeid	2.5 g/L	3.33	3.33	93.34	11.11
Bio-Arc	2.5 g/L	6.67	6.67	86.66	15.55
Rhizolex-T	2 g/k seed	0.00	3.33	96.67	0.00
Vitavax 200	2 g/k seed	6.67	3.33	90.00	5.55
Maxiam	2 g/k seed	10.00	6.67	83.33	8.88
<i>Allium cepa</i>	50%	3.33	3.33	93.34	5.55
<i>Datura spp.</i>	50%	10.00	10.00	80.00	15.55
<i>Eucalyptus globules</i>	50%	6.67	6.67	86.66	11.11
<i>Mentha viridis</i>	50%	13.34	10.00	76.66	20.00
Control	-	45.00	35.00	20.00	55.55
L.S.D. at 5%	-	6.60	7.14	10.13	18.23

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2.2. Biochemical changes:

2.2.1. Peroxidase and polyphenoloxidase activity:

Data presented in Table (6) indicate that tomato plants grown from seeds soaked in the solutions of different inducers resulted in an increase of peroxidase and polyphenoloxidase activity compared to the untreated control. Bio-Zeid gave the highest increment in peroxidase (95.5%) followed by Bio-Arc (86.4%) and Rizolex-T (82.3%). Furthermore, *Datura* spp. gave the highest increase in polyphenoloxidase activity

followed by Bio-Zeid (166%) thus Rizolex-T (165%) compared to check treatment.

2.2. Chitinase enzyme activity:

Data in Table (7) recorded that treatment of tomato seeds treatment resulted in an increase in chitinase activity compared to the untreated check. *Allium cepa* gave the highest increasing (6.40 activity/min.) followed by Bio-Zeid (6.30 activity/min.) and Rizolex-T (5.95 activity/min.) compared to other treatment.

Table (6). Determination of peroxidase and polyphenoloxidase activity in tomato plants with different treatments, under root-rot disease conditions (2015).

Treatment	Conc.	Peroxidase activity (activity/min.)	% increase	Polyphenoloxidase activity (activity/min.)	% increase
Bio-Zeid	2.5 g/L	4.30	95.5	2.00	166
Bio-Arc	2.5 g/L	4.10	86.4	1.98	165
Rizolex-T	2.0 g/L	4.00	82.3	1.98	165
Vitavax 200	2.0 g/L	3.95	80.7	1.75	145
<i>Allium cepa</i>	50%	3.50	59.6	1.66	138
<i>Datura</i> spp.	50%	3.40	55.6	2.20	183
Untreated	-	2.20	-	1.20	-

Table (7). Determination of chitinase activity in tomato plants with different treatments, under root-rot disease conditions (2015).

Treatment	Concentration	Chitinase / min. activity	% increase
Bio-Zeid	2.5 g	6.30	96.9
Bio-Arc	2.5 g	5.90	95.2
Rizolex-T	2.0 g	5.95	95.7
Vitavax 200	2.0 g	5.70	49.5
<i>Allium cepa</i>	50%	6.40	97.0
<i>Datura</i> spp.	50%	5.50	49.0
Untreated	-	3.20	35.0

DISCUSSION

Root-rot disease of tomato caused by *Fusarium solani* is one of the most important and widespread disease of the cultivated tomato. *Fusarium solani* is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, causes major economic losses by inducing necrosis and root-rot in many crop plants (Khalil *et al.*, 2009).

In vitro tests, results obtained indicated that all tested biocides effectively inhibited the mycelial growth of *Fusarium solani*. Bio-Zeid (*Trichoderma album*) used was the most effective bio-agent followed by Bio-Arc (*Bacillus megaterium*), Clean-Root and plant guard. In pots, the obtained results showed that seed treatment with the biocides decreased the percentage of pre- and post-emergence damping-off and root-rot percentage caused by *F. solani*. All treatment significantly reduced the disease incidence. These results are similar to those obtained by Landa *et al.* (2004), Omar *et al.* (2006) and Barhate *et al.* (2015). Effect of the fungicides on mycelial growth was increased by increasing concentrations of the fungicides. Rhizolex-T completely inhibited growth of *F. solani* at 10 and 200 ppm. Seed treatment with fungicides showed a significant decrease in percentage of pre- and post-emergence damping-off and increased percentage of healthy survivals. Rhizolex-T and Vitavax 200 were the most effective fungicide followed by Maxiam. All fungicides significantly decreased disease severity compared with the control. This is in agreement with results obtained by Singh *et al.* (1993), Narnavar and Kalekar (1997) and Musmada *et al.* (2009). Obtained results reported here in indicated that the tested plant extracts tested inhibited linear growth. Increasing concentration of the plant extracts was associated with great reduction in mycelial growth. Moreover, *Allium cepa* extracts was proven to be the most effective one. Through all plant extracts decreased percentage of pre- and post-emergence

damping-off and increased percentage of healthy survival plants. *Eucalyptus globules* showed reasonable effect while, *Datura* spp. and *Mentha viridis* extracts were the least effective in this regard. The results obtained are in agreement with those reported Kumar *et al.* (2011) and Priyanka *et al.* (2014). Further comprehensive studies are needed the methods of using plant extracts in disease control with practical application.

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كفاءة المبيدات الحيوية والمبيدات الفطرية والمستخلصات النباتية على الفطر

فيوزاريوم سولاني في نبات الطماطم

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الملخص العربي

يُعتبر مرض عفن جذور الطماطم المُتسبب عن فطر الفيوزاريوم سولاني من أهم الأمراض التي تُصيب محصول الطماطم تحت الظروف المُعدية، ويُسبب خسائر كبيرة سنوياً لزراعات الطماطم. وأُجريت تلك الدراسة بغرض مُكافحة هذا المرض باستخدام مُبيدات حيوية (Bio-Zeid, Bio-Arc and Clean-Root) ومُبيدات فطرية (Rizolex-T, Vitavax 200 and Maxiam) ومستخلصات نباتية (الكافور Camphor والنعناع Mint والداثوره Datura والبصل Onion) ودراسة تأثيرهم على التغيرات الحيوية في نباتات الطماطم. وقد وجد في المعمل أن المُبيدات الحيوية قادرة على تثبيط نمو فطر *F. solani* وكان أكثر هذه المُبيدات هو المُبيد Bio-Zeid. وجميع المستخلصات النباتية (الكافور - البصل - النعناع - الداثوره) لديها قدرة على تثبيط الفطر، وكلما زاد التركيز زاد تأثيرها المُثبط وكان مُستخلص البصل أكفأها. وكذلك المبيدات لفطرية لديها القدرة علي تثبيط نمو الفطر وكان أكثرها فاعلية مُبيد الريزولكس-تي. كما وجد في الصوبة أنه عند مُعاملة البذور بالمبيدات الحيوية أدى إلى خفض نسبة الإصابة بالمرض مُقارنةً بغير المُعامل في تجارب الأصص. وكذلك مُعاملة البذور بالمستخلصات النباتية أدى لأنخفاض نسب حدوث المرض وكان أكفأ هذه المُعاملات البصل ثم الداثوره. وكذلك وجد أن مُعاملة البذور بالمبيدات الفطرية أدى إلى انخفاض نسبة الإصابة بالمرض وكان الريزولكس-تي والفيثافاكس ٢٠٠ أكثرها تأثيراً. كما أظهرت دراسة التغيرات الكيميائية الحيوية كمؤشرات عن المُقاومة المُستحثة زيادة كبيرة في نشاط إنزيم البيروكسيديز والبولي فينول أوكسيديز وإنزيم الشيتينيز. وكان أكثر المُعاملات تأثيراً المبيد الحيوي بيو زيد ومُستخلص البصل وكذلك المبيد الفطري ريزولكس-تي مُقارنةً بالكنترول.