CHAPTER 4

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSIONS

4.1. *In vitro* experiment

Widespread use of pesticides has significant drawbacks including cost, handling hazards, pesticide residues, and threats to human health and environment **(Paster and Bullerman, 1988; Arcury *et al*., 2002).** For many years, a variety of different chemicals has been used as antifungal agents to inhibit the growth of plant pathogenic fungi.

The antifungal activity of ascorbic acid, benzoic acid, Bion®, citric acid, salicylic acid, Ridomil gold®, Tridex®, Plant guard®, Micronized soreil\Samark® and Vitavax-200®were tested *in vitro*. CDA medium supplemented with four different concentrations of each compound was inoculated separately with the pathogens (*A. solani* and *F. solani*). Fungal growth diameters (GD) were determined and growth reduction % (GR %) were then estimated for each tested chemical concentration.

4.1.1 Effect of some antioxidants and some fungicides on mycelia growth of *Altenaria solani*

4.1.1.1 The effect of some antioxidants on mycelial growth of *Altenaria solani*

Results presented in Tables (5 and 6)and illustrated in Figure (2) show the response of *Altenaria solani* to the inhibitory effect of the evaluated antioxidants.

It is clear that the linear growth of *A. solani*decreased significantly as the concentrations of all treatments increased.Salicylic acid was the most efficient tested compound in this regard, as it caused complete reduction (100%) of the fungal growth at 150 ppm (with EC50 values of 76.8 ppm) followed by citric and benzoic acids at 200 ppm (with EC_{50} values of 86.6 and 101.8 ppm, respectively).

On the other hand, both ascorbic acid and Bion® were failed to cause complete inhibition even at the highest tested concentrations of 300 and 1000 ppm, respectively.

The inhibitory effects of the antioxidants were in the range of 21.39 to 100.0% (salicylic acid), 32.22 to 100.0% (citric acid), 6.07 to 100.0% (benzoic acid), 37.08 to 82.15% (ascorbic acid), and 33.16 to 78.99% (Bion®) (Table 5 and Fig. 2).

Statistical analysis cleared that there were significant differences in average linear growth values between the evaluated antioxidants. The lowest average linear growth was achieved by salicylic acid (2.1 cm) followed by citric acid (2.53 cm). There were no significant differences between ascorbic acid and Bion® which gave average linear growth of 2.79 and 3.13, respectively, while the least effective evaluated compound in reducing mycelial growth was benzoic acid that gave the highest general mean of linear growth (3.20 cm).

The inhibitory effect of some antioxidants to the growth of *A. solani* were investigated by **Tofali *et al.* (2003); Abdel-Sayed (2006)** and **Abada *et al.* (2008b)**.Several investigators reported that the antioxidants may control seed and soil-

borne fungal diseases **(Shahda, 2001 and Dmitrier *et al*., 2003)**, as well as foliar fungal diseases **(Hassan *et al*., 2006)**.

Organic acids are known for years for their antibacterial and antifungal properties which have been widely used in foodstuff industry and agriculture **(De Muynck *et al.*, 2004; Laitila *et al.*, 2002; Lavermicocca *et al.,* 2003; Pao *et al.,* 2008; Sathe *et al.,* 2007; Sholberg and Gaunce 1995 & 1996; Tripathi and Dubey 2004)**. Also, **El-Saidy and Abd El-Hai (2011)** found that the acids effectively controlled fungi.

Table (5): Effect of different concentrations of certain evaluated antioxidants on growth of *A. solani*

Treatment	Concentration (ppm)	*Average colony diameter (cm)	** Average diameter for treatment (cm)	Mycelial Growth Reduction (%)
Ascorbic acid	Control	6.41	-	0.00
	50	4.03	2.79^{ab}	37.08
	100	3.88		39.50
	200	2.10		67.24
	300	1.14		82.15
Benzoic acid	Control	7.51	-	0.00
	50	7.06	3.20^{a}	6.07
	100	4.59		38.91
	150	1.17		84.47
	200	0.00		100.00
Bion®	Control	7.88	-	0.00
	200	5.27	3.13^{ab}	33.16
	400	3.09		60.80
	800	2.51		68.13
	1000	1.66		78.99
Citric acid	Control	6.41	-	0.00
	50	4.34	2.53^{bc}	32.22
	100	3.61		43.66
	150	2.18		66.02
	200	0.00		100.00
Salicylic acid	Control	6.94	-	0.00
	50	5.46	2.1^{c}	21.39
	100	2.93		57.73
	150	0.00		100.00
	200	0.00		100.00

LSD 0.05 = 0.59

Values followed by the same letter(s) within each column don't differ significantly.

* Average diameter was calculated for each tested concentration based on 3 replicates.

** Average diameter for treatment was calculated based on 12 replicates.

Table (6): EC_{50} and EC_{95} values and response/concentration regression equations of the evaluated antioxidants for *A. solani*

Treatment	EC_{50} (ppm)* (Confidence limits)	EC_{95} (ppm)* (Confidence limits)	Regression equation Y= a + b x
Ascorbic acid	101.6 (83.7─123.2)	1047.9 (575.3─1920.1)	Y= -3.3 + 1.6 x
Benzoic acid	101.8 (95.4─108.6)	188.3 (170.1─208.5)	Y= -12.4 + 6.2 x
Bion®	336.4 (271.5─416.5)	3575.5 (1926.8─6658.5)	Y= -4.05 + 1.6 x
Citric acid	86.6 (77.4─96.8)	303.2 (238.8─385.4)	Y= -5.9 + 3.02 x
Salicylic acid	76.8 (71.2─82.9)	154.8 (138.2─173.4)	Y= -10.2 + 5.4 x

*EC_{50} and EC_{95} = Effective concentration for 50% and 95% growth inhibition, respectively.

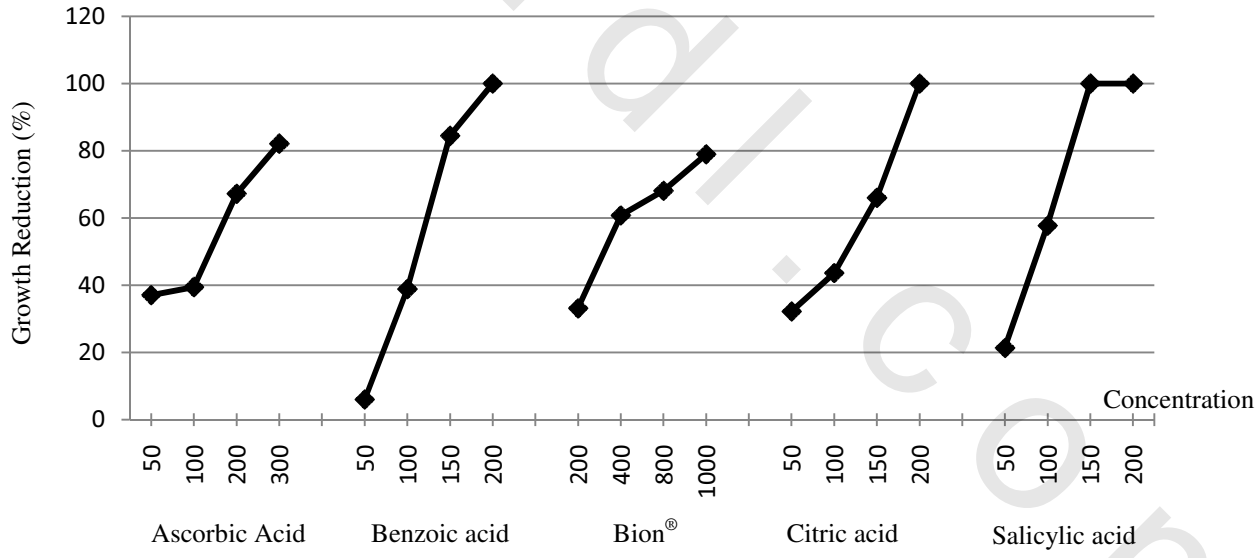

Figure (2): Mycelial growth reduction (%) on the linear growth of *A. solani* in response to different concentrations ofapplied antioxidants on CDA medium.

The efficacy of salicylic acid as antifungal substances against various plant pathogenic fungi has been discussed in many investigations. In this concern, the results obtained by **Ismail *et al.* (1988)** clarify that among some phenolic compounds, salicylic acid had an inhibitory effect on germ tube length and spores germination of *Fusarium oxysporum* f.sp. *lycopersic*i and *Aspergillus fumigatus*. Also, **Shashi-Chauhan and Chauhan (1989)** reported that salicylic acid was highly toxic to the mycelial growth of most of deterophytic fungi (*Trichophyton mentagrophytes, T. tonsuran, T. violaceum, T. rubrum, Microsporum gypseum* and *Epidermophyton floccosum*). They added that the toxicity increased as the concentration of the drug increased in the medium. In addition, **Dwivedi (1990)** recorded that concentrations higher than 200 µg/ml (130 mM) of salicylic acid *in vitro*, inhibited mycelial growth of *Pythium aphanidermatum.*

4.1.1.2 The effect of some fungicides on mycelial growth of *A. solani*

Presented results in Tables (7 and 8)and illustrated Figure (3) show the response of *Altenaria solani* to the inhibitory effect of some tested fungicides.

Data showed that all the tested bio-and chemical fungicides, (Plant guard®, Ridomil Gold®, Micronized soreil\Samark® and Tridex® 80%) caused significant reduction of the linear growth of *A. solani*, when compared with the untreated check treatment. This reduction was gradually increased as the tested concentration increased. These results agreed with those recorded by **Hawamdeh and Ahmed (2001)** who reported that fungicides had significantly reduced the colony growth of early blight pathogen, comparing with control. Tridex® and Ridomil Gold® were the most efficient fungicides, since they caused complete inhibition to the fungus linear growth when they were used at 400 ppm (with EC_{50} values of 63.6 and 92.2 ppm, respectively)and inhibiting the mycelia extension of the pathogen with different percentages at all levels of concentrations. Meanwhile, both Plant guard® and Micronized soreil\Samark® failed to cause complete inhibition even at the highest tested concentrations of 2500 and 1000 ppm, respectively (with high EC_{50} values of 1913.1, 607.8 ppm, respectively).

The inhibitory effect of theevaluated fungicides were in the range of 42.30 to 100.0% (Tridex® 80%), 34.33 to 100.0% (Ridomil Gold®), 18.20 to 74.90% (Micronized soreil\Samark®) and 9.65 to 63.60% (Plant guard®) (Table, 7 and Fig. 3).

Differences in average linear growth values between the fungicides were significant. The lowest average linear growth was achieved by Tridex® (2.18 cm) followed by Ridomil gold®(2.88 cm). There were no significant differences between Micronized soreil\Samark® and Plant guard® which gave the higher general mean of average linear growth (4.20 and 4.43, respectively).

On the basis of EC_{50} values, Tridex® and Ridomil gold® were superior potent compounds against *A. solani* as their EC_{50} values were compared with EC_{50} values of Micronized soreil/Samark® and Plant guard® (Tables 7 and 8).

It is thus obvious from the results summarized above that the best tested fungicide was Tridex® which showed maximum inhibition of *A. solani* at 400 ppm.

The effectiveness of Mancozeb in controlling early blight was confirmed by **Singh *et al.* (2001).** Also, **Choulwar *et al.* (1989), Sinha and Prasad (1991), Choulwar and Datar (1992)** and **Devanathan and Ramanujam (1995)** reported that Mancozeb was

most effective tested chemical against *A. solani.* Moreover, among non-systemic fungicides Mancozeb was found to be effective under *in vitro* conditions **(Prasad and Naik, 2003).**

Also data revealed that the tested biocide (Plant guard®) reduced the mycelia linear growth of *A. solani* as compared with control. The maximum inhibition in growth was recorded at the higher concentration of $8.81x10^2$ spores/ml (63.60% inhibition).

Table (7): Effect of different concentrations of the applied fungicides on linear growth of *A. solani*

Treatment	Concentration	*Average colony diameter (cm)	**Average diameter for treatment (cm)	Mycelial Growth Reduction (%)
Plant guard®	Control	7.60	-	0.00
	$3.68 x10^{2***}$	6.87	4.43[a]	9.65
	$5.47 x10^{2***}$	4.27		46.86
	$7.22 x10^{2***}$	3.82		49.71
	$8.81 x10^{2***}$	2.77		63.60
Ridomil Gold®	Control	6.92	-	0.00
	50	4.54	2.88[b]	34.33
	100	3.79		45.25
	200	3.20		70.62
	400	0.00		100.00
Micronized soreil\ Samark®	Control	7.88	-	0.00
	250	6.44	4.20[a]	18.20
	500	5.58		29.20
	750	2.80		64.45
	1000	1.98		74.90
Tridex®	Control	8.07	-	0.00
	50	4.66	2.18[c]	42.30
	100	2.55		68.36
	200	1.50		81.37
	400	0.00		100.00

LSD 0.05 = 0.412

Values followed by the same letter(s) within each column don't differ significantly.

* Average diameter was calculated for each tested concentration based on 3 replicates.

** Average diameter for treatment was calculated based on 12 replicates.

*** Spores/ml

Table (8): $EC_{50}$and EC_{95} values and response/concentration regression equations of the evaluated bio-and chemical fungicides for *A. solani*

Treatment	EC_{50} (ppm)* (Confidence limits)	EC_{95} (ppm)* (Confidence limits)	Regression equation Y= a + b x
Plant guard®	687.1** (633.5─745.4)	1825.3** (1376.6─2421)	Y= -10.99 + 3.9 x
Ridomil Gold®	92.2 (79.8─106.6)	456.1 (339.9─613.4)	Y= -4.7 + 2.4 x
Micronized soreil/ Samark®	607.8 (545.2─677.6)	2353.2 (1689.4─3280.4)	Y= -7.8 + 2.8 x
Tridex®	63.6 (53.1─76.2)	325.2 (244.04─434.3)	Y= -4.2 + 2.3 x

* EC50 and EC_{95} = Effective concentration for 50% and 95% growth inhibition, respectively.
** Spores/ml.

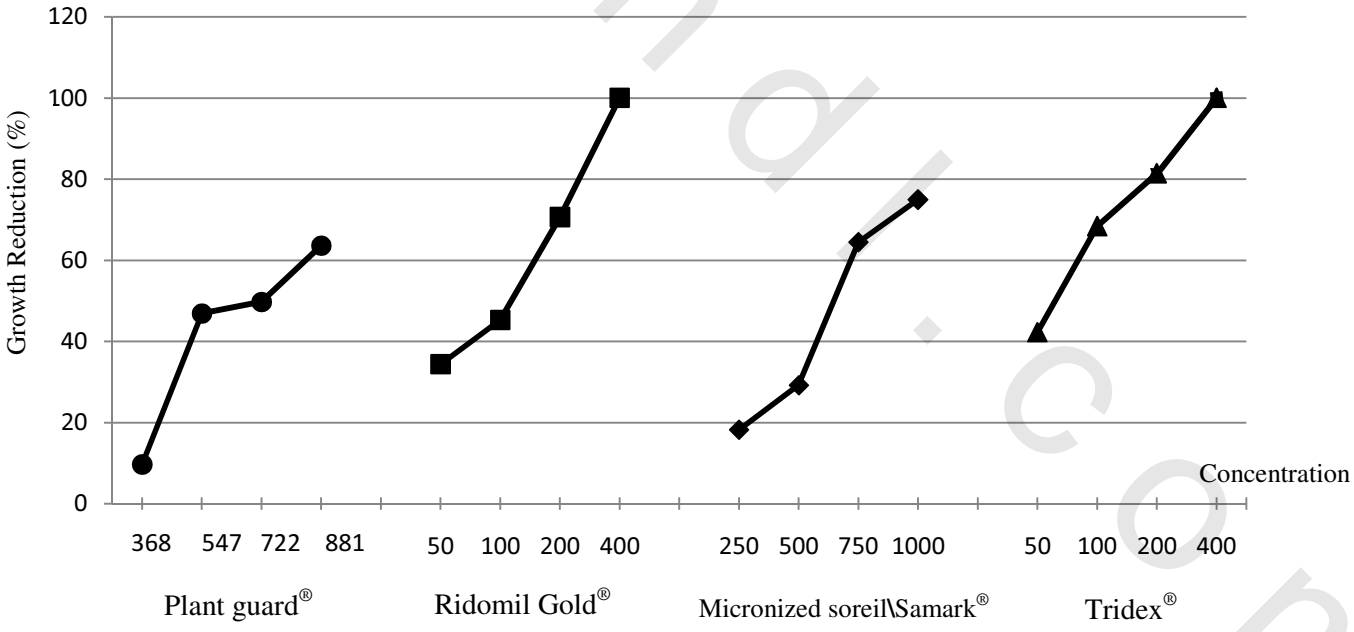

Figure (3): Mycelial growth reduction (%) on the linear growth of *A. solani* in response to different concentrations ofbio-and chemical fungicides applied on CDA medium.

The results suggest that an exposure to high concentration of biofungicide was high toxic to fungal growth as compared to the untreated check. Plant guard® (containing the fungus *Trichoderma harzianum*) was far less effective, as the inhibition range was 9.65-63.60% showing high EC_{50} estimated by 687.1 spores/ml.

Biological activity of antagonist fungi and bacteria may partially be associated with production of antibiotic **(Etebarian *et al.,* 2000)**.Moreover, **Elad (1996)** stated that mechanisms of the antagonism of *Trichoderma* spp. against different pathogens may be due to mycoparasitism, competition and antibiosis. Furthermore, the antagonistic effect of *Trichoderma* spp. may be due to faster mycelia growth than pathogenic fungi **(Wei *et al*., 1999; Melo and Foull, 2000)**.

4.1.2 Effect of some antioxidants and some fungicides on mycelia growth of *Fusarium solani*

4.1.2.1 The effect of some antioxidants on mycelial growth of *Fusarium solani*

Data presented in Tables (9 and 10) and Figure (4) showed that as the applied antioxidant concentrations increased the mycelia linear growth of *F. solani* decreased under *in-vitro* conditions. The tested fungi showed more sensitivity against salicylic acid comparing with the rest of the applied antioxidants at the all used concentrations. Moreover, the fungal growth was completely inhibited by salicylic acid at concentration of 200 ppm. Data also show that the inhibition of *F. solani*growth reached to 95.96% and 92.55% at the concentrations of 200 ppm and 300 ppm of citric acid and ascorbic acid, respectively.

The inhibitory effect of the evaluated antioxidants were in the range of 25.62 to 100% (salicylic acid), 18.29 to 95.96% (citric acid), 24.53 to 92.55% (ascorbic acid), 20.44 to 80.33% (Bion®), and 6.64 to 77.24% (benzoic acid) (Table 9 and Fig. 4).

Among the five tested antioxidants, the minimum mean of mycelial growth was found for salicylic acid treatment (2.75 cm) when it was tested against *F. solani*. This was followed by citric acid (3.34 cm), ascorbic acid (3.47 cm) and Bion® (3.54 cm) with no significant differences between them. On the other hand, benzoic acid was appeared to be poor for controlling or inhibiting the mycelia growth where it gave the highest mean of mycelial growth estimated by 5.32 cm, nevertheless, the diameter of the control colony was as high as 8.14 cm.

The lowest EC_{50} (effective concentration for 50% growth inhibition) value was achieved by salicylic acid (78.2 ppm). Based on EC_{50} values, these five antioxidants could be arranged in a descending order as follows; salicylic, citric, ascorbic and benzoic acids and Bion® (Table 10).

Salicylic acid had been found to be active, as antimicrobial agent. **Guo *et al*. (1993)** reported that the toxicity of salicylic acid to *Fusarium oxysporum* f.sp. *vasinfectum*was 16.5 times more than that of normal fungicide Carbendazim. Moreover, salicylic acid

could inhibit spore germination and mycelial growth of *Fusarium oxysporum* f. sp. *cumin*i **(Mandavia *et al*., 2000)**.

4.1.2.2 The effect of some fungicides on the mycelial growth of *F. solani*

The effect of three fungicides, (Plant guard®, Micronized soreil/Samark® and Vitavax-200®) were *in vitro* evaluated for their inhibitory effect on the linear growth of *F. solani* and the results are presented in Table (11 and 12) and Fig. 5. Results showed that Vitavax-200® was effective in inhibiting the growth of mycelia extension of the pathogen at all levels of the tested concentrations. The mycelial extension decreased as fungicide concentrations increased.

The complete inhibition of mycelia growth by Vitavax-200® (100%) was achieved at 200 ppm, while the level of inhibition reached 95.59% at 100 ppm and the lowest EC_{50} (23.5 ppm) proved its effectiveness for inhibiting the mycelial growth.

Table (9): Effect of different concentrations of the applied antioxidants on linear growth of *F. solani*

Treatment	Concentration (ppm)	*Average colony diameter (cm)	** Average diameter for treatment (cm)	Mycelial Growth Reduction (%)
Ascorbic acid	Control	8.14	-	0.00
	50	6.14	3.47[b]	24.53
	100	4.76		41.48
	200	2.39		70.64
	300	0.61		92.55
Benzoic acid	Control	8.64	-	0.00
	50	8.07	5.32[a]	6.64
	100	7.28		15.77
	150	3.98		53.96
	200	1.97		77.24
Bion®	Control	7.68	-	0.00
	200	6.11	3.54[b]	20.44
	400	3.67		52.26
	800	2.89		62.40
	1000	1.51		80.30
Citric acid	Control	7.99	-	0.00
	50	6.53	3.34[b]	18.29
	100	4.20		47.43
	150	2.30		71.21
	200	0.34		95.96
Salicylic acid	Control	8.56	-	0.00
	50	6.37	2.75[c]	25.62
	100	3.97		53.66
	150	0.68		92.08
	200	0.00		100.00

LSD 0.05 = 0.564

Values followed by the same letter(s) within each column don't differ significantly.
* Average diameter was calculated for each tested concentration based on 3 replicates.
** Average diameter for treatment was calculated based on 12 replicates.

Table (10): $EC_{50}$and EC_{95} values and response/concentration regression equations of the evaluated antioxidants for *F. solani*

Treatment	EC_{50} (ppm)* (Confidence limits)	EC_{95} (ppm)* (Confidence limits)	Regression equation Y= a + b x
Ascorbic acid	106.6 (93.8─121.2)	475.9 (358.2─633.5)	Y= -5.1 + 2.5 x
Benzoic acid	143.1 (132─155.2)	359.3 (289.7─445.7)	Y= -8.9 + 4.1 x
Bion®	450.7 (389.7─521.3)	2733.9 (1797.5─4165.4)	Y= -5.6 + 2.1 x
Citric acid	94.97 (86.9─103.8)	257.4 (216.1─306.7)	Y= -7.5 + 3.8 x
Salicylic acid	78.2 (71.7─85.4)	183.6 (160.4─210.2)	Y= -8.4 + 4.4 x

* EC_{50} and EC_{95} = Effective concentration for 50% and 95% growth inhibition, respectively.

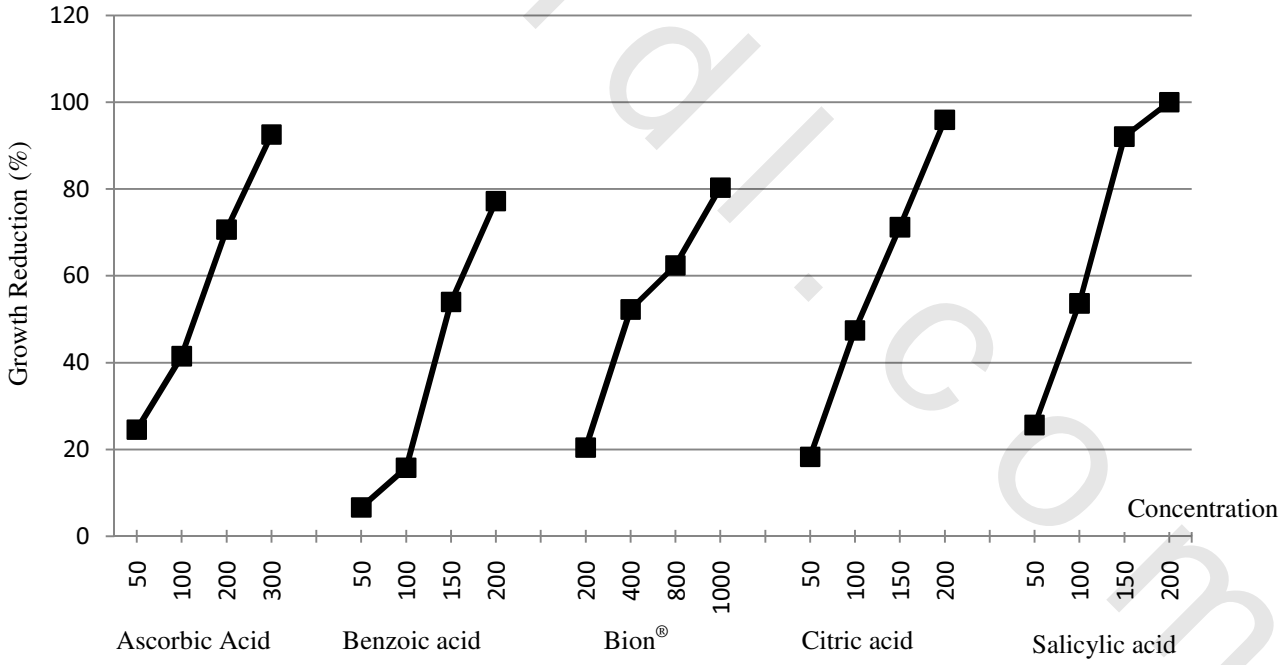

Figure (4): Mycelial growth reduction (%) on the linear growth of *F. solani* in response to different concentrations ofapplied antioxidant on CDA medium

Vitavax-200® inhibited the radial growth of *F. solani* by 24.15-100%, while Micronized soreil/Samark® and Plant guard® were less effective on mycelial growth at all concentrations giving inhibition ranges of 13.14-62.56% and 6.76-61.70%, respectively. This suggests that Vitavax-200® is more effective in suppressing the pathogen.**Ahmad *et al.* (1996)** proved that Vitavax® have significant suppressive effect on the growth of *F. solani*. Similar inhibitory effects of Vitavax® fungicide on *Fusarium oxysporum* were recorded by **Sultana and Abdul Ghaffar (2013)**. They reported that Vitavax® at 100 ppm showed inhibition ratio that reached 92.2% and at 500 ppm it gave 100% inhibition of pathogen growth.

The mean of colony diameter (2.37 cm) that achieved by Vitavax-200® show high level of activity and significance compared with Micronized soreil/Samark® and Plant guard® (4.87 and 5.02, respectively). The results showed that there were significant differences between the mean values of colony diameter resulted from using concentrations of 100 ppm and 200 ppm of Vitavax-200® (P 0.24>0.05).

The effectiveness of Vitavax® has been already reported against the pathogenic fungi *Fusarium oxysporum* **(Chavan *et al.*, 1977** and **Ahmad *et al.*, 1996)**. Carboxin has been reported to reduce glucose oxidation to 50%, acetate oxidation to 70-90% as well as reducing the synthesis of DNA, RNA and protein by up to 60-90%. These effects inhibit fungal growth to about 90% **(Ragsdale and Sisler, 1970)**.

Table (11): Effect of different concentrations of the evaluated bio-and chemical fungicides on linear growth of the *F. solani*

Treatment	Concentration (ppm)	*Average colony diameter (cm)	** Average diameter for treatment (cm)	Mycelial Growth Reduction (%)
Plant guard®	Control	7.89	-	0.00
	3.68 x10^{2}***	7.36	5.02[a]	6.76
	5.47 x10^{2}***	5.38		31.84
	7.22 x10^{2}***	4.31		45.37
	8.81 x10^{2}***	3.02		61.70
Micronized soreil/ Samark®	Control	8.11	-	0.00
	250	7.04	4.87[a]	13.14
	500	5.90		27.26
	750	3.49		56.94
	1000	3.04		62.56
Vitavax-200®	Control	8.32	-	0.00
	10	6.31	2.37[b]	24.15
	50	2.81		66.21
	100	0.37		95.59
	200	0.00		100.00

LSD 0.05 = 0.82
Values followed by the same letter(s) within each column don't differ significantly.
* Average diameter was calculated for each tested concentration based on 3 replicates.
** Average diameter for treatment was calculated based on 12 replicates.
*** Spores/ml

Table (12): $EC_{50}$and EC_{95} values and response/concentration regression equations of the evaluated bio-and chemical fungicides for *F. solani*

Treatment	EC_{50} (ppm)* (Confidence limits)	EC_{95} (ppm)* (Confidence limits)	Regression equation Y= a + b x
Plant guard®	748.04** (691.7─808.99)	1751.6** (1366.4─2245.95)	Y= -12.8 + 4.5 x
Micronized soreil/ Samark®	729.7 (643.2─827.8)	3153.2 (2059.6─4832.8)	Y= -7.4 + 2.6 x
Vitavax-200®	23.5 (19.5─28.2)	126.5 (97.5─164.96)	Y= -3.08 + 2.3 x

* EC_{50} and EC_{95} = Effective concentration for 50% and 95% growth inhibition, respectively.
** Spores/ml.

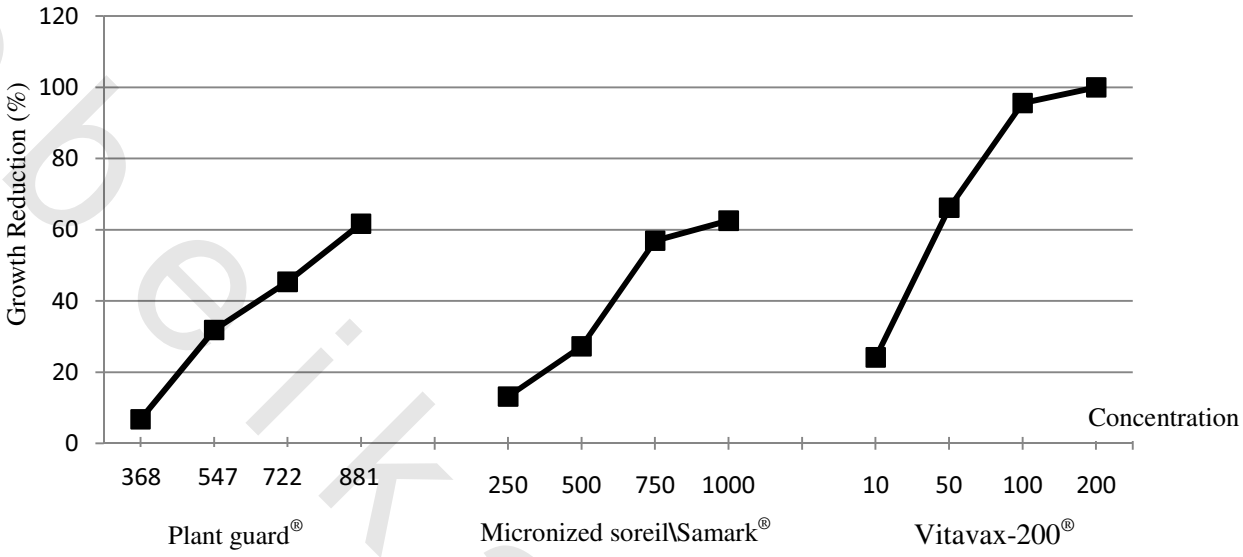

Figure (5): Mycelial growth reduction (%) on the linear growth of *F. solani* in response to different concentrations ofbio-and chemicalfungicides applied on CDA medium

4.2.Control of the root rot and early blight diseases under greenhouse conditions

Many strategies to control the fungal pathogens have been investigated in the field (**Biondi *et al.,* 2004** and **Khan *et al.,* 2007**). Currently, the most effective method for preventing soil-borne diseases is to apply chemical fungicides which could be harmful to other living organisms and reduce useful soil microorganisms (**Khalifa *et al.,* 1995** and **Lewis *et al.,* 1996**). Therefore, public concern is focused on alternative methods of pest control, which can play a role in integrated pest management systems to reduce dependence on chemical pesticides (**Sutton, 1996**).

It is frequently mentioned that using antioxidants as resistance inducers become known as a safety method of reducing the severity of many diseases including early blight of tomato (**Reuveni *et al.,* 1993 & 1997; Kessmann *et al.,* 1994; Spletzer and Enyedi, 1999; Abdel-Sayed, 2006; Gado, 2006 and Abada *et al.,* 2008b**).

The aim of this experiment was to evaluate the efficacy of the tested compounds for controllingtwo tomato diseases (root rot and early blight) and also the ability of these compounds to act asplant defense inducers against these diseases.The experiment was carried out in pots maintained under greenhouse conditions.

4.2.1 Effect of different treatments on diseases severity and reduction percentages

The efficacy of some antioxidants (ascorbic, benzoic, citric and salicylic acids and Bion®) in addition to a biocide (Plant guard®) and four chemical fungicides (Ridomil gold®, Tridex®, Micronized soreil\Samark® and Vitavax-200®) as separate (alone) or sequential treatment with salicylic acid on some tomato diseases (root rot and early blight) and diseases severity was investigated.

4.2.1.1 First season

4.2.1.1.1 Root rot (incited by *F. solani*)

Effect of the performed treatments on the root rot disease (disease severity)and reduction percentagesis shown in Table (13) and Fig.(6). It was clear that all the evaluated treatmentsreduced disease severity of the root rot disease.Also, the tested chemical fungicides were more efficient than the tested antioxidants.

Vitavax-200®was the most effective compound against *F. solani*causing 84.9% reduction of disease severity, followed by the treatment of salicylic acid +½ dose of Vitavax-200® (78.8%) andMicronized soreil/ Samark® (63.6%). Meanwhile, salicylic acid was the most effective testedantioxidant (51.5%) followed by the biocideBion® (45.6%).

The mean of disease severity (10% and 14.58%) that achieved by using Vitavax-200® alone andafter the sequential treatment of salicylic acid+½ Vitavax-200®, respectivelyshow high level of activity and significance compared with the all tested antioxidants. The results showed that there were significant differences between the mean values of disease severity resulted from the tested chemical fungicides alone or theirsequential treatments with antioxidants and tested antioxidants alone or biocide (Plant guard®).

These results seem to confirm those of **Agostini *et al.* (2003)**who reported that the induced plant resistance products were effective for disease control but they may be more useful in an integrated program with standard fungicides.

4.2.1.1.2 Early blight (incited by *A. solani*)

Data presented in Table (14) and Fig. (7)indicated that all the applied treatments significantly reduced tomato early blight disease caused by *A. solani* compared to untreated control. In addition, the most effective treatment in this regard was Ridomil Gold®, followed by Tridex® then salicylic acid +½ Ridomil Gold®, which achieved disease reductions estimated by 87.5, 80.4 and 76.8%, respectively. The respective averages of disease severity for these treatments were 8.33, 13.10 and 15.48%, respectively compared with 66.67% for untreated plants. Also, data revealed that chemical fungicides were more efficient than the evaluated antioxidants in this regard. It is well known that fungicides, especially systemic ones are more efficient in management of many fungal diseases

including early blight **(Parvez *et al.,* 2003)**. Meanwhile, **Razvi (1995)** reported that Mancozeb has been very effective fungicide against early blight having protective and eradicative properties.

Table (13): Effect of some antioxidants,bio-and chemical fungicides on the disease severity of root rotcaused by*F. solani*(first season).

Treatment	Disease severity (%)				Reduction (%)
	R1	R2	R3	Mean	
antioxidants					
Ascorbic acid	37.5	50	37.5	41.67^{bcd}*	39.4
Benzoic acid	56.25	37.5	56.25	50.00^{bc}	27.3
Bion®	37.5	25	50	37.50^{bcd}	45.6
Citric acid	50	50	56.25	52.08^{ab}	24.2
Salicylic acid	25	37.5	37.5	33.33^{cd}	51.5
Bio-and chemical fungicides					
Plant guard®	25	50	56.25	43.75^{bc}	36.7
Micronized soreil/ Samark®	12.5	37.5	25	25.00^{de}	63.6
Vitavax-200®	12.5	6.25	12.5	10.42^{e}	84.9
Sequential treatment					
Salicylic acid +½ Vitavax-200®	12.5	12.5	18.75	14.58^{e}	78.8
control	75	75	56.25	68.75^{a}	-

LSD 0.05 = 16.72

*Values followed by the same letter(s) within each column are not significantly different

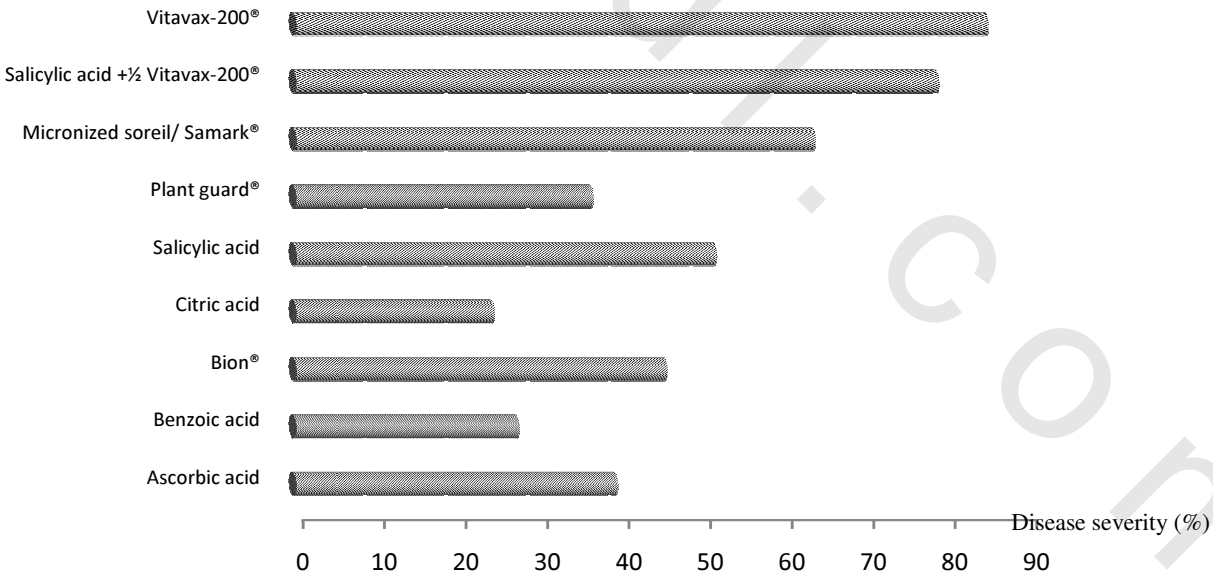

Figure (6): Effect of the performed treatments with different tested compounds on the reduction percentages of root rot disease incited by *F. solani* (first season).

On the other hand, Bion® was the most effective antioxidant achieving disease reduction estimated by 62.5%,andwas not significantas compared with salicylic acid that fulfilled 58.9% disease reduction followed by ascorbic acid (53.6% reduction).The other two antioxidants showed moderate effect.

Table (14): Effect of some antioxidants, bio-and chemical fungicides on the disease severity of early blightcaused by*A. solani*(first season).

Treatment	Disease severity (%)				Reduction (%)
	R1	R2	R3	Mean	
antioxidants					
Ascorbic acid	28.57	42.86	21.43	30.95^{bc}*	53.6
Benzoic acid	42.86	21.43	57.14	40.48^{b}	39.3
Bion®	21.43	32.14	21.43	25.00^{bcd}	62.5
Citric acid	57.14	21.43	32.14	36.90^{b}	44.6
Salicylic acid	21.43	32.14	28.57	27.38^{bcd}	58.9
Bio-and chemical fungicides					
Plant guard®	21.43	32.14	42.86	32.14^{bc}	51.8
Micronized soreil/ Samark®	14.29	21.43	35.71	23.81^{bcd}	64.3
Ridomil Gold®	3.57	7.14	14.29	8.33^{d}	87.5
Tridex®	7.14	10.71	21.43	13.10^{cd}	80.4
Sequential treatment					
Salicylic acid +½ Ridomil Gold®	21.43	17.86	7.14	15.48 ^{cd}	76.8
control	57.14	71.43	71.43	66.67^{a}	-

LSD 0.05 = 18.32

*Values followed by the same letter(s) within each column are not significantly different

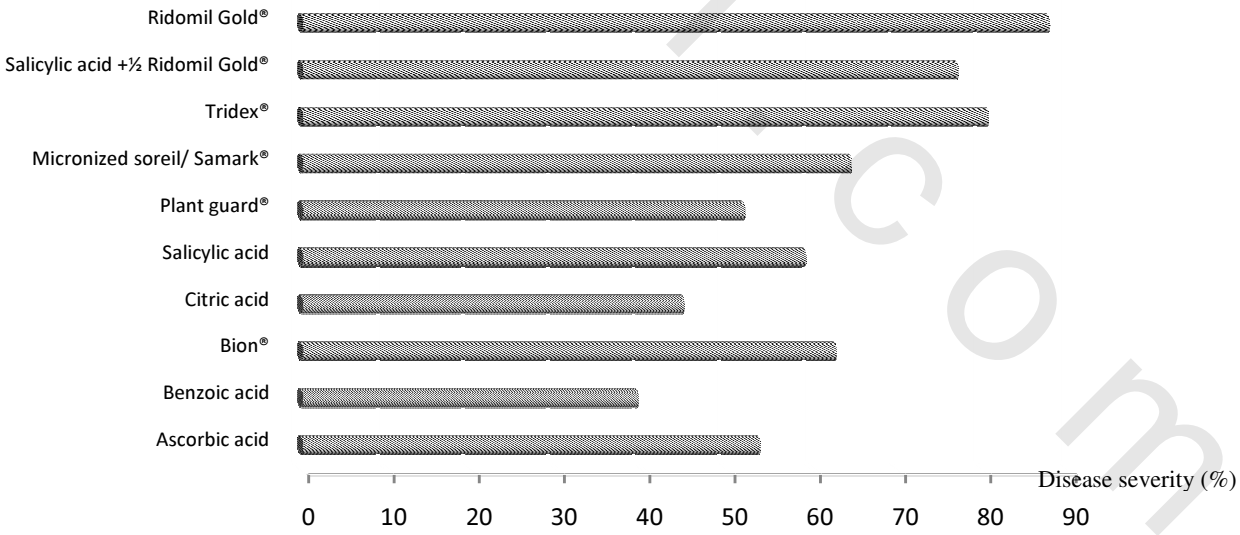

Figure (7): Effect of the performed treatments with different tested compounds on the reduction percentages of early blight disease, incited by *A. solani* (first season).

4.2.1.2 Second season

4.2.1.2.1 Root rot (incited by *F. solani*)

The demonstrated results in Table (15) and Figure (8) exhibit the effect of the used treatments on the disease severity and reduction percentages of the root rot disease incited by *F. solani* on tomato. Almost, the same trend as that of 1st season was obtained during the second one.

It is obvious that, infested root discoloration was reduced by the all applied treatments as compared with untreated check plants. Vitavax-200® exhibited high reduction percentage estimated by 89.2%, followed by the sequential treatment of salicylic acid +½ Vitavax-200® (83.8%), Micronized soreil/ Samark® (70.3%), Bion® (64.7%), salicylic acid (56.8%), ascorbic acid (48.7%), Plant guard® (43.2%) and citric acid (37.8%), while benzoic acid showed less efficacy as compared with the other evaluated treatments where it gave the lowest reduction of 27%.

Regarding the mean of disease severity, there were no significant differences between Vitavax-200® and salicylic acid +½ Vitavax-200® (both gave mean of disease severity calculated by 8.33% and 12.5%, respectively) or between Micronized soreil/ Samark® and Bion® (both recording mean of disease severity calculated by 22.92% and 27.08%, respectively).

Those above-mentioned results showed that Bion® was the most effective tested antioxidant.These results seemed to be in agreement with those of Benhamou and Belanger (1998) who reported that Bion® increased plant resistance against *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato plants. Also, the presented results was in harmony with many researchers**(Benhamou and Belanger, 1998; Cole, 1999; Anfoka, 2000)** who reported that Bion® at low rates activated resistance in many crops against a broad spectrum of diseases, it translocated systemically in plants and acts as functional analog of SA in natural SAR signaling pathway **(Kessmann *et al.*, 1994; Lawton *et al.*, 1996; Oostendorp *et al.*, 2001).**

4.2.1.2.2 Early blight (incited by *A. solani*)

Data in Table (16) and Figure (9) revealed that all of the evaluated treatments were found to have an effect on the severity of the early blight disease under greenhouse conditions.It is obvious that, as achieved in the first season, Ridomil Gold® pronounced the highest efficacy against the targeted disease reducing the severity by 88.89%, followed by Tridex® (75.93%), salicylic acid +½ Ridomil Gold® (68.52%), Micronized soreil/ Samark® (59.26%), salicylic acid (55.56%), Bion® (53.70%), ascorbic acid (48.15%), Plant guard® (38.89%), benzoic acid (25.93%) and citric acid (20.37%).

Table (15): Effect of some antioxidants, bio-and chemical fungicides on the disease severity of root rotcaused by*F. solani*(second season).

Treatment	Disease severity (%)				Reduction (%)
	R1	R2	R3	Mean	
antioxidants					
Ascorbic acid	56.25	37.5	25.0	39.58^{bc}*	48.7
Benzoic acid	37.5	56.25	75.0	56.25^{ab}	27.0
Bion®	18.75	25.0	37.5	27.08^{cd}	64.7
Citric acid	37.5	56.25	50.0	47.92^{bc}	37.8
Salicylic acid	25.0	37.5	37.5	33.33^{bcd}	56.8
Bio-and chemical fungicides					
Plant guard®	25.0	56.25	50.0	43.75^{bc}	43.2
Micronized soreil/ Samark®	37.5	18.75	12.5	22.92^{cd}	70.3
Vitavax-200®	6.25	0.00	18.75	8.33^{d}	89.2
Sequential treatment					
Salicylic acid +½ Vitavax-200®	6.25	18.75	12.5	12.50^{d}	83.8
control	56.25	100	75	77.08^{a}	-

LSD 0.05 = 23.4

*Values followed by the same letter(s) within each column are not significantly different.

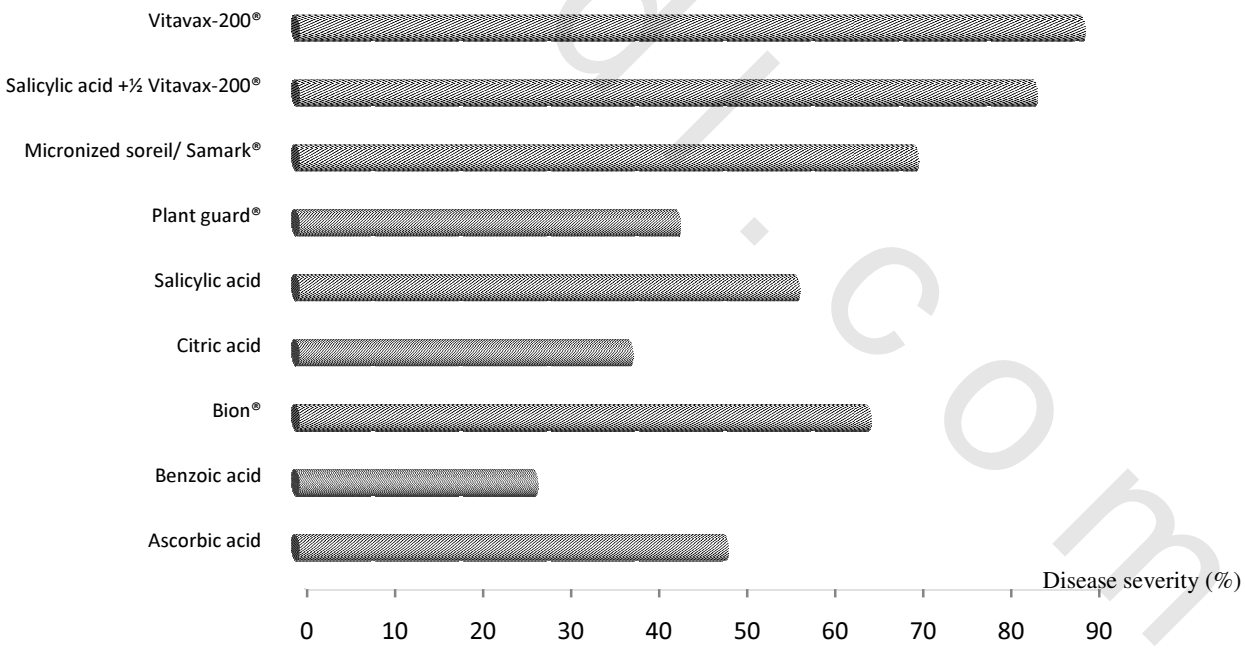

Figure (8): Effect of the evaluated treatments with different tested compounds on the reduction percentages of root rot disease, incited by *F. solani* (second season).

According to the mean of disease severity, the less mean was observed by the most efficient fungicide Ridomil Gold® recording 7.14% disease severity. Also, there were no significant differences between Tridex® and salicylic acid +½ Ridomil Gold® (both treatment gave mean of disease severity determined by 15.48% and 20.24%, respectively). Furthermore, there were no significant differences between Micronized soreil/ Samark®, salicylic acid and Bion® whereas they recorded 26.19%, 28.57% and 29.76% disease severity, respectively.

According to the above-mentioned results, Plant guard® was showing a weak effectin controlling pathogens compared with most of the other treatments and that could bepossibly due to the longer time taken by conidia to germinate **(Hjeljord *et al*., 2002)**.

Table (16): Effect of some antioxidants, bio-and chemical fungicides on the disease severity of early blight caused by*A. solani*(second season).

Treatment	Disease severity (%)				Reduction (%)
	R1	R2	R3	Mean	
Antioxidants					
Ascorbic acid	21.43	42.86	35.71	33.33^{bc}*	48.15
Benzoic acid	42.86	57.14	42.86	47.62^{ab}	25.93
Bion®	53.57	14.29	21.43	29.76^{bcd}	53.70
Citric acid	57.14	42.86	53.57	51.19^{ab}	20.37
Salicylic acid	21.43	57.14	7.14	28.57^{bcd}	55.56
Bio-and chemical fungicides					
Plant guard®	53.57	21.43	42.86	39.29^{bc}	38.89
Micronized soreil/ Samark®	17.86	32.14	28.57	26.19^{bcd}	59.26
Ridomil Gold®	7.14	0.00	14.29	7.14^{d}	88.89
Tridex®	21.43	7.14	17.86	15.48^{cd}	75.93
Sequential treatment					
Salicylic acid +½ Ridomil Gold®	21.43	32.14	7.14	20.24^{cd}	68.52
control	71.43	57.14	64.29	64.29^{a}	-

LSD 0.05 = 22.73

*Values followed by the same letter(s) within each column are not significantly different.

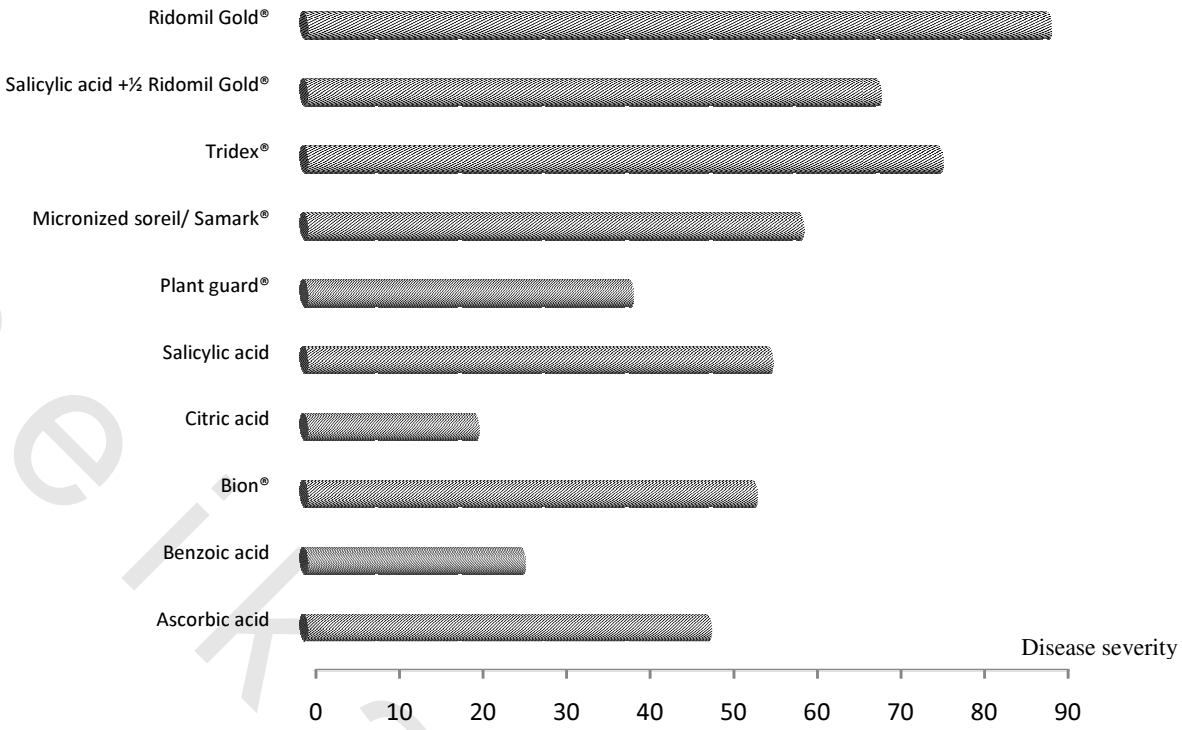

Figure (9): Effect of the evaluated treatments with different tested compounds on the reduction percentages of early blight disease, incited by *A. solani* (second season).

4.2.2 Determination of enzymes activity

The aim of this experiment was to determine the activity of some enzymes responsible for diseases resistance in control and sprayed tomato plants with biocide, antioxidants and fungicides alone or as a sequential treatment with antioxidants under greenhouse conditions against the early blight disease incited by *A. solani*. Activities of the selected enzymes were determined 3 and 7 days after inoculation.

4.2.2.1 Peroxidase activity

Peroxidase activity was determined in tomato leaves inoculated with *A. solani*, the causal agent of tomato early blight. Plants were treated with some antioxidants, bio-and chemical fungicides then they were inoculated with *A. solani*. Results are presented in the following section:

4.2.2.1.1 First season

Perusal data in Table (17) and Figure (10) revealed that enzyme activity increased with time elapsed after inoculation at all the tested treatments compared with that of control plants.However, increasing rates of activity differed according to the tested treatment.

After three days post inoculation, significant increase in enzyme activity was obtained, in particular in those plants pre-treated with combination of salicylic acid with half recommended rate of Ridomil Gold®,Bion®, salicylic acid, Ridomil Gold®and Tridex®(3.78, 3.17, 2.69, 2.47 and 2.34 folds, respectively over that of control).

The peroxidase enzyme activity after seven days post-inoculation showed significant increase in all treated plants compared with that activity in control.Combination of salicylic acid with half recommended rate of Ridomil Gold®exhibited the highest values of peroxidase activity (4-folds over that of control), whereas Bion® showed the highest peroxidase activity among the tested antioxidants followed bysalicylic acid (3.39 and 3.22 folds of control, respectively).Chemical fungicides often act as inducers of systemic resistance in plants against the pathogens. The present results revealed that Ridomil Gold® was the most effective fungicidefor increasing the activity of the enzyme followed by Tridex®, whereas they recorded peroxidase activity estimated by 2.99 and 2.66 fold over that of control, respectively. Also, ascorbic acid and Micronized soreil/ Samark® showed moderate effect whereas they gave peroxidase activity calculated by 2.2 and 2.09 folds over that of control, respectively.

Table (17): Effect of some antioxidants, bio-and chemical fungicides on peroxidase (PO) activities in tomato leaves inoculated with *A. solani* at different periods following inoculation (first season).

Treatment	Enzyme activity (units/mg protein)** Time after inoculation (days)		Mean
	3	7	
Antioxidants			
Ascorbic acid	0.166^{cde}	0.222^{cd}*	0.194
Benzoic acid	0.108^{e}	0.132^{ef}	0.120
Bion®	0.273^{ab}	0.342^{ab}	0.307
Citric acid	0.121^{de}	0.159^{def}	0.140
Salicylic acid	0.231^{bc}	0.325^{b}	0.278
Bio-and chemical fungicides			
Plant guard®	0.112^{e}	0.184^{de}	0.148
Micronized soreil/ Samark®	0.126^{de}	0.211^{cd}	0.169
Ridomil Gold®	0.212^{bc}	0.302^{b}	0.257
Tridex®	0.201^{bcd}	0.269^{bc}	0.235
Sequential treatment			
Salicylic acid +½ Ridomil Gold®	0.325^{a}	0.404^{a}	0.364
Control	0.086^{e}	0.101^{f}	0.094
LSD (0.05)	0.078	0.072	-

*Values followed by the same letter(s) within each column are not significantly different.

** Each value reported is the average of three replicates.

Hence, the mean of the enzyme activity throughout the inspection period showed that the treatment of combination of salicylic acid with half recommended rate of Ridomil Gold® exhibited the highest mean estimated by 0.364 units, followed by Bion® (0.307 U), salicylic acid (0.278 U), Ridomil Gold® (0.257 U) and Tridex® (0.235 U).

Bion® and salicylic acid have been developed as systemic acquired resistance activator with no antimicrobial properties, but with increased crop resistance to a wide range of diseases.These activators trigger the systemic acquired resistance signal transduction pathway in several plant species **(Lawton *et al.*, 1996; Kessmann *et al.*, 1994)** by switching on a wide range of well characterised SAR genes **(Hammerschmidt *et al.*, 2001)**, primarily those encoding PR-proteins **(Durrant and Dong, 2004; Ton *et al.*, 2005)**, cell wall hydroxyproline-rich glycoprotein (HRGP), andproduction of peroxidases and phytoalexins **(Van Loon, 1997; Kuc, 1995)**.

Also, **Gorlach *et al.* (1996)** and **Brisset *et al.* (2000)** reported that Bion® is the first product of the new generation of crop protectants (benzothiadiazoles) that has been shown to be effective through resistance activation.

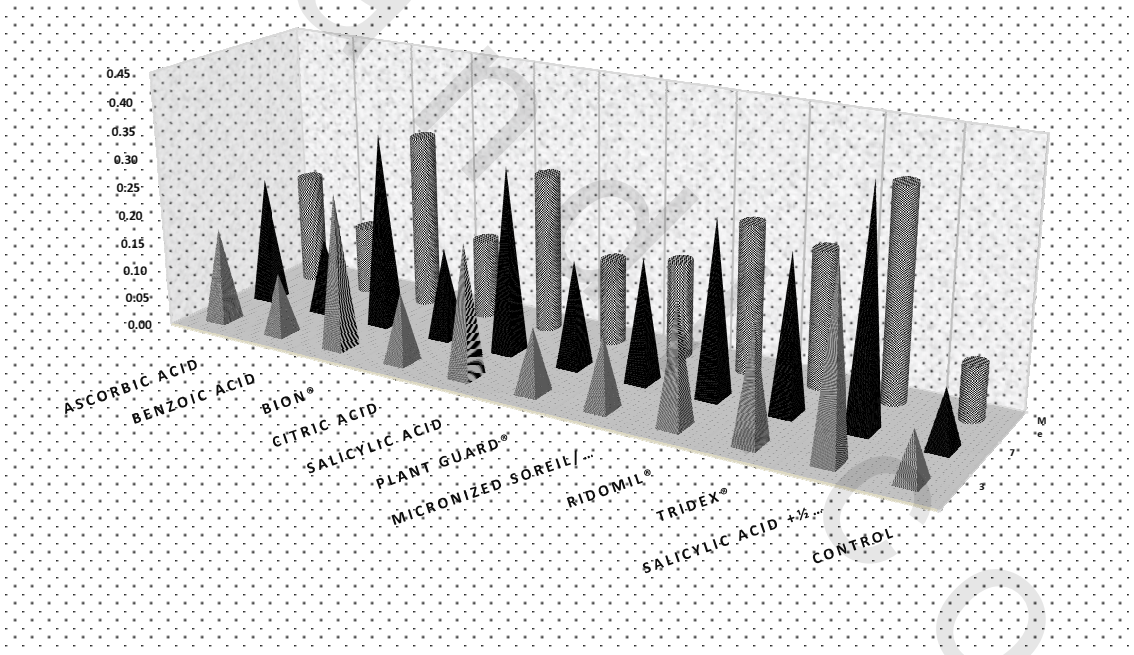

Figure (10): General mean and changes in peroxidase activity in tomato leaves treated with some antioxidants, bio-and chemical fungicides and inoculated with *A. solani*, at different periods following inoculation

4.2.2.1.2 Second season

Results in Table (18)and Figure (11) showed that pretreatment of plants with the tested compounds before inoculation with *A. solani* significantly increased peroxidase activity which has been determined through the inspection period. However, increasing rates, compared with peroxidase activity incontrol significantly differed according to the tested compound and time elapsed after inoculation.

Three days after inoculation, as for first season, significant increase in peroxidase activity was detected in that sequential treatment of salicylic acid with half recommended rate of Ridomil Gold®(3.91folds over that of control) followed byboth salicylic acid and Bion® whereas they recorded peroxidase activity reached to 3.02 and 2.86 folds of control.Those were followed by Ridomil Gold® giving 2.41 peroxidase activity folds of control. Meanwhile, there were no significant differences betweenTridex®, ascorbic acid, Plant guard® and Micronized soreil/ Samark®which gave peroxidase activity calculated by 2.25, 2.49, 2.18 and 2.13, respectivelyfolds over that of control.

After sevendays of inoculation, combination of salicylic acid with half recommended rate of Ridomil Gold®exhibited the highest values of peroxidase activity (3.59 folds over that of control). Again, salicylic acid ranked the 1st in peroxidase activity among the other evaluated antioxidants (2.82 folds of control), Bion® came in the 2nd rank giving 2.72 folds of control. However, there were insignificant differences between Ridomil Gold® and Tridex®, where they came in the 4th rank giving 2.66 and 2.53 foldsover the control, respectively. Citric acid and benzoic acid showed less peroxidase activity compared with the other tested compounds.

Generally, it was evident that combination of salicylic acid with half recommended rate of Ridomil Gold®exhibited the highest mean of peroxidase activity throughout all the tested intervals after inoculation compared with other treatments. Moreover, salicylic acid came second in inducing peroxidase activity, then came Bion® in the 3rd rank.

Therefore, the results are in agreement with those of**El-Khallal (2007)** and **Mandal *et al.* (2009)**, since they reported that the activity of peroxidase was sharply increased in response to foliar spray of salicylic acid.

Table (18): Effect of some antioxidants, bio-and chemical fungicides on the peroxidase (PO) activities in tomato leaves inoculated with *A. solani* at different periods following inoculation (second season).

Treatment	Enzyme activity (units/mg protein)** Time after inoculation (days)		Mean
	3	7	
Antioxidants			
Ascorbic acid	0.229^{de}*	0.243^{de}	0.236
Benzoic acid	0.168^{f}	0.199^{e}	0.183
Bion®	0.297^{bc}	0.323^{bc}	0.310
Citric acid	0.182^{ef}	0.212^{e}	0.197
Salicylic acid	0.314^{b}	0.336^{b}	0.325
Bio-and chemical fungicides			
Plant guard®	0.227^{de}	0.256^{cde}	0.242
Micronized soreil/ Samark®	0.222^{de}	0.272^{be}	0.247
Ridomil Gold®	0.251^{cd}	0.317^{bcd}	0.284
Tridex®	0.234^{de}	0.302^{bcd}	0.268
Sequential treatment			
Salicylic acid +½ Ridomil Gold®	0.407^{a}	0.427^{a}	0.417
Control	0.104^{g}	0.119^{f}	0.094
LSD (0.05)	0.052	0.068	-

*Values followed by the same letter(s) within each column are not significantly different.

** Each value reported is the average of three replicates.

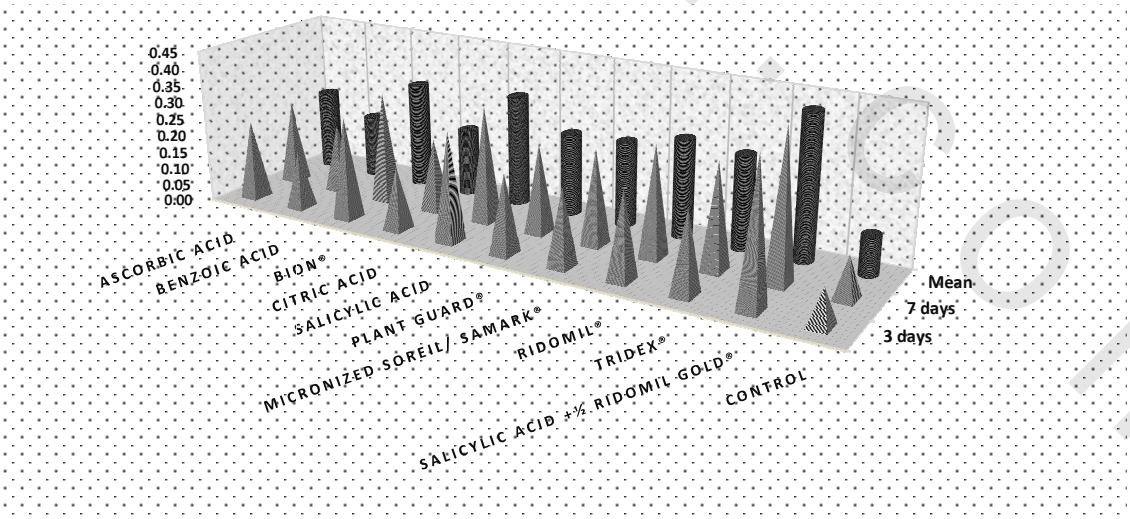

Figure (11): General mean and changes in peroxidase activity in tomato leaves treated with some antioxidants, bio-and chemical fungicides and inoculated with *A. solani*, at different periods following inoculation

4.2.2.2 Polyphenol oxidase activity:

The changes in activities of polyphenol oxidase were monitored on 3 and 7 days after challenge inoculation of *A. solani*. Plants were pre-treated with the tested antioxidants, bio-and chemical fungicides before inoculation with *A. solani*. Results are presented in the following section:

4.2.2.2.1 First season

Data presented in Table (19) and Figure (12) showed that most the treatments increased the activity of polyphenol oxidase enzyme compared with control. Increasing rates differed according to the tested treatment and time after inoculation with *A. solani*.

A significant increase in polyphenol oxidase activity was detected in plants pre-treated with sequential treatment salicylic acid with half recommended rate of Ridomil Gold®after 3 days post inoculation attaining highest PPO activities (0.065 units/mg protein), followed by salicylic acid, Bion® and ascorbic acid wherease they recorded polyphenol oxidase activity calculated by 0.054, 0.043 and 0.033 units/mg protein, respectively. There were no significant differences between citric acid and benzoic acid since they gave the least PPO activities of 0.011and 0.009 units/mg protein, respectively.

Gradual increase in PPO activity was observed after seven days post inoculation with *A. solani*. Pretreatment with the antioxidants salicylic acid, Bion® and ascorbic acid or chemical fungicides Ridomil Gold® and Tridex® significantly induced an increase of PPO activity compared with control, attaining (0.244, 0.240, 0.170, 0.162 and 0.092 units/mg protein). While treatment with salicylic acid with half recommended rate of Ridomil Gold® resulted in the highest PPO activities (0.311 units/mg protein) after seven days of inoculation.

The general mean of PPO activity showed that salicylic acid with half recommended rate of Ridomil Gold® recorded the highest mean of PPO activity which calculated by 0.188 units, followed by salicylic acid (0.149 U), Bion® (0.141 U) and ascorbic acid (0.102 U), while the least mean was recorded in benzoic acid (0.014 U).

4.2.2.2.2 Second season

Perusal data in Table 20 and Figure 13 revealed that PPO activities were limited throughout all the treatments. However, a little of significant differences were detected among most of them.

Treatment with salicylic acid with half recommended rate of Ridomil Gold® resulted in the highest PPO enzyme activity increase after three days post inoculation, attaining maximum values (0.078units/mg protein), insignificantly followed by salicylic acid that gave PPO activity reached to 0.055 units/mg protein. Moreover, no significant differences were recorded among Bion®, ascorbic acid and Ridomil Gold® which gave 0.044, 0.043 and 0.036units/mg protein, respectively.

After seven days post-inoculation, there were no significant differences between salicylic acid with half recommended rate of Ridomil Gold® and salicylic acid as separate since both showed the highest PPO activities among the tested treatments inducing 0.227 and 0.156units/mg protein, respectively.

Table (19): Effect of some antioxidants, bio-and chemical fungicides on the Polyphenol oxidase (PPO) activities in tomato leaves inoculated with *A. solani* at different periods following inoculation (first season).

Treatment	Enzyme activity (units/mg protein)*		Mean
	Time after inoculation (days)		
	3	7	
Antioxidants			
Ascorbic acid	0.033^{d}	0.170^{c}	0.102
Benzoic acid	0.009^{fg}	0.019^{e}	0.014
Bion®	0.043^{c}	0.240^{b}	0.141
Citric acid	0.011^{fg}	0.025^{de}	0.018
Salicylic acid	0.054^{b}	0.244^{b}	0.149
Bio-and chemical fungicides			
Plant guard®	0.017^{ef}	0.076^{de}	0.046
Micronized soreil/ Samark®	0.021^{e}	0.054^{de}	0.037
Ridomil Gold®	0.024^{de}	0.162^{c}	0.093
Tridex®	0.023^{e}	0.092^{d}	0.058
Sequential treatment			
Salicylic acid +½ Ridomil Gold®	0.065^{a}	0.311^{a}	0.188
Control	0.007^{g}	0.008^{e}	0.008
LSD (0.05)	0.009	0.065	-

Values followed by the same letter(s) within each column don't differ significantly.
* Each value reported is the average of three replicates.

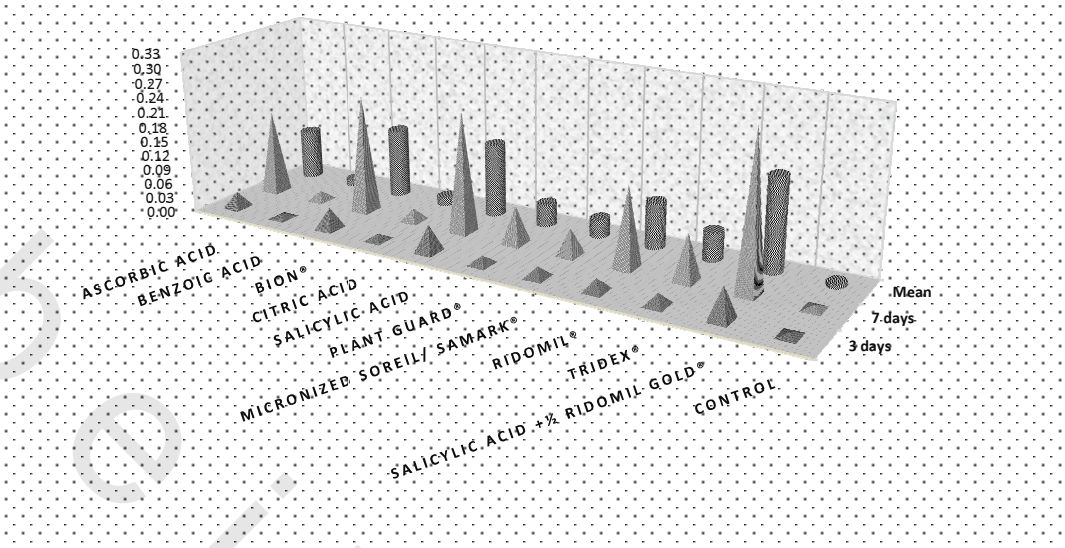

Figure (12): General mean and changes in polyphenol oxidase activity in tomato leaves treated with some antioxidants, bio-and chemical fungicides and inoculated with *A. solani*, at different periods following inoculation

Polyphenol oxidase activity as the general mean showed that salicylic acid with half recommended rate of Ridomil Gold® was more efficient in inducing PPO activities in tomato leaves inoculated with *A. solani* than the other tested treatments recorded 0.152 units, followed by salicylic acid (0.105 U), Bion® came in the 3rd order giving 0.094 U, ascorbic acid was in the 4th rank recording 0.086 U, while the least effective one was benzoic acid giving 0.012 U.

Data showed that treatment of tomato seedlings with salicylic acid with half recommended rate of Ridomil Gold® or treatment of salicylic acid as separateresulted in significant increase in the activity of polyphenol oxidases. These results were in accordance with the finding of**Ahmed (2010)**, who concluded that combination treatments (Mancozeb plus and ascorbic acid in the first season as sequential treatment or Equation pro and salicylic acid as mixing treatment) significantly increased the activity of polyphenol oxidase (PPO) in potato leaves inoculated with *A. solani*.

Table (20): Effect of some antioxidants, bio-and chemical fungicides on the Polyphenol oxidase (PPO) activities in tomato leaves inoculated with *A. solani* at different periods following inoculation (second season).

Treatment	Enzyme activity (units/mg protein)* Time after inoculation (days)		Mean
	3	7	
Antioxidants			
Ascorbic acid	0.043^{bc}	0.130^{b}	0.086
Benzoic acid	0.003^{d}	0.021^{d}	0.012

Bion®	0.044^{bc}	0.144^{b}	0.094
Citric acid	0.006^{d}	0.026^{cd}	0.016
Salicylic acid	0.055^{ab}	0.156^{ab}	0.105
Bio-and chemical fungicides			
Plant guard®	0.019^{cd}	0.041^{cd}	0.030
Micronized soreil/ Samark®	0.014^{cd}	0.034^{cd}	0.024
Ridomil Gold®	0.036^{bc}	0.108^{bc}	0.072
Tridex®	0.024^{cd}	0.072^{bcd}	0.048
Sequential treatment			
Salicylic acid +½ Ridomil Gold®	0.078^{a}	0.227^{a}	0.152
Control	0.003^{d}	0.011^{d}	0.007
LSD (0.05)	0.027	0.077	-

Values followed by the same letter(s) within each column don't differ significantly.
* Each value reported is the average of three replicates.

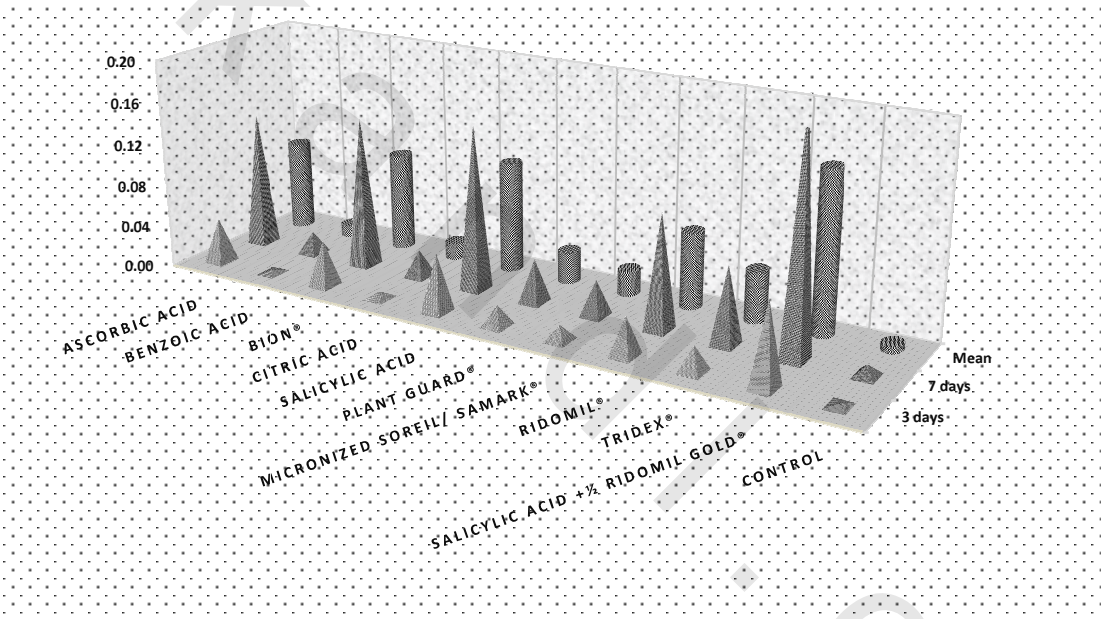

Figure (13): General mean and changes in polyphenol oxidase activity in tomato leaves treated with some antioxidants, bio-and chemical fungicides and inoculated with *A. solani*, at different periods following inoculation

4.2.3 Determination of selected fungicides residue in tomato fruits

The two selected fungicides for residue analysis were Vitavax-200® (carboxin + thiram) and Ridomil Gold MZ® 68% (mefenoxam + mancozeb) since they both exhibited high efficiency against the root rot and early blight disease incited by *F. solani* and *A. solani*, respectively.

According to the Agricultural Pesticides Committee **(APC)**, Ministry of Agriculture and Land Reclamation in Egypt, Pre-harvest interval (PHI) of Vitavax-200®

for root rot in tomato seedlings is still not available (NA) while the Pre-harvest interval (PHI) ofRidomil Gold® is 11 days for early blight in tomato.

4.2.3.1 Residue of Vitavax-200® in tomato fruits

Concerning health hazards, the maximum residue limits (MRL) for each of Vitavax-200® active ingredients (carboxin and thiram) was 0.1 mg/kg on tomato fruits established by European Union pesticide database **(EU, 2013)**.

Dissipation pattern of carboxin (as one component of Vitavax-200®) residue in tomato fruits under greenhouse condition is shown in Table (21) and Figure (14). Noticeably, the detected residue levels of carboxin indicated that the residue levels were inversely proportional to time (in days) post-treatment. The results showed that the deposits concentration of carboxin after three days post-treatment were 0.13 mg/kg (ppm), then sharply decreased to 0.01 mg/kg after ten days of application revealing 92.3 % loss.

On the other hand, the residue value of thiram(as the second component of Vitavax-200®) was 0.37 mg/kg after three days, then it decreased down to reach 0.12 mg/kg after ten days from application with a loss of 67.6% but still slightly higher than the recommended maximum residue limits(MRL).

According to the detected residues of each of carboxin and thiram (thecompenents of Vitavax-200®), it could be concluded that carboxin residue level highly declined to a fading residue level after 10 days post-treatment, while thiram residue level persisted slightly above its MRL. So, it is highly recommended that the pre-harvest interval (PHI) of Vitavax-200® treatment on tomato must be more than 10 days to be safely consumed.

4.2.3.2 Residue of Ridomil Gold® in tomato fruits

Data in Table (21) and Figure (14) indicate the determined residue values of mefenoxam (Metalaxyl-M)and mancozeb (the compenents of Ridomil Gold®) in tomato fruits at two different intervals (3 and 10 days) after application. The European Union MRL_S for mefenoxam and mancozeb in tomato are 0.2 and 3 mg/kg, respectively

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The results showed that mefenoxam (as the first or main component of Ridomil Gold®) dissipated rapidly after application where the residue values of mefenoxam after three days post treatment was 0.07 mg/kg. The degradation continued to decrease down to 0.02mg/kg with loss of 71.4% after ten days from application. The estimated residue levels of mefenoxam after 3 and/10 days post treatment were below its MRL (0.2 mg/kg) of European Union in tomato.

The results are in highly agreement with **Milgroom and Fry (1988)** who observed that metalaxylresidues decreased rapidly in the first two days after application. Meanwhile, **Malhat (2012)** reported that metalaxyl disappears rapidly in tomato fruit and field under natural conditions and exhibited first-order kinetic dissipation.

The deposits of mancozeb (the second component of Ridomil Gold®)in tomato fruits was found to be 0.36mg/kg after three days from application by the recommended dosage. Up to 10 days post-treatment, mancozeb showed a high persistence where the degradation was quite slow since the amount was only 0.35 mg/kg after ten days from application showing a weak loss or degredation of only 2.8%.

Table (21): Residue of fungicides detected in tomato fruits under greenhouse condition

Fungicide	Active ingredient	Detected residues (mg/kg) Time after treatment (days)		Loss (%)	Persistence (%)	MRL* (mg/kg)
		3	10			
Vitavax-200®	Carboxin	0.13	0.01	92.3	7.7	0.1
	Thiram	0.37	0.12	67.6	32.4	0.1
Ridomil Gold®	Mefenoxam	0.07	0.02	71.4	28.6	0.2
	Mancozeb	0.36	0.35	2.8	97.2	3

* MRL=Maximum residue limit **(EU, 2013)**

Similar results were obtained by **Rani *et al.* (2013)**, they reported that the dissipation of metalaxyl was much higher with the time than the dissipation of mancozeb. Also, they found that the amount of metalaxyl residue reached to be 0.06 mg/kg after one days of spray and 0.02 mg/kg after three days. Moreover, they concluded that Ridomil MZ at 0.25 % and 0.50 % concentration were lower than the MRL value at 0 day.

Whether after 3 or 10 days post-treatment, the detected residue values of mancozeb were below its European Union MRL values in tomato. So, the tomato fruits could be safely consumed during this period.

It can thus be concluded that tomato fruits could be safely consumed after three days of application according to the recommended maximum residue limit (MRL) for each of Ridomil Gold® active ingredients (mefenoxam and mancozeb). These results were in harmony with those obtained by **Rani *et al.* (2013)**, **Ahuja and Pande (2004)**. Furthermore, the residues of Ridomil Gold® are safer than Vitavax-200® on tomatoes if they were consumed after 3 days of fungicide application.

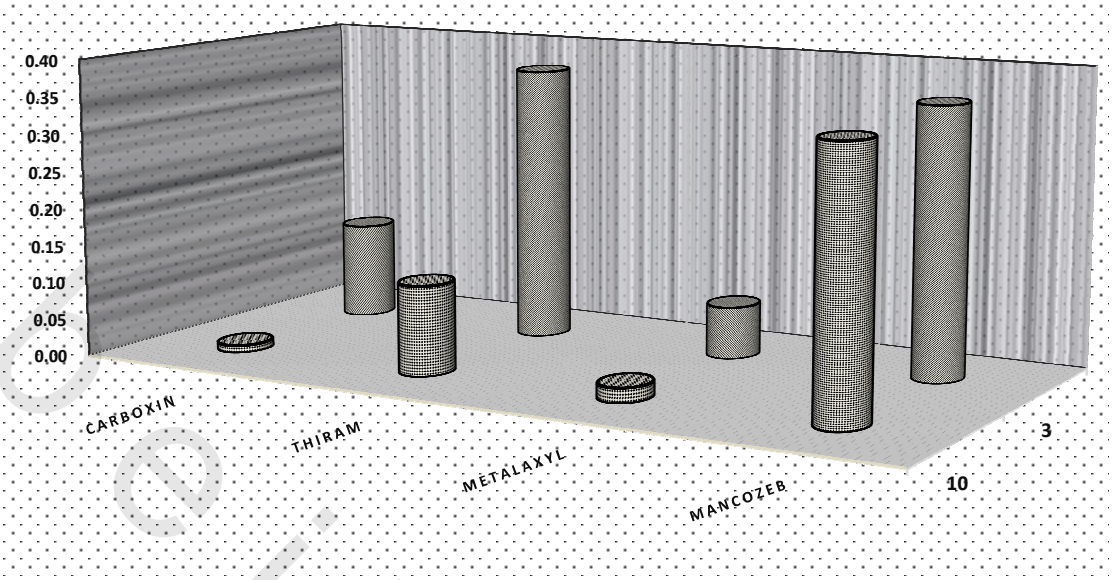

Figure (14): Dissipation of tested fungicides in tomato fruits at different periods post treatment

4.3.Conclusion

Because high disease pressure and high crop value require frequent applications of chemical pesticides, significant environmental pollution and the spread of resistant pathogen strains are among the main problems encountered the farmers. This situation has prompted the search for alternative of fungicides that could be efficient either for conventional disease management programs or for integration with other methods.

A new promising alternative to control fungal diseases is to make use of the plant's own defence response, which can confer a high level of durable protection for the plants through systemic acquired resistance (SAR). This can be achieved by the use of biological and chemical inducers that in turn can elicit substantial increases in the expression of defence genes in the plants **(Hammerschmidt et al. 2001; Conrath et al. 2001)**.

The present investigation demonstrates that some antioxidants and a biofungicide have the potential to inhibit the growth of two tested pathogenic fungi (*A. solani and F. solani*). In addition, the use of antioxidants in a sequential treatment with the fungicides could be suggested and recommended to be applied especially for freshly plant products, in order to produce high quality products due to disease control and prevention with no or low fungicide residues.

This study presents a new approach for the management control of tomato fungal diseases depends on the activation of the defense of the plant against pathogenby using sequential treatment salicylic acid as inducer and suppression of the fungal pathogenicity by applying half recommended rate of fungicides (Ridomil Gold® or Vitavax-200®) as a chemical control .