CHAPTER 5

SUMMARY

5. SUMMARY

The objective of the present investigation was to evaluate the inhibitory activity of certain antioxidants to be used as fungicides alternatives on the growth of two fungi,*Alternaria solani* and *Fusarium solaniin vitro*. The evaluated antioxidants were ascorbic, benzoic, citric and salicylic acids and the plant activator Bion® 50 WG. Also a biofungicide(Plant guard®) and certain chemical fungicides, namelyRidomil gold MZ® 68%, Micronized soreil/Samark® 70% WP (Sulfur), Tridex® 80% and Vitavax-200® were also evaluated. Also the aim of the studying was to evaluate the efficiency of the alternation of spraying theprevious fungicides plus prior antioxidant as separate orsequential treatments for management of some tomato diseases (early blight and root rot) *in vivo*. Moreover, to identify some associated biochemical changes which lead to induction of plant defense against the pathogen.

5.1. *In vitro* experiment

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.

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

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

It is clear that the linear growth of *A. solani*decreased significantly as the concentrations of all treatments increased.Salicylic acid was (100%) the most efficient tested compound in this regard, as it caused complete reduction of the fungal growth at 150 ppm (with EC_{50} 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®).

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).

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

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. 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 the evaluated 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®).

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 thoseEC_{50} values of Micronized soreil/Samark® and Plant guard®.

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

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

5.1.2.1 The effect of some antioxidants on the mycelial growth of *Fusarium solani*

The tested fungi showed more sensitivityagainst 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).

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®.

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

Results showed that Vitavax-200® was effective in inhibiting the growth of mycelia 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. 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.

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®.

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

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

5.2.1 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.

5.2.1.1 First season

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

It was clear that all the evaluated treatments reduced 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%) and Micronized soreil/ Samark® (63.6%). Meanwhile, salicylic acid was the most effective tested antioxidant (51.5%) followed by the biocide Bion® (45.6%).

The mean of disease severity (10% and 14.58%) that achieved by using Vitavax-200® alone and after the sequential treatment of salicylic acid+½ Vitavax-200®, respectively show 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 their sequential treatments with antioxidants and tested antioxidants alone or biocide (Plant guard®).

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

Data 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.

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

5.2.1.2 Second season

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

Results exhibited 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 treatmt 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).

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

Data 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 achievedin 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%).

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.

5.2.2 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.

5.2.2.1 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:

5.2.2.1.1 First season

Data 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 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 by salicylic acid (3.39 and 3.22 folds of control, respectively). The present results revealed that Ridomil Gold® was the most effective fungicide for 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.

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).

5.2.2.1.2 Second season

Results 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 in control 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® (3.91 folds over that of control) followed by both 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 between Tridex®, ascorbic acid, Plant guard® and Micronized soreil/ Samark® which gave peroxidase activity calculated by 2.25, 2.49, 2.18 and 2.13, respectively folds over that of control.

After seven days 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 inoculationcompared with other treatments. Moreover, salicylic acid came second in inducing peroxidase activity, then came Bion® in the 3rd rank.

5.2.2.2 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 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 0.011 and 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

Data 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 (0.078 units/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 0.036 units/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 0.156 units/mg protein, respectively.

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 separate resulted in significant increase in the activity of polyphenol oxidases.

5.2.3 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 diseases incited by *F. solani* and *A. solani*, respectively.

5.2.3.1 Residue of Vitavax-200® in tomato fruits

The detected residue levels of carboxin indicated (as one component of Vitavax-200®) 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 (the compenents of Vitavax-200®), it could be concluded that carboxin residue level was 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

The results showed that mefenoxam (as thefirst 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 deposits of mancozeb (the second component of Ridonil 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%.

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), and the residues of Ridomil Gold® are more safer than Vitavax-200® on tomatoes if they were consumed after 3 days of fungicide application.