

Induction of Pathogenesis-Related (PR) Proteins as A plant Defense Mechanism for Controlling the Cotton Whitefly *Bemisia tabaci*

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ABSTRACT

The activities of four pathogenesis-related (PR) proteins (*Beta*-1, 3-glucanase, chitinase, polyphenol oxidase and peroxidase) were determined in both *Bemisia tabaci* infested and non-infested tomato plants. *Beta*-1, 3-glucanase activity in non-infested and 24, 48, 72 and 96 h infested tomato plants with *B. tabaci*, were spectrophotometrically determined. The results show that the activity of the enzyme was significantly increased in comparison to control. The activity of β -1,3-glucanases was slightly pronounced one day after infestation and reached maximum level after three days (3.3-folds than control). The accumulation of chitinase started to increase with time one day after infestation (1.2 folds over control). Chitinase specific activity increased significantly after 48 h of whitefly feeding and continued in elevation with time until it reached the maximum in the fourth day of feeding. In all treatments, polyphenol oxidase activity after 24, 48, 72 or 96 h of whitefly infestation increased more than control. The activity increased substantially after 24 h of feeding of whitefly (4.3 folds than control). The maximum rate of activity of peroxidase was exhibited after 48 h exposure to whitefly that it had recorded 1.8 folds greater than 0-time and followed by 24 h whitefly-infested plants with 1.6-folds increase in peroxidase activity compared to control then 72 h and 96 h after infestation (1.35 and 1.27 folds greater than control respectively). The PR proteins play an important defensive role against whiteflies. *Pseudomonas chlororaphis* (non-pathogenic bacteria) reduced the mean numbers of whitefly adults/cm² by about 36.5%. These results provide clear evidence that tomato plants resistance is based on a variety of defense systems against several pests. The treatment of tomato plants with *Pseudomonas* spp. caused rapid trigger systemic defenses against herbivores and pathogens.

Keywords: *Bemisia tabaci*, Pathogenesis-Related(PR) Proteins, Defense, *Pseudomonas* spp.

INTRODUCTION

Many investigations revealed that one of the plant defense tools, that help in protecting them from pathogens or insects attack is lifting concentrations of secondary compounds or assortment of host proteins, many of which are termed PR proteins (Carr and Klessig, 1989; Bol *et al.*, 1990; Linthorst, 1991). Plants react to different natural environmental challenges with diverse biochemical changes. For case, they commonly hoist concentrations of secondary compounds in reaction to pathogen infection or insect herbivory (Karban and Baldwin, 1997). Other plant reactions incorporate lignification production of PR proteins (Vance *et al.*, 1980; Diaz and Merino, 1998). The first PR-protein was discovered in 1970. Since 1970s, a number of PR proteins have been recognized in various plant species (Liu and Xue, 2006). PR proteins are thought to play a part in plant defense (Van Loon, 1999). The term PR proteins was coined in 1980 for proteins produced by the host plant, but induced only by pathogens, pests, or other stress-related circumstances (Antoniw and White, 1980). So, any host protein actuated by any type of infectious agent or comparable condition would be known as PR protein. The PR proteins are known to be communicated in reaction to various external

stimuli, counting pathogens, injuring, chemical elicitors and hormones (Brederode *et al.*, 1991; Inbar *et al.*, 1998). PR proteins are belonged to many families exemplifying β -1,3-glucanases, chitinases, thaumatin, proteinases, peroxidases, ribonuclease-like proteins, or proteins of unknown function (Van Loon, 1999), and it differs in its accumulation among depending on the kind of stimulant and the host plant diversity. In spite of a ceaselessly developing information about the structure of PR proteins and how they work, their contribution in plant defense is not completely caught on. In tomato plants, whiteflies alone have been shown to induce the production of specific PR proteins (β -1,3-glucanase, chitinase, P2, and P4) to accumulate (Mayer *et al.*, 1996). In tomato plants, it is inconspicuous how the level of PR protein aggregation as a reaction of whitefly feeding compares to accumulation when whiteflies acquired a plant virus.

On the other hand, some plant growth promoting microorganisms could promote protective activity and encourage plant resistance against soil borne pathogens (Whipps, 2001). useful micro-organisms that promote plant validity, through the raise of plant resistance against biotic stresses include bacteria, such as *Pseudomonas* spp. Also, direct connection with plant pathogens, bio agents announced to induce systemic resistance

in plants (Srivastava *et al.*, 2010). Ramamoorthy *et al.* (2002) reviewed that *Pseudomonas fluorescens* was found to protect tomato plants from wilt disease. Activities of peroxidase and polyphenol oxidase spread in bacterized tomato root tissues exposed to the pathogen at one day after pathogen challenge and obtain the peak at the 4th day. So also, β -1,3 glucanase and chitinase were produced at higher levels at 3-5 days of inoculation in bacterized plants. During the present study, we aimed to develop a plan of an IPM program for *B. tabaci* under greenhouse conditions.

MATERIALS AND METHODS

1- Laboratory culture of the cotton whitefly *Bemisia tabaci*:

The cotton whitefly *Bemisia tabaci* (Gennadius) (Order: Hemiptera, Aleyrodidae) was reared on healthy tomato plants. The mother colony was established by late Prof. Mourad El-Helaly and has been reared under greenhouse conditions at Department of Applied Entomology since 1960s. In 2000, the mother colony of whiteflies was re-identified by Dr. Jon Martin, Insect/Plant Division, Department of Entomology, The Natural History Museum, UK.

2- Induction of PR proteins in tomato plants.

2.1- Experimental design:

Tomato seeds were sown in trays, then transferred singly to pots. When the seedlings had 7 leaves, 15 tomato plants were arranged in a completely randomized design in five groups. Each three plants represent a treatment. The first group (control plants) was kept in an insect-proof greenhouse at 30±5°C, 65±5 RH and under natural light conditions. Tomato plants of the other four groups were covered by glass lantern and each plant was exposed to whitefly at the rate of 50 adults for 24, 48, 72 and 96 hours.

2.2- Total protein determination:

Total protein content was determined by the method of Bradford (1976), using bovine serum albumin (BSA) as standard. Fifty mg of commassie blue G-250 were dissolved in 25 ml ethanol 95 % then added to 50 ml O-phosphoric acid 85%. The mixture was diluted to 500 ml with H₂O and filtered through filter paper (whatman no.1). One tenth (0.1) ml of plant extract samples were added to 5 ml of reaction media. The tubes were well shaken using a vortex and were allowed to stand for 10 min. at room temperature after which the developed color was measured at 595 nm.

2.3- Enzyme Extraction:

One gram of leaf samples was collected from both *B. tabaci* infested and non-infested plants, and crushed with pre-cooled mortar and pestle in 5 ml of 0.05 M sodium acetate buffer (pH 5.0) in the presence of 0.3 gm polyvinyl pyrrolidone (PVP) and centrifuged at 16000g for 15 min at 4°C. The

supernatant was used in enzyme assay as crude enzyme extract.

2.4- Assay of β -1,3-glucanase activity:

β -1,3-glucanase activity was colorimetrically assayed by the laminaria dinitrosalicylate method (Saikia *et al.*, 2005). The reaction mixture consisted of 62.5 μ L of 0.04% Laminarin and 62.5 μ L of enzyme extract. The reaction was carried out at 40°C for 10 min. The reaction was stopped by adding 375 μ L of dinitrosalicylic acid and heated for 5 min. on boiling water, Eppendorf were shaken using a vortex and its absorbance was measured at 500 nm. The enzyme activity was expressed as Ug⁻¹ fresh weight (quantity of enzyme that liberates one μ M glucose per a minute under experimental conditions).

2.5- Assay of chitinase activity:

To determine the chitinase activity in both *B. tabaci* infested and non-infested plants, one ml of the enzyme extract was added to one ml of 1% colloidal chitin in 0.05 M citrate phosphate buffer (pH 6.6) and mixed by shaking in test tube then kept in water shaking bath at 37°C for 75 min. The reaction was then stopped by adding one ml of dinitrosalicylic acid (Monreal and Reese, 1969) and heated for 5 min., then cooled and centrifuged at 3000 rpm for 5 min. to get rid of chitin before measuring O.D. at 540 nm. Chitinase activity was defined as Ug⁻¹ fresh weight (μ M N-Acetylglucosamine liberated per a minute).

2.6- Assay of polyphenol oxidase activity:

Polyphenol oxidase activity was determined according to Mayer *et al.* (1965). Two hundred μ L of the enzyme extract was added to 1.5 ml of 0.1 M Phosphate buffer (pH 7). To start the reaction 200 μ L of 0.01 M catechole in phosphate buffer (pH 7) was added and the activity was expressed as change in absorbance at 495 nm/min.

2.7- Assay of peroxidase activity:

The reaction mixture consisted of 0.5 ml of enzyme extract and 0.5 ml of 1% H₂O₂. 1.5 ml of 0.05 ml pyrogallol was added to every sample separately and incubated at room temperature. The enzyme activity was expressed as the change in absorbance at 420 nm at 1 min. intervals (Hammerschmidt *et al.*, 1982).

2.8- Statistical analysis:

Statistical analysis of the obtained data and all the probable comparison combination were analyzed in a Randomized Complete Blocked Design (RCBD) by using SAS (1997) at probability level of 0.05.

3- Using of non-pathogenic bacteria (*Pseudomonas* spp.) to induce PR proteins in tomato plants:

3.1- Preparation of bacterial culture:

Isolates of *P. fluorescens* and *P. chlororaphis* were kindly provided by Dr. Nader Abd Elwahab, Associate Professor of Plant Pathology, Department

of Plant Pathology, Faculty of Agriculture, Alexandria University. Isolates were grown in King's B agar plates for 48 h at 28°C. The bacterial isolates were multiplied in King's B broth for 48 h at 28°C in 500 ml flasks suspended for two days and turbidity was adjusted calorimetrically to approximately 10^9 Colony Forming Unit CFU/ml. Fifty ml of bacterial suspension were applied to each pot.

3.2- Experimental design:

To assess the ability of soil born rhizosphere bacteria to induce PR proteins in tomato plants and the possibility of utilization this phenomenon as defense mechanism against whitefly, tomato plants bearing each 7 leaves were divided into four groups. The first group was healthy tomato plants reared in a whitefly-free greenhouse. The second group was tomato plants which were artificially wounded daily for a week and reared in a whitefly-free greenhouse. The third group was tomato plants infested with *B. tabaci* adults for a week. The fourth group was tomato plants that were infected with *P. chlororaphis* or *P. fluorescens* and reared in a whitefly-free greenhouse. Each treatment was replicated five times. Tomato plants of all treatments were aligned in a completely randomized design in greenhouse at 30 ± 5 C°, 65 ± 5 RH under natural light conditions. Whitefly adults were collected from mother colony and released at the center of the greenhouse. Numbers of adult/cm² on each plant were daily recorded. The experiment was repeated for four successive days.

3.3- Statistical analysis:

Obtained data were analyzed in factorial (two factors) design using ANOVA (SAS, 1997) and the mean numbers of *B. tabaci* adults/cm² were compared by using the least significant difference test (LSD) at probability level of 0.05.

RESULTS

1- The effect of *B. tabaci* infestation on β -1, 3-glucanase activity in tomato plants:

Table 1: Time-course activity of β -1,3-glucanase and protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.

Parameters	Time in hours				
	0-time	24	48	72	96
Glucanase activity*	15.96±1.04 E	23.05±0.41 D	31.96±0.38 C	53.37±0.30 A	37.69±0.66 B
Protein content**	0.6143±0.02 C	0.7375±0.01 B	0.7964±0.01 B	0.8393±0.02 AB	0.9161±0.06 A
Specific activity***	25.98±1.32 C	31.29±1.15 C	40.15±0.88 B	63.63±1.12 A	41.51±3.03 B

Values are means \pm S.E of 3 replicas.

Means followed by the same letter(s) within the same row are not significantly different at 0.05 probability level.

* The enzyme activity was expressed as Ug⁻¹ fresh weight (quantity of enzyme that liberates one μ M glucose per a minute under experimental conditions).

** The protein content was expressed as mg g⁻¹ fresh weight.

*** Specific activity = enzyme activity \div protein content.

Beta-1,3-glucanase activities, specific activity and protein content for infested and non-infested leaf samples are illustrated in Table 1 and Figures 1, 2, 3 & 4. In all treatments, activities of β -1,3-glucanase significantly increased than control. Increase in β -1,3-glucanase activity was exhibited in plants after one day of infestation.

The activity of β -1,3-glucanase reached maximum level after three days (3.3 folds over control) and that was also observed 24 and 48 h. after infestation that the activity was 1.4 and 2 folds respectively (Figures, 1&4), then withdraw in the fourth day though it remained more active than control.

As shown in Table 1 and Figures 2 & 4, exposure of tomato plants to feeding by whitefly caused an increment in the leaf protein content in infested plants with time when compared to non-infested plants. The total protein content increased from 0.61 mg g⁻¹ fresh weight in control plants to 0.91 mg g⁻¹ fresh weight in 96 h. infested plants. Although there was increment in leaf protein content with time; there was no significant differences among days 1, 2, and 3 after infestation or between days 3 and 4. The increment in leaf protein content in infested plants suggested that there might be stimulation of some plant proteins occurring simultaneously, with the apparent induction of PR proteins.

Like the enzyme activity, specific activity of β -1,3-glucanase followed the same pattern (Table, 1 and Figures, 3 & 4); it reached maximum level after three days of infestation and it was significantly higher than any treatment, then it has retracted in the fourth day and kept more significantly higher than control. There were no significant differences in specific activity between plants that exposed to whitefly for 48 or 96 h. Also, no significant difference was observed between 24 h. whitefly-exposed plants and 0-time (control).

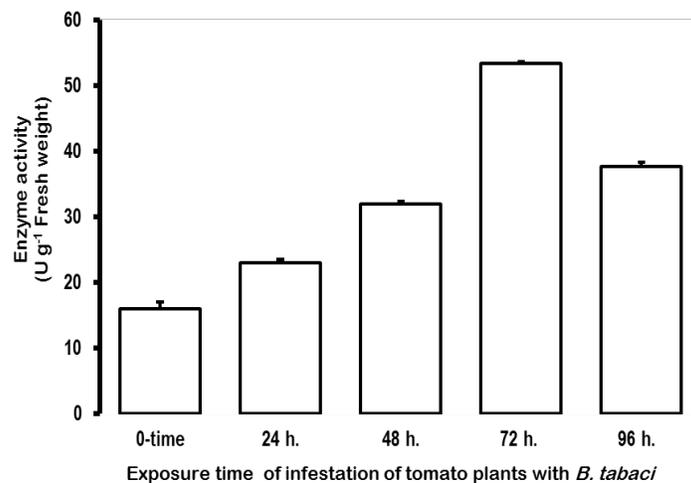


Figure 1: The effect of time-course on the enzyme activity of β -1,3-glucanase in the infested tomato plants with the cotton whitefly *B. tabaci*.

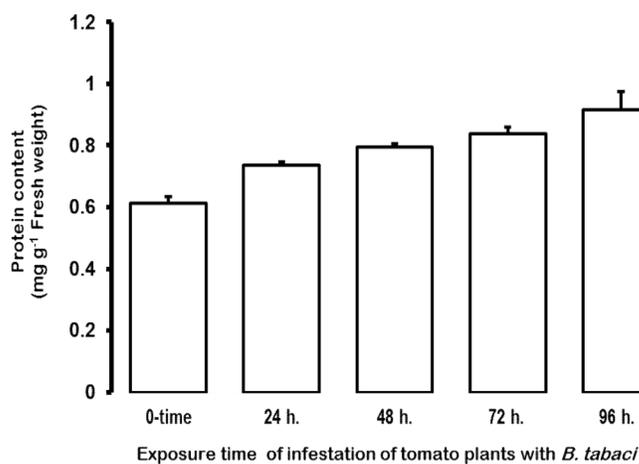


Figure 2: The effect of time-course on the leaf protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.

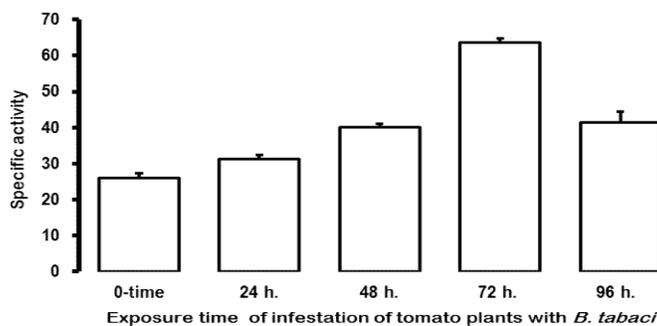


Figure 3: The effect of time-course on the specific activity of β -1,3-glucanase in infested tomato plants with the cotton whitefly *B. tabaci*.

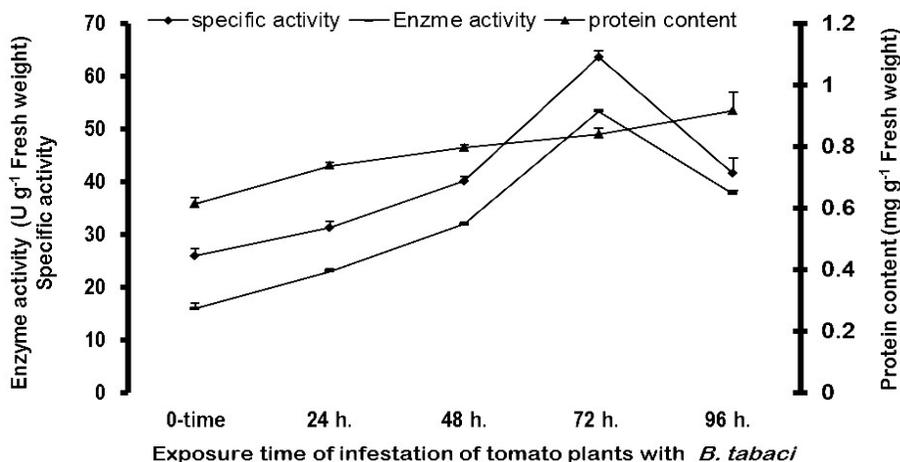


Figure 4: The effect of time-course on enzyme activity and the specific activity of β -1,3-glucanase, and on leaf protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.

2- The effect of *B. tabaci* infestation on chitinase in tomato plants:

Chitinase activity, specific activity and protein content which detected in whitefly-infested and non-infested leaf samples are shown in Table (2) and Figures (5 to 8).

The activity of chitinase of tomato leaves was found to be correlated with plant-whitefly interaction. The accumulation of chitinase started to increase with time one day after infestation (1.2

folds more than control). It had been recorded 0.006 U g⁻¹ fresh weight of 24 h. infested tomato leaf, then gradually increased after 48, 72 h. (3.6 and 4.3 folds increase compared to control, respectively) and continued to accumulate and reached the maximum increase of 0.033 U g⁻¹ fresh weight of tomato leaf (6.1 folds more than control) at the fourth day. Except the 24 h infested plants, in all the treatments, the activity of chitinase increased significantly ($p = 0.05$) than control (Figures, 5&8).

Table 2: Time-course activity of chitinase and protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.

Parameters	Time in hours				
	0-time	24	48	72	96
Chitinase activity	0.0054±3.18 D	0.0066±7.58 D	0.0197±9.01 C	0.0230±1.38 B	0.0330±8.65 A
Protein content	1.17±0.03 D	1.28±0.018 C	1.34±0.015 BC	1.39±0.023 AB	1.43±0.023 A
Specific activity	0.0046±3.38 C	0.0041±1.18 C	0.0146±6.67 B	0.016±1 B	0.023±1.46 A

Values are means± S.E of 3 replicas.

Means followed by the same letter(s) within the same row are not significantly different at 0.05 probability level.

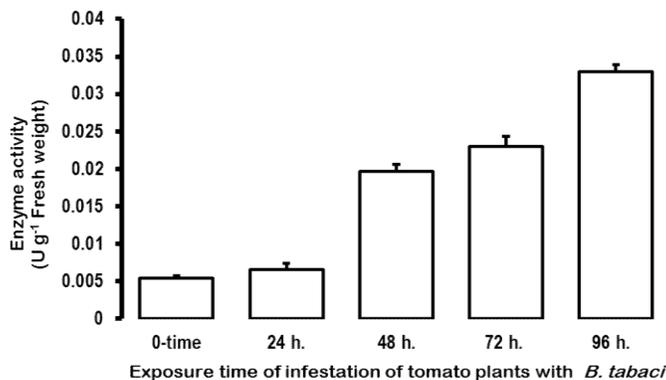
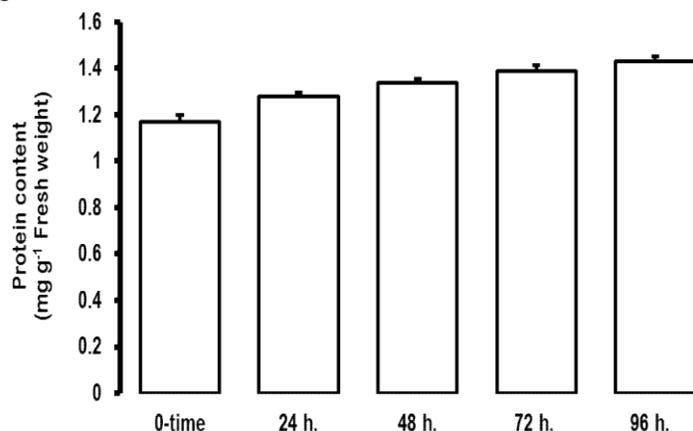


Figure 5: The effect of time-course on the enzyme activity of chitiase in the infested tomato plants with the cotton whitefly *B. tabaci*.

As shown in Table (2) and Figures (6 & 8), due to feeding by whitefly, total protein content in tomato leaf tissues was increased slowly at a steady pace. The total protein content was 1.28 ± 0.018 mg g⁻¹ fresh weights one day after infestation which was 1.09 folds more than control (1.17 ± 0.03 mg g⁻¹ fresh weight) (Fig.6). Protein content continued to climb by the time and reached the maximum (96 h after infestation; 1.43 ± 0.02 mg g⁻¹ fresh weight) but there were no significant differences between plants that exposed to whitefly for 96 h and 72 h. or between 72 h. and 48 h whitefly-infested plants (Fig. 6).

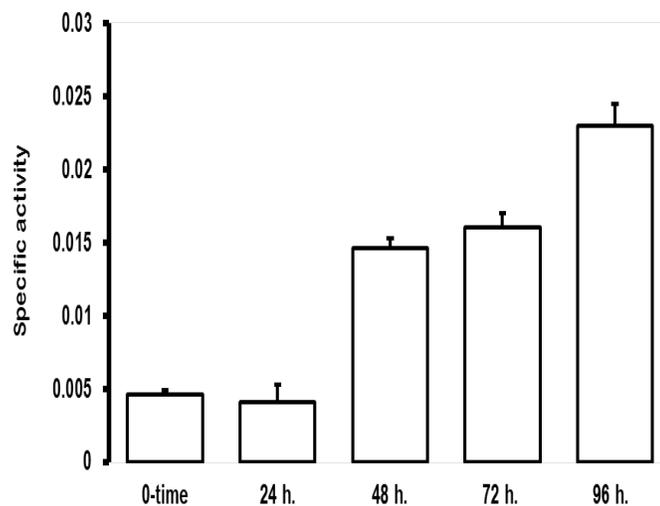
Such as chitinase activity, specific activity has the same trend. It increased significantly after 48 h. of whitefly feeding and continued in elevation with

time until it reached the maximum in the fourth day of feeding. Comparing to 0-time, specific activity of chitinase was significantly higher in plant exposed to whitefly for 48, 72 and 96 h. (Table 2 and Figures, 7 & 8); it was observed that specific activity of chitinase decreased after one day of infestation (0.0041) but there were no significant differences between plants that exposed to whitefly for 24 h and control, also there was no significant difference between plants that exposed to whitefly for 48 h and 72 h. in their specific activities (0.0146 and 0.0160 respectively). The chitinase specific activity of tomato plants exposed to whitefly for 96 h was significantly higher than others.



Exposure time of infestation of tomato plants with *B. tabaci*.

Figure 6: The effect of time-course on the leaf protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.



Exposure time of infestation of tomato plants with *B. tabaci*

Figure 7: The effect of time-course on the specific activity of chitinase in the infested tomato plants with the cotton whitefly *B. tabaci*.

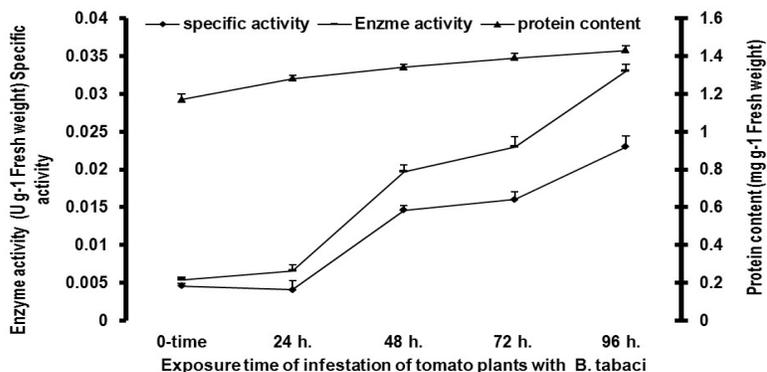


Figure 8: The effect of time-course on enzyme activity and the specific activity of chitinase, and on leaf protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.

3- The effect of *B. tabaci* infestation on polyphenol oxidase activity in tomato plants:

Polyphenol oxidase activity, specific activity and protein content for whitefly infested and non-infested tomato leaf samples are illustrated in Table (3) and Figures (9, 10 & 11). Table (3) showed that in all treatments, polyphenol oxidase activity after 24, 48, 72 or 96 h. of whitefly infestation increased more than 0-time. It was found that the activity increased substantially after 24 h. of feeding of whitefly (26 U g⁻¹ fresh weight = 4.3 folds more than control). Subsequently the enzyme activity retreated gradually after 48, 72 and 96 h. of infestation (2.7, 2.1 and 1.5 folds more than control respectively).

There were significant differences among all treatments in Polyphenol oxidase activity. No significant difference was observed in enzyme activities between 96 h whitefly-exposed plants and non-infested plants.

Specific activity of polyphenol oxidase had the same trend of the enzyme activity (Table, 3 and figures 10 & 11). It was found that specific activity reached the highest value after 24 h of feeding (20.26) and it was significantly higher than any exposure time, then it retracted significantly during the 2nd, 3rd and 4th days after infestation (12.23, 9.14 and 6.34, respectively).

Table 3: Time-course activity of polyphenol oxidase and protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.

Parameters	Time in hours				
	0-time	24	48	72	96
Polyphenol activity	6 ± 0.76 D	26 ± 0.87 A	16.33 ± 0.93 B	12.67 ± 0.88 C	9.17 ± 1 D
Protein content	1.17 ± 0.03 D	1.28 ± 0.018 C	1.34 ± 0.015 BC	1.39 ± 0.023 AB	1.43 ± 0.023 A
Specific activity	5.19 ± 0.72 D	20.26 ± 0.46 A	12.23 ± 0.79 B	9.14 ± 0.69 C	6.34 ± 0.7 D

Values are means ± S.E of 3 replicas.

Means followed by the same letter(s) within the same row are not significantly different at 0.05 probability level.

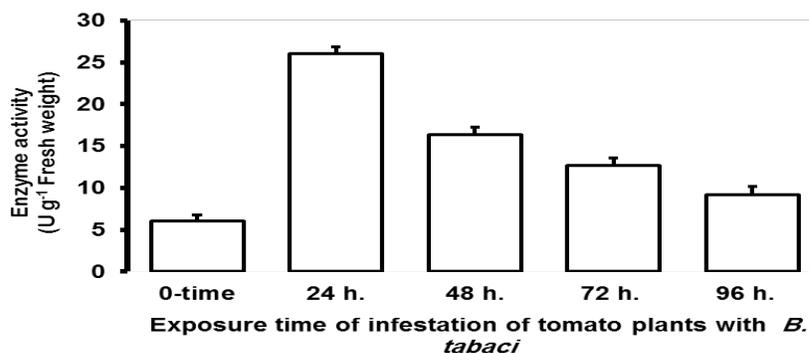


Figure 9: The effect of time-course on the enzyme activity of polyphenol oxidase in the infested tomato plants with the cotton whitefly *B. tabaci*.

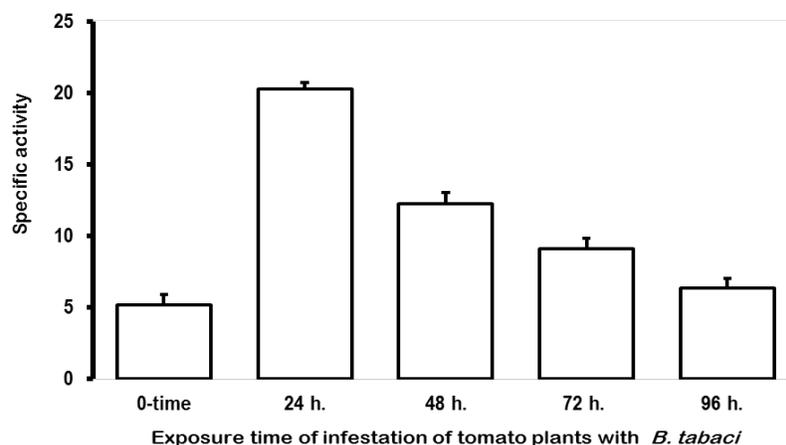


Figure 10: The effect of time-course on the specific activity of polyphenol oxidase in infested tomato plants with the cotton whitefly *B. tabaci*.

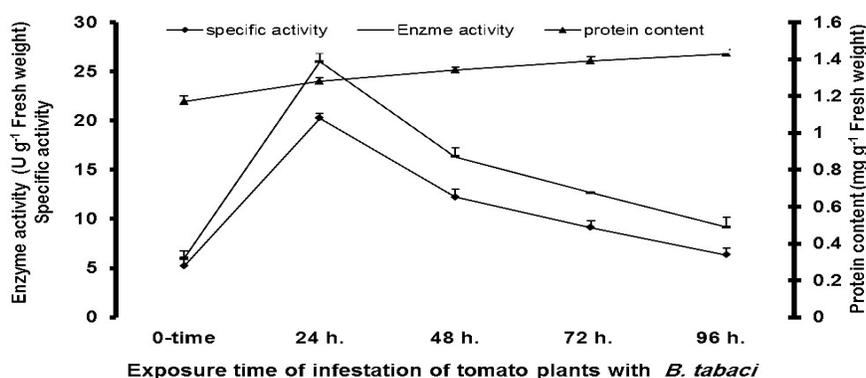


Figure 11: The effect of time-course on enzyme activity and the specific activity of polyphenol oxidase, and on leaf protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.

There were significant differences among all treatments in polyphenol oxidase specific activities but there was no significant difference found on specific activity between 0-time treatment and 96 h infested plants.

4- The effect of *B. tabaci* infestation on peroxidase activity in tomato plants:

Table (4) and figures (12 & 14) illustrate the peroxidase activity in tomato plants induced by

whitefly feeding. The maximum rate of activity exhibited after 48 h exposure to whitefly. It had recorded $1648.67 \pm 37.02 \text{ U g}^{-1}$ fresh weight which equivalent 1.8 folds greater than 0-time and followed by 24 h whitefly-infested plants with 1.6 folds increase in peroxidase activity compared to control then 72h. and 96 h. after infestation (1.35 and 1.27 folds greater than 0-time respectively).

Table 4: Time-course activity of peroxidase in the infested tomato plants with the cotton whitefly *B. tabaci*.

Parameters	Time in hours				
	0-time	24	48	72	96
Peroxidase activity	924.67±31.02 D	1518±60.1 B	1648.67±37.02 A	1252.67±31.29 C	1179.33±20.72 C
Protein content	1.17±0.03 D	1.28±0.018 C	1.34±0.015 BC	1.39±0.023 AB	1.43±0.023 A
Specific activity	795.1±47.21 B	1183.72±53.68 A	1234.31±41.21 A	904.62±37.37 B	827.31±24.09 B

Values are means ± S.E of 3 replicas.

Means followed by the same letter(s) within the same row are not significantly different at 0.05 probability level.

Statistically, peroxidase activities in tomato plants which exposed to whitefly feeding for 24, 48 and 72 h were significantly different compared to the control (0-time). There was no significant

difference between 72 h and 96 h whitefly-exposed plants in the enzyme activity

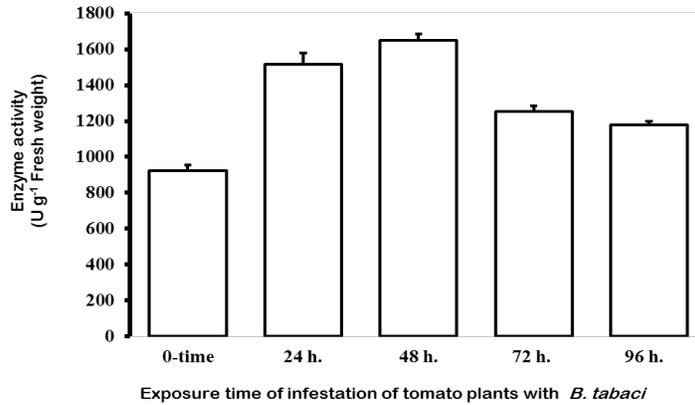


Figure 12: The effect of time-course on the enzyme activity of peroxidase in the infested tomato plants with the cotton whitefly *B. tabaci*.

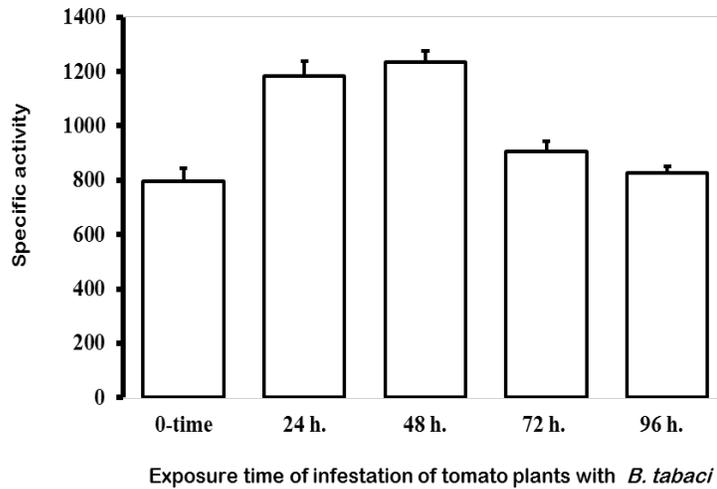


Figure 13: The effect of time-course on the specific activity of peroxidase in the infested tomato plants with the cotton whitefly *B. tabaci*.

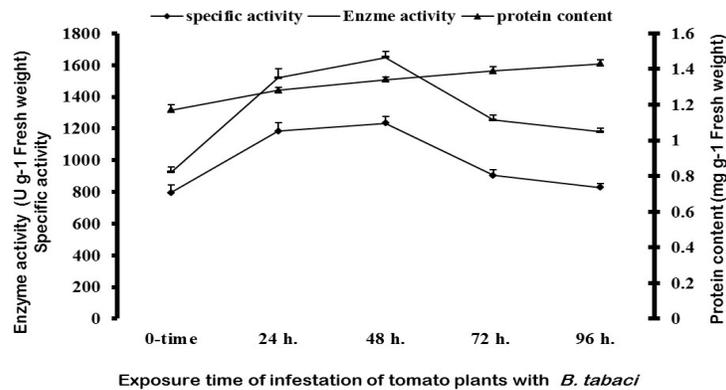


Figure 14: The effect of time-course on enzyme activity and the specific activity of peroxidase, and on leaf protein content in the infested tomato plants with the cotton whitefly *B. tabaci*.

The peroxidase specific activity reached 1183.72 Ug^{-1} fresh weight after 24 h of whitefly feeding on tomato leaves. It elevated to the maximum of 1234.31 \pm 41.21 (1.5 folds greater than control) in 48 h infested-tomato plants (Figures, 13& 14), then it decreased after 72, and 96 h. of infestation (1.1 and 1.04 folds respectively more than control).

There was no significant difference between peroxidase specific activity in 24 and 48 h. infested tomato plants. Also, no significant differences were observed among specific activities of 72, 96 h. whitefly-exposed plants and control.

Figure (15) illustrates the comparative activities of β -1, 3-glucanase, chitinase, polyphenol oxidase and peroxidase within four days of *B. tabaci* infestation. The activity of β -1, 3-glucanase increased gradually to reach to the maximal value at the 3rd day of *B. tabaci* infestation. The activity of chitinase increased gradually in a positive correlation with the time of whitefly infestation. The activity of polyphenol oxidase reached to the maximal value after 24 h of *B. tabaci* infestation then gradually decreased. The peroxidase activity

slightly increased and reached the maximal value after 48 h. of *B. tabaci* infestation then gradually decreased.

5- PR proteins in tomato plant induced resistance to *B. tabaci* infestation.

Table (5) illustrates that the maximum increase of the mean number of whitefly adults/cm² of tomato leaf (7.8 \pm 0.1 adults/cm²) was recorded in untreated tomato plants after 48 h. of whitefly infestation. The obtained results revealed a remarkable decrease in the mean numbers of whitefly adults/cm² of tomato leaf to reach the minimal mean number of whitefly adults/cm² of tomato leaf (7.1 \pm 0.13 adults/cm²) in untreated tomato plants after 96 h of whitefly infestation. The mean number of whitefly adults/cm² of untreated tomato plants was 7.4 \pm 0.16 adults/cm² (Figure, 16). The mean numbers of whitefly adults/cm² of artificially wounded or pre-infested tomato plants with *B. tabaci* were significantly decreased to reach 6.6 \pm 0.14 adults/cm² or 6.5 \pm 0.15 adults/cm² respectively (Table 5 and Figure 16).

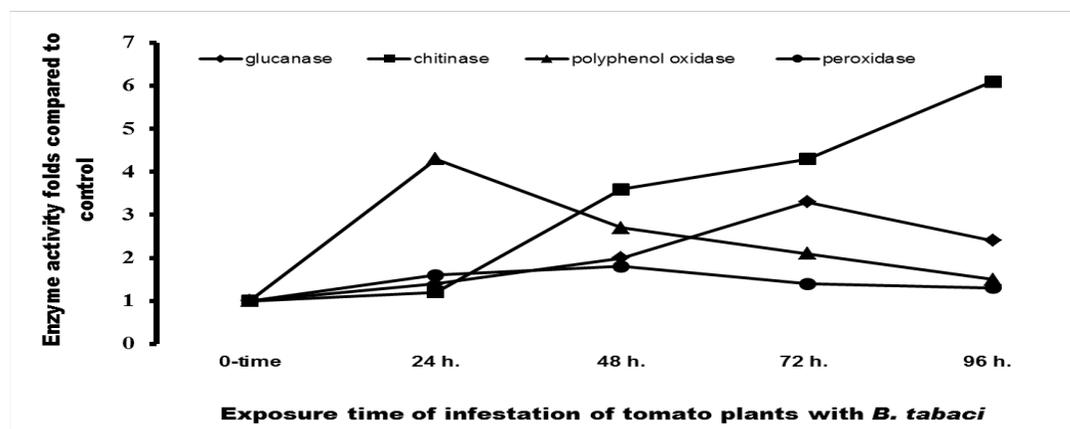


Figure 15: The effect of time-course on enzyme activity of β -1, 3-glucanase, chitinase, polyphenol oxidase and peroxidase in the infested tomato plants with the cotton whitefly *B. tabaci*.

Table 5: The effect of artificially wounded, whitefly pre-infested and pretreated tomato plants with *Pseudomonas* spp. on the mean numbers of whitefly adults/cm².

Treatment	Time in hours				Means
	24	48	72	96	
Control	7.6 \pm 0.35	7.8 \pm 0.1	7.2 \pm 0.24	7.1 \pm 0.13	7.4 \pm 0.16 A
Wounded	6.3 \pm 0.3	6.7 \pm 0.3	6.4 \pm 0.19	6.9 \pm 0.19	6.6 \pm 0.14 B
Pre-infested	6.5 \pm 0.37	6.4 \pm 0.34	6.2 \pm 0.29	6.9 \pm 0.19	6.5 \pm 0.15 B
<i>P. fluorescens</i>	4.8 \pm 0.15	5.4 \pm 0.22	5.7 \pm 0.18	4.7 \pm 0.19	5.1 \pm 0.14 C
<i>P. chlororaphis</i>	4.9 \pm 0.1	4.3 \pm 0.16	4.8 \pm 0.19	4.7 \pm 0.14	4.7 \pm 0.13 D
Means	6.3 \pm 0.41 A	6.3 \pm 0.73 A	6.2 \pm 0.5 A	6.4 \pm 0.57 A	

Values are means \pm S.E of 4 replicas.

Means followed by the same letter(s) are not significantly different at 0.05 probability level.

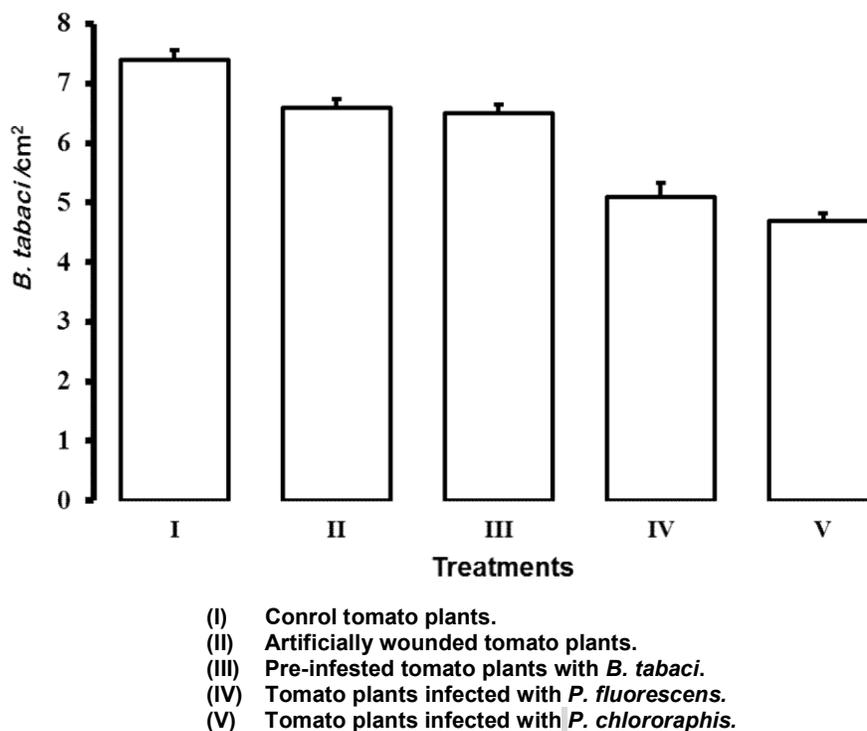


Figure 16: The effect of artificially wounded, whitefly pre-infested and pretreated tomato plants with *Pseudomonas* spp. on the mean numbers of whitefly adults/cm².

Treatment of tomato plants with *P. fluorescens* reduced the mean numbers of whitefly adults/cm² by 31.1%, while *P. chlororaphis* reduced the mean numbers of whitefly adults/cm² by 36.5% (Table 5 and Figure 16). As shown in Table 5, significant differences in the mean numbers of whitefly adults/cm² of tomato leaf were observed among all treatments except between artificially wounded and pre-infested tomato plants with *B. tabaci*.

From the obtained results, it seems clearly that the PR proteins play an important defensive role against whiteflies. Artificially wounded tomato plants reduced the mean numbers of whitefly adults/cm² by about 10.8%, while the biological agent *P. chlororaphis* (non-pathogens bacteria) reduced the mean numbers of whitefly adults/cm² by about 36.5%.

DISCUSSION

Jimencz *et al.*, (1995) was the first to report on PR proteins resulting from feeding by *B. tabaci*. The present results are in conformity with the findings obtained by Inbar *et al.*, (1999b). They declared that feeding of leaf miner and *B. argentifolii* induced local and systemic production of putative defensive proteins, and lysozymes in tomato plants. Mayer *et al.*, (1997) proved that the induction of β -1,3-glucanases and other PR-proteins in tomato plants infested with *Bemisia argentifolii* begun 2 to 3 days after feeding starts and enzyme levels

increased for 3 to 4 weeks. Also, Antony and Palaniswami (2006) reported that cassava plants fed upon by whitefly *B. tabaci* showed increased levels of PR proteins as compared to non-infested plants. The enzyme specific activities increased from 2 to 7 folds and protein content in leaf extracts decreased in whitefly-infested plants, compared to non-infested plants. Also, Bi and Felton. (1995) reported that the activity of several enzymes increased after insect infestation. Inbar *et al.* (1999a) declared that feeding of whiteflies induced high levels of PR proteins that were considered to play defensive roles against insect pests. Srinivasan and Uthamasamy, (2004) reported that the induction of PR proteins such as chitinase, and β -1,3-glucanase in tomato began after 72-96 h. of feeding by whitefly *B. tabaci*.

A companion with our results, Taggar *et al.*, (2012) demonstrated that *B. tabaci* infestation increased the activities of peroxidase activity in black gram, (*Vigna mungo*) and suggested that the enhanced activities of the enzymes might contribute to bio-protection of black gram plants against *B. tabaci* infestation. Puthoff *et al.*, (2010) reported that *B. tabaci* and *Trialeurodes vaporariorum* evoked similar changes in tomato wound- and defense-response gene expression. The levels of RNAs ethylene-regulated genes that encode the basic β -1,3-glucanase (*GluB*) and basic chitinase (*Chi9*), were abundant in infested leaves from the

time nymphs initiated feeding. In addition, *GluB* RNAs accumulated in apical non-infested leaves. PR protein-1 RNAs also accumulated after whitefly feeding. McKenzie *et al.*, (2002) declared that PR protein (β -1,3-glucanase, chitinase, peroxidase, P2 and P4) response was much vigorous when it was attacked by whiteflies hosting tomato mottle virus (ToMoV) than by whitefly alone.

Graham and Stichlen, (1994) reported that induction of PR proteins such as chitinases, peroxidases and β -1-3-glucanase has bad impact on pathogens and insect pests. Chitinase debases chitin, a considerable ingredient of insect cell. Chitinase may extremely influence insects by harming the peritrophic membrane (chitin-based structures) that provides a boundary to ingested pathogens and other material that pose a threat to the insect. Chitinases can moreover act as inhibit amylase activity and meddled with assimilation of plant parts (Ary *et al.*, 1989). It may also increase vulnerability of the insects to biological control agents. Chitinase activity may interfere with insect growth and nourishing, encourage microbial infection, and finally cause death (Shapiro *et al.*, 1987; Wang *et al.*, 1996). Following pathogen and insect attack, tomato plants start in induction of Peroxidases. Peroxidases are implicated in production and polymerization of phenolics and hypersensitive reactions, limiting the chance of disease dispersal (Bowles, 1990). Peroxidases moreover have passive influence on food edible and protein accessibility to herbivorous insects (Duffey and Stout, 1996).

The harmfulness of phenolics such as chlorogenic acid has been habitually ascribed to their tendency to be oxidized enzymatically by means of polyphenol oxidase (PPO), or peroxidase (POD) a process which improves the anti-insect action of phenolics by creating highly reactive *o*-quinones (Felton *et al.*, 1989; Appel, 1993). This oxidation forms quinones that covalently tie to proteins, in this way constraining their bioavailability as nutrients, and may form reactive oxygen species (e.g., superoxide radical and H₂O₂) that harm fundamental supplements or indispensable molecules such as lipids, proteins, and nucleic acids; this is the basis of the anti-nutritive function of PPO against insects. (Felton *et al.*, 1989, 1992; Appel, 1993; Summers and Felton, 1994). Cysteine, lysine, histidine, and methionine residues are preferentially alkylated, which prevents their assimilation (Felton *et al.*, 1992, Hurrell and Finot 1984). As these are essential amino acids, a diet rich in PPO can lead to nutritional deficiencies and a suppression of larval growth (Felton *et al.* 1989, Duffey and Stout 1996). In tomato, the importance of PPO in defense was confirmed by the finding that it is induced by the tomato herbivore defense signal systemin (Constabel *et al.*, 1995).

Endorsement with present study, it is well established that insect injury as well as pathogen onslaught can obviously induce PR proteins (Stout *et al.*, 1994; Stout *et al.*, 1995; Stout and Duffey, 1995). Dowd and Lagrimini (2006) found that the number of adult whiteflies of *T. vaporariorum* per plant was significantly reduced on high peroxidase infested plants compared to wild type plants. Wounding and herbivore damage cause rapid increases in jasmonic acid (Stout and Bostock, 1999; Reymond *et al.*, 2000), triggering systemic defenses against herbivores and pathogens. Plants are equipped with a range of defense mechanisms against herbivorous insects. Moreover, feeding damage to plants by insect herbivores induces the production of plant volatiles, which are attractive to the herbivore's natural enemies (Girling *et al.*, 2008).

On the other hand, plants can gain immunity against diseases through various biological factors including necrotizing pathogens, non-pathogens and soil born rhizosphere bacteria and fungi (Van loon *et al.*, 1998). Enkerli *et al.*, (1993) found that chitinase activity was increased systemically by the infection of tomato plants with *Phytophthora infestans*. Ren, and West (1992) reported that *Pseudomonas* pretreated plants resulted in an additional increase in PR proteins. Fluorescent pseudomonads are non-pathogenic rhizobacteria (Saravanan *et al.*, 2004; Karthikeyan *et al.*, 2006) and various isolates of *Pseudomonas fluorescens*, repressed the soil borne pathogens via several suggested mechanisms (Karthikeyan *et al.*, 2006). Previous studies showed the ability of *Pseudomonas* spp. to promote a few biochemical metabolic changes enhancing insect control. Ardebili *et al.*, (2011) reported that tomato seed bacterization by *P. fluorescens* remarkably prompt peroxidase, polyphenoloxidase and superoxid dismutase activities in root tissues. It is well established that *Pseudomonas* spp. increased physical and mechanical strength of the host cell-wall and causing biochemical and physiological changes leading to synthesis of PR proteins (van Loon, 1999; Kim, *et al.*, 2001; Ramamoorthy and Samiyappan, 2001).

These results provide clear evidence that tomato plants resistance is depend on a set of protecting systems against different pests. Wounding, insect infestation and infection of tomato plants with *Pseudomonas* spp. cause rapid trigger systemic defenses against herbivores and pathogens. In addition, these results provide several insights into the integration and coordination of the induced defenses of tomato plants against multiple pests and suggest that the expression of resistance against some pests may compromise resistance to others. The obtained results of the present study will help in developing a plan of pest management

program for *B. tabaci* under greenhouse conditions. When tomato plants pretreated with the biological agent *Pseudomonas chlororaphis* (non-pathogenic bacteria), the numbers of *B. tabaci* adults/cm² on tomato plant leaves were dramatically reduced.

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المخلص العربي

حث إنتاج البروتينات المرتبطة بالمرضات كآلية دفاع للنباتات ودورها في مكافحة ذبابة القطن

Bemisia tabaci البيضاء

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تمت دراسة تأثير الإصابة بذبابة القطن البيضاء *B. tabaci* على نشاط بعض البروتينات المرتبطة بالمرضات *polyphenol oxidase* و *chitinase* و *Beta-1, 3-glucanase* و *pathogenesis-related (PR) proteins* و *peroxidase* في نباتات الطماطم بعد ٢٤ و ٤٨ و ٧٢ و ٩٦ ساعة من الإصابة ومقارنتها بنباتات غير مصابة. عموماً يزداد نشاط إنزيم β -1,3-glucanase معنوياً مقارنة بالمرجع (الكنترول) حيث يزداد بشكل طفيف بعد اليوم الأول من الإصابة، ويصل إلى المستوى الأقصى بعد ثلاثة أيام (يزداد نشاط الإنزيم ٣,٣ ضعف مقارنة بالكنترول). بدأ تراكم إنزيم *chitinase* في الإزداد بعد مرور يوم من الإصابة (٢,٢ ضعف عن الكنترول). ثم إزداد تدريجياً بعد ٤٨، ٧٢ ساعة (٣,٦ و ٤,٣ ضعف مقارنة بالكنترول، على الترتيب) وأستمر في التراكم ليصل إلى الزيادة القصوى في أوراق الطماطم حيث بلغ ما يعادل ٦,١ ضعف عن الكنترول وذلك في اليوم الرابع. يزداد نشاط إنزيم الـ *polyphenol oxidase* في جميع المعاملات، أي بعد ٢٤ و ٤٨ و ٧٢ و ٩٦ ساعة من الإصابة بالذبابة الأبيض عنه في النباتات غير المصابة (الوقت صفر). قدرت الزيادة في النشاط بعد ٢٤ ساعة من تغذية الذباب الأبيض حيث بلغت ٤,٣ ضعف عن الكنترول. تم تسجيل أعلى معدل لنشاط الـ *peroxidase* بعد مرور ٤٨ ساعة من التعرض لحشرات الذباب الأبيض بما يعادل ١,٨ ضعف أكثر من الزمن صفر، يليه ٢٤ ساعة من إصابة النباتات بالذبابة الأبيض بـ ١,٦ ضعف زيادة في النشاط مقارنة بالكنترول ثم ٧٢ ساعة و ٩٦ ساعة بعد الإصابة (١,٣٥ و ١,٢٧ ضعف أكثر من الزمن صفر، على الترتيب).

لوحظ حدوث انخفاض معنوي في متوسط عدد الذباب الأبيض في الطماطم التي تم عدوها بـ *Bacterioides* لاحتواءها بـ *Pseudomonas chlororaphis* حيث إنخفض متوسط عدد الذباب الأبيض على الطماطم التي تم عدوها بحوالي ٣٦,٥% مقارنة بالكنترول.

مما تقدم يتضح أن البروتينات المرتبطة بالمرضات تلعب دوراً هاماً في مكافحة الذبابة البيضاء، حيث أدت إلى خفض متوسط أعداد الحشرات بمقدار ٣٦,٥%. هذه النتائج تعطي دليل قاطع على أن مقاومة النباتات للحشرات تعتمد على نظام معقد. كما أدت معاملة نباتات الطماطم بأنواع من البكتريا *Pseudomonas spp.* إلى ظهور سريع لنظام دفاعي ضد الآفات الحشرية. هذه النتائج قد تساعد في تطوير خطة برنامج الإدارة المتكاملة للذبابة البيضاء تحت ظروف الزراعات المحمية.