

Evaluation the Efficacy of Some Phenolic Compounds in Controlling Bacterial Spot disease and Biochemical Changes associated in Pepper Plants under Greenhouse Conditions

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ABSTRACT

Treating pepper plants with phenolic compounds *i.e.* pyrogallol, catechol, caffeic, tannic and cinnamic significantly decreased bacterial spot disease of pepper plants. Treated pepper leaves 2 days before inoculation with *Xanthomonas vesicatoria* was more effective in reducing disease incidence and severity than inoculated plants with *X. vesicatoria* at once with spraying phenolic compounds. Spraying pepper leaves 2 days before inoculation, exhibited that, pyrogallol was the most effective treatment which reduced disease incidence and disease severity by 85.71 and 91.99% respectively followed by catechol and cinnamic which were reduced disease incidence with 80.96 and 76.19% and severity by 89.33 and 86.67% respectively. As for biochemical changes, the obtained results showed that, PO, PPO, PAL, chitinase and β -1,3-glucanase activities increased as a result of spraying pepper plants with the tested phenolic compounds compared to untreated control. As well as, all treatments led to an induction of PO isoenzymes in treated pepper plants. The data also illustrated that two unique bands were detected and specific to Pyrogallol 2 and Pyrogallol 3 and one unique band was detected as affected to Pyrogallol 1. Polyacrylamide gel electrophoresis of protein showed that 15 protein bands with molecular weights ranging from 122 to 25 kDa are contained in pepper plants. New protein bands expressed as a result of treating pepper plants with phenolic inducers. Four new bands found between 25 and 56 Kda and a fifth band at 84 Kda.

Keywords: Pepper plants - Bacterial spot - Phenolic compounds - Pyrogallol - Catechol - Caffeic - Tannic - Cinnamic

INTRODUCTION

Pepper (*Capsicum annum* L) is one of the most essential, widespread and preferred vegetable crops cultured in Egypt for local consumption and exportation. Bacterial spot caused by *Xanthomonas vesicatoria* is a common disease of pepper which causes early defoliation and losses due to reduced quantity of fruits and yield of unmarketable fruits. Control is generally based on sanitation procedures, crop rotation, use of disease-free seeds and copper-based compounds (Schwartz & Gent, 2007 and Li, 2012). In the 1950s, streptomycin was used comprehensively but resistant bacterial strains developed and rendered it ineffective (Quezado-Duval *et al.*, 2003 and Stall & Thayer, 1962). A similar phenomenon was detected with copper (Stall *et al.*, 1986). Phenolic compounds in plants are intensely complicated in the interaction between the pathogen and the plant. They are toxic to pathogenic organisms and their post-infection production and accumulation are more intense in resistant plant cultivars than in susceptible ones (Mikulic *et al.*, 2007). Many studies have pointed out the antimicrobial effectiveness of certain phenolic classes, such as coumaric and caffeic acid, flavonoids and coumarins (Amborabe *et al.*, 2002 and Baidez *et al.*, 2006). Tannic acid and catechol reduced the linear growth of *Fusarium solani*, *Verticillium alba-atrum* and *V. dahliae* and inhibited spore germination (Mansour, 2005). Both catechol and pyrogallol were found to have antibacterial effects on *Pseudomonas putida* and *Corynebacterium xerosis* (Kocacaliskan *et al.*, 2006). Tannins are found within leaves, wood, flowers and seeds of plants. One major function of tannins is to provide protection against

microbial pathogens and insects (Dixon *et al.*, 2005 and Lattanzio *et al.*, 2006).

The defense gene products include peroxidase (PO) and polyphenoloxidase (PPO) that catalyse the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexin and phenolics biosynthesis (Anand *et al.*, 2009). PAL is the key enzyme in the production of the basic molecule used for the biosynthesis of most phenols. Peroxidase and polyphenoloxidase are able to oxidize phenols, yielding highly toxic quinones, which can potentially prevent fungal germination (Lattanzio *et al.*, 2006). Both chitinase and β -1,3-glucanase have been intensively studied for their accumulation in infected plant tissues and their functions in plant defense reactions in different plant-microbe interactions (Van Loon, 1997).

Therefore, this study was conducted to study the ability of some of phenolic compounds to induce resistance of pepper plants against bacterial spot disease caused by *Xanthomonas vesicatoria* and the enzyme activities.

MATERIALS AND METHODS

Plant material: healthy pepper plants of the sensitive cultivar California wonder (30 days age) and after 15 days of transplanting were used.

Phenolic Compounds: pyrogallol, catechol, caffeic, tannic and cinnamic were used at concentration of 4 mM.

Pathogenic bacteria: *Xanthomonas vesicatoria* was isolated from diseased pepper plants growing in open field in Egypt and identified by consulting Bradbury (1984), Lelliott & Stead (1987) and Vauterin *et al.*, (1995). Inoculum of bacterium was prepared by grown

on KB at 28°C for 24hr. Bacterial cells were suspended in 0.01M magnesium sulfate (pH 7.2) and the bacterial suspension was adjusted to 10^8 cfu ml⁻¹ (Optical Density 660=0.06).

1-Greenhouse experiment

Plants 45 days age were divided to two groups. First groups were treated with phenolic compounds and inoculated with a bacterial suspension 2 hours after treatment. Second group was treated with chemical inducers and inoculated with a bacterial suspension 2 days after treatment. Control Plants were treated with water then inoculated with bacteria at the same time.

Plants were inoculated with pathogenic bacterial suspensions (1×10^8 cfu) and sprayed through stomata leaves of pepper plants by a high pressures prayer. After that, all plant treatments were covered by plastic sheet for 24 hrs. All inoculated plants were maintained in humid chamber and disease severity was recorded after 10 days from inoculation (Abbasi *et al.*, 2002). Disease severity was recorded using the Horsfall-Barratt scale (Horsfall and Barratt, 1945) as following: 0= 0 represented a sparse plant canopy, 1=1-10 necrotic spots on the leaves/plant, 2= 11-20 necrotic spots on the leaves/plant, 3= 21-30 necrotic spots on the leaves/plant, 4= 31-40 necrotic spots on the leaves/plant, 5= 41-50 necrotic spots on the leaves/plant, 6= 51-60 necrotic spots on the leaves/plant and 7= >61 lack of epinasty on new growth.

$$\text{Efficacy (\%)} = \frac{\text{Treatment - Control}}{\text{Control}} \times 100$$

2-Determination the enzyme activities

Enzyme extraction

Leaf samples were collected (from first group of plants T1 two days after treatment, two days after treating plants with phenolic compound without inoculation from second group T2 and also 2 days after inoculation from second group T3 were immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2 mL of sodium phosphate buffer, 0.1 M (pH 7.0) at 4°C. The homogenate was centrifuged at 4°C for 20 minute 4000 ×g. Protein extract prepared from the leaves were used to estimate PO, PPO, PAL, β-1,3-glucanase and chitinase (Anand *et al.*, 2007).

Determination of Peroxidase (PO):

Peroxidase activity was determined according to the method described by Allam and Hollis (1972). Peroxidase activity was expressed as the increase in absorbance at 425 nm/g fresh weight/minutes.

Determination of Polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by Matta and Dimond (1963). Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/min.

Determination of phenylalanine ammonia lyase (PAL):

Activity of PAL was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid as described by Dickerson *et al.*, (1984). The optical density (O.D.) value was recorded at 290 nm and enzyme activity was expressed as μmol trans-cinnamic acid min⁻¹ g⁻¹ protein.

Determination of Chitinase:

Determination the activity of chitinase was carried out according to the method of Boller and Mauch, (1988). Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/g fresh weight tissue/60 minutes.

Determination of β-1,3-Glucanase:

Determination the activity of the β-1,3-glucanase was carried out according to the method of (Sun *et al.*, 2006). β 1-,3-glucanase was expressed as mM glucose equivalent released /g fresh weight tissue /60 minutes.

3-Activity gel electrophoresis

Peroxidase.

Activity gel electrophoresis of peroxidase was carried out to study the expression pattern of different isoforms of PO with different treatments. For native anionic polyacrylamide gel electrophoresis according to the method of Sindhu *et al.*, 1984.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Eighty-microliters (80 μL of protein) of leaves samples were subjected to SDS-polyacrylamide gel electrophoresis was performed in 12 % acrylamide slab gels following the system of Laemmli (1970) to identify their protein profiles. Gels were photographed scanned, analyzed using Gel Doc VILBER LOURMAT system.

RESULTS

1-Effect of foliar application with some phenolic compounds on bacterial spot disease of pepper plants

Data in Table1 show that pepper plants with treated the tested phenolic compounds significantly decreased bacterial spot disease. Treated pepper leaves 2 days before inoculation with *Xanthomonas vesicatoria* was more effective in reducing disease incidence and severity % than inoculated plants with *X. vesicatoria* at once with spraying phenolic compounds. As for spraying pepper leaves 2 days before inoculation exhibited that pyrogallol was the most effective treatment which reduced disease incidence and disease severity by 85.71 and 91.99% respectively, followed by catechol and cinnamic where reduced disease incidence with 80.96 and 76.19% and disease severity by 89.33 and 86.67% respectively. Also, pyrogallol, catechol and cinnamic were the most effective treatments when plants treated with inducers and inoculated with *X. vesicatoria* at the same time.

Table 1. Bacterial leaf spot disease of pepper plants as affected with foliar spraying with some phenolic compounds

| Treatment | Disease incidence % | | Disease severity % | | Efficacy (%) | | | |
|--------------|---------------------|-------|--------------------|-------|---------------------|--------|--------------------|--------|
| | T1 | T2 | T1 | T2 | Disease incidence % | | Disease severity % | |
| | | | | | T1 | T2 | T1 | T2 |
| Pyrogallol | 13.33 | 10.00 | 5.71 | 2.86 | -78.95 | -85.71 | -82.37 | -91.99 |
| Catechol | 16.67 | 13.33 | 6.66 | 3.81 | -73.68 | -80.96 | -79.43 | -89.33 |
| Caffeic | 26.33 | 20.00 | 8.10 | 5.24 | -58.42 | -71.43 | -74.98 | -85.33 |
| Tannic | 30.00 | 23.67 | 9.05 | 6.19 | -52.63 | -66.19 | -72.05 | -82.67 |
| Cinnamic | 23.67 | 16.67 | 7.62 | 4.76 | -62.62 | -76.19 | -76.47 | -86.67 |
| Control | 63.33 | 70.00 | 32.38 | 35.71 | 0.00 | 0.00 | 0.00 | 0.00 |
| L.S.D. at 5% | 9.20 | 9.87 | 4.76 | 4.24 | ----- | ----- | ----- | ----- |

T1= Treated plants with phenolic compounds and *X. vesicatoria* at the same time.
 T2= Inoculated plants with *X. vesicatoria* 2 days post treating with phenolic compounds.

Effect of application some phenolic compounds on peroxidase and polyphenoloxidase activities of pepper plants.

Data in Table 2 show that PO and PPO activities increased as a result of spraying pepper plants with phenolic compounds compared to untreated control.

Treated plants with phenolic compounds 2 days before inoculation with *X. vesicatoria* led to increasing activity of PO and PPO. In addition to the activity of PO and PPO was increased post inoculation. On the other hand, plants treated with phenolic compounds and *X.*

vesicatoria at the same time increased the activities of PO and PPO compared with control but less than when plants treated before inoculation. Generally, while the highest increase in PO activities was recorded by pyrogallol, caffeic and catechol respectively in all cases, tannic recorded the least increase in PO activities in all cases.

While as for PPO, catechol, pyrogallol and caffeic recorded the highest increase in PPO activities, the least increase was recorded with cinnamic on PPO activities at all cases.

Table 2. Peroxidase and polyphenoloxidase activities in response to foliar application with some phenolic compounds of pepper plants.

| Treatment | PO | | | PPO | | | Efficacy (%) | | | | | |
|------------|-------|-------|-------|-------|-------|-------|--------------|--------|--------|--------|--------|--------|
| | T1 | T2 | T3 | T1 | T2 | T3 | T1 | PO T2 | T3 | T1 | PPO T2 | T3 |
| Pyrogallol | 71.76 | 81.78 | 92.00 | 48.78 | 63.27 | 74.32 | 31.50 | 105.74 | 310.90 | 245.22 | 180.08 | 193.87 |
| Catechol | 66.12 | 77.25 | 84.99 | 49.95 | 68.58 | 75.87 | 21.17 | 94.34 | 279.59 | 253.50 | 203.59 | 200.00 |
| Caffeic | 66.42 | 77.94 | 86.52 | 39.52 | 55.98 | 60.84 | 21.72 | 96.08 | 286.42 | 179.69 | 147.81 | 140.57 |
| Tannic | 64.83 | 72.54 | 79.26 | 29.43 | 30.51 | 51.03 | 18.80 | 82.49 | 254.00 | 108.28 | 35.06 | 101.78 |
| Cinnamic | 68.10 | 73.68 | 83.40 | 27.09 | 29.16 | 41.22 | 24.79 | 85.36 | 272.49 | 91.72 | 29.08 | 62.99 |
| Control | 54.57 | 39.75 | 22.39 | 14.13 | 22.59 | 25.29 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

T1= Treated plants with phenolic compounds and *X. vesicatoria* at the same time, then samples were taken 2 days later
 T2= Treated plants with phenolic compounds, then samples were taken 2 days later
 T3= Inoculated plants with *X. vesicatoria* 2 days post treating with phenolic compounds, then samples were taken 2 days later

Changes in phenylalanine ammonia lyase (PAL) activities in treated pepper plants with some phenolic compounds.

Results in Table 3 reveal that, PAL was greatly increased in the pepper plants sprayed with phenolic compounds compared with control. In addition, the highest activity of PAL was recorded

when plants treated with phenolic compounds and *X. vesicatoria* at the same time.

Plants sprayed with pyrogallol recorded the highest increase in activity of PAL enzyme in all cases followed by catechol and cinnamic. Meanwhile, caffeic recorded the least increase in PAL activity.

Table 3. Changes in phenylalanine ammonia lyase (PAL) activities in treated pepper plants with some phenolic compounds.

| Treatment | PAL | | | Efficacy (%) | | |
|------------|--------|--------|--------|--------------|-------|-------|
| | T1 | T2 | T3 | T1 | T2 | T3 |
| Pyrogallol | 645.69 | 409.52 | 483.19 | 86.25 | 21.94 | 36.26 |
| Catechol | 439.13 | 383.51 | 416.02 | 26.67 | 14.19 | 17.31 |
| Caffeic | 358.24 | 369.07 | 389.29 | 3.33 | 9.89 | 9.78 |
| Tannic | 404.46 | 370.51 | 393.63 | 16.67 | 10.32 | 11.00 |
| Cinnamic | 421.79 | 374.85 | 397.96 | 21.67 | 11.61 | 12.22 |
| Control | 346.68 | 335.85 | 354.62 | 0.00 | 0.00 | 0.00 |

T1= Treated plants with phenolic compounds and *X. vesicatoria* at the same time, then samples were taken 2 days later
 T2= Treated plants with phenolic compounds, then samples were taken 2 days later
 T3= Inoculated plants with *X. vesicatoria* 2 days post treating with phenolic compounds, then samples were taken 2 days later

Changes in chitinase and β -1,3-glucanase enzyme activity in treated pepper plants with some phenolic compounds.

Data in Table 4 indicate that, treating pepper plants with phenolic compounds led to induce an increase on the activities of chitinase and β -1,3-glucanase compared with the control in all cases.

Regarding the chitinase activities, treated plants with phenolic compounds without inoculation led to increasing activities of chitinase. Moreover, the activities of chitinase was increased post inoculation with *X. vesicatoria*. Whereas, treated plants with phenolic compounds and *X. vesicatoria* at the same time, led to increasing the activities of chitinase but less

than the other cases. Generally, the highest activities of chitinase was induced by catechol, caffeic and pyrogallol in all cases respectively, while, cinnamic recorded the least increase in chitinase activity.

As for β -1,3-glucanase, the highest increase in β -1,3-glucanase activities was recorded by cinnamic and pyrogallol when plants treated with phenolic compounds and *X. vesicatoria* at the same time. In the other contrary, catechol and tannic recorded the highest increase in β -1,3-glucanase activities when plants treated pre-inoculation, but this increase was decreased after inoculation nevertheless high compared with control.

Table 4. Changes in chitinase and β -1,3-glucanase enzymes activity in treated pepper plants with the tested phenolic compounds.

| Treatment | Chitinase | | | β -1,3-glucanase | | | Efficacy (%) | | | | | |
|------------|-----------|------|-------|------------------------|-------|-------|--------------|-------|--------|--------|--------|--------|
| | T1 | T2 | T3 | T1 | T2 | T3 | T1 | T2 | T3 | T1 | T2 | T3 |
| Pyrogallol | 7.58 | 7.76 | 10.73 | 23.90 | 16.27 | 13.75 | 44.38 | 42.91 | 179.43 | 138.76 | 105.43 | 76.06 |
| Catechol | 8.87 | 9.88 | 12.39 | 19.37 | 26.50 | 23.69 | 68.95 | 81.95 | 222.66 | 93.51 | 234.60 | 203.33 |
| Caffeic | 8.53 | 8.71 | 11.13 | 19.58 | 17.64 | 14.47 | 62.48 | 60.41 | 189.84 | 95.60 | 122.73 | 85.28 |
| Tannic | 7.17 | 7.27 | 10.53 | 19.51 | 20.88 | 15.55 | 36.57 | 33.89 | 174.22 | 94.91 | 163.64 | 99.10 |
| Cinnamic | 6.38 | 6.61 | 10.49 | 28.51 | 15.48 | 11.81 | 21.52 | 21.73 | 173.18 | 184.82 | 95.45 | 51.22 |
| Control | 5.25 | 5.43 | 3.84 | 10.01 | 7.92 | 7.81 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

T1= Treated plants with phenolic compounds and *X. vesicatoria* at the same time, then samples were taken 2 days later

T2= Treated plants with phenolic compounds, then samples were taken 2 days later

T3= Inoculated plants with *X. vesicatoria* 2 days post treating with phenolic compounds, then samples were taken 2 days later

Effect of foliar application of pepper with some phenolic compounds on peroxidase isoenzymes.

The profiling of peroxidase isoenzymes in treated pepper plants revealed that, all treatments led to an induction of PO isoforms. Three common PO isoenzymes were expressed in all the samples including the control. While the fourth isoenzymes was differed between all samples, this band was absent in control 1,

cinnamic 2 and control 2. The data clearly indicate that two unique bands were detected and specific to pyrogallol 2 and pyrogallol 3. And also one unique band was detected to Pyrogallol 1. The increased intensity of the induced PO was found in pyrogallol treated plants. Finally, it can be concluded that pyrogallol 1, pyrogallol 2 and pyrogallol 3 have the same loci and enzyme activity as shown in Table 5 and Figure 1.

Table 5. Peroxidase isoenzymes in pepper leaves treated with phenolic compounds.

| Treatment | T1 | | | | | | T2 | | | | | | T3 | | | | | |
|------------|------------------|---|---|---|---|---|------------------|---|---|---|---|---|------------------|---|---|---|---|---|
| | Peroxidase bands | | | | | | Peroxidase bands | | | | | | Peroxidase bands | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| Pyrogallol | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Catechol | + | - | + | + | + | - | + | - | + | + | + | - | + | - | + | + | + | - |
| Caffeic | + | - | + | + | + | - | + | - | + | + | + | - | + | - | + | + | + | - |
| Tannic | + | - | + | + | + | - | + | - | + | + | + | - | + | - | + | + | + | - |
| Cinnamic | + | - | + | + | + | - | + | - | + | + | - | - | + | - | + | + | + | - |
| Control | + | - | + | + | - | - | + | - | + | + | - | - | + | - | + | + | + | - |

T1= Treated plants with phenolic compounds and *X. vesicatoria* at the same time, then samples were taken 2 days later

T2= Treated plants with phenolic compounds, then samples were taken 2 days later

T3= Inoculated plants with *X. vesicatoria* 2 days post treating with phenolic compounds, then samples were taken 2 days later

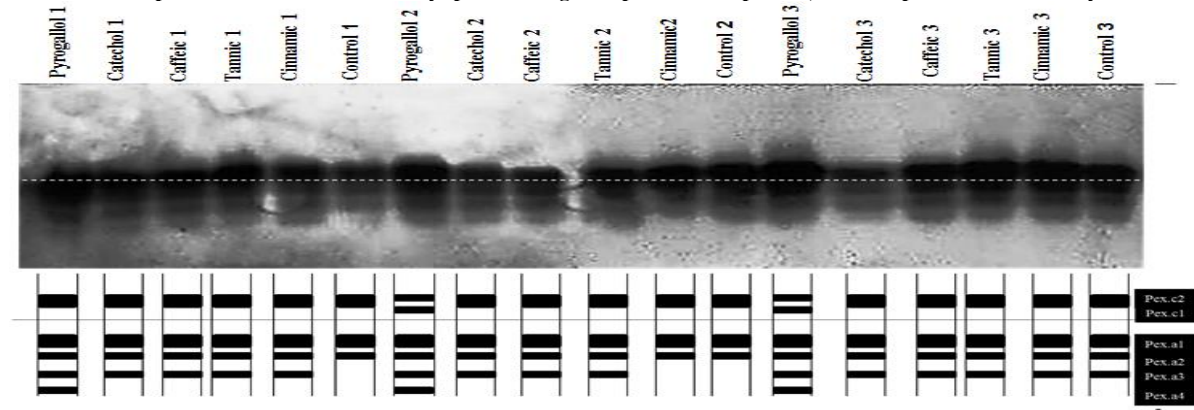


Fig. 2. Peroxidase isoenzymes in pepper leaves treated with phenolic compounds.

Effect of foliar application of pepper with some Phenolic compounds on PAGE of protein.

Concerning the results of SDS (PAGE) presented in Table 6 and demonstrated in Fig. 2 show that 15 protein bands with molecular weights ranging from 122 to 25 kDa are contained in pepper plants. New protein bands expressed as a result of treating pepper plants with phenolic inducers. Four new bands were appeared between 25 and 56 Kda and a fifth band at 84 Kda. The data clearly indicate that the unique bands with 25 kDa

was specific to pyrogallol 1 while the unique bands with 51 kDa was specific to tannic 1, cinnamic 1, cinnamic 2 and cinnamic 3. Moreover, the unique bands with 84 kDa was absent from all treatment at the T1 while was appeared in all treatments at T2 and T3 expect control at T3. The obtained results confirmed that new protein bands with low molecular weight had a progressive relationship on reducing disease severity of bacterial spot.

Table 6. Molecular weights of fractionated protein profiles of pepper leaves treated with phenolic compounds

| Band No | M. WKDa | Unique | T1 | | | | | | T2 | | | | | | T3 | | | | | |
|---------|---------|--------|------------|----------|---------|--------|----------|---------|------------|----------|---------|--------|----------|---------|------------|----------|---------|--------|----------|---------|
| | | | Pyrogallol | Catechol | Caffeic | Tannic | Cinnamic | Control | Pyrogallol | Catechol | Caffeic | Tannic | Cinnamic | Control | Pyrogallol | Catechol | Caffeic | Tannic | Cinnamic | Control |
| 1 | 122 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | 108 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3 | 100 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 4 | 94 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 5 | 84 | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 77 | | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 7 | 73 | | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 8 | 68 | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 9 | 60 | | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + |
| 10 | 56 | + | - | - | - | + | + | - | - | - | + | - | - | - | - | - | - | + | - | - |
| 11 | 51 | | + | + | + | + | + | + | + | + | - | - | + | - | + | - | - | - | - | + |
| 12 | 34 | + | - | + | - | + | + | - | + | + | + | - | - | - | + | - | + | - | - | - |
| 13 | 30 | | + | + | + | + | + | - | + | + | + | - | + | - | + | + | - | - | + | - |
| 14 | 27 | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | 25 | + | - | - | - | - | - | - | + | - | - | + | + | - | + | + | + | + | + | - |
| Total | | | 9 | 9 | 8 | 10 | 10 | 8 | 11 | 10 | 10 | 9 | 9 | 8 | 10 | 10 | 9 | 9 | 9 | 8 |

T1= Treated plants with phenolic compounds and *X. vesicatoria* at the same time, then samples were taken 2 days later

T2= Treated plants with phenolic compounds, then samples were taken 2 days later

T3= Inoculated plants with *X. vesicatoria* 2 days post treating with phenolic compounds, then samples were taken 2 days later

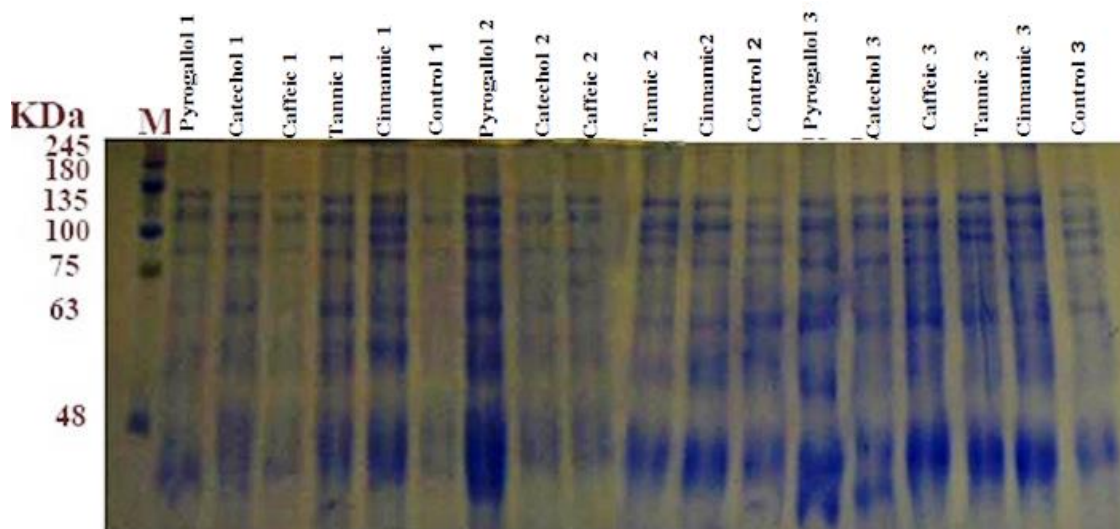


Fig .2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS- PAGE) analysis of total protein extracted from pepper leaves treated with phenolic compounds

DISCUSSION

Bacterial spot caused by *Xanthomonas vesicatoria* is a common disease of pepper which causes

early defoliation and losses due to reduced quantity of fruits and yield of unmarketable fruits. Control method is generally based on sanitation procedures, crop rotation, use of disease-free seeds and copper-based

compounds (Schwartz & Gent, 2007 and Li, 2012). In the present study results indicate that pepper plants treatment with some phenolic compounds significantly decreased bacterial spot disease. Spraying pepper leaves two days before inoculation with *Xanthomonas vesicatoria* was more effective in reducing disease incidence and disease severity than plants inoculated with *X. vesicatoria* at once with spraying phenolic compounds.

The obtained results in the current study supported by the finding of many authors, According to Ali, (2011) treating the tomato plants with ascorbic acid and salicylic acid reduced disease severity of *X. vesicatoria* and increasing the activity of peroxidase and polyphenoloxidase enzymes. Treating pepper plants with SA-based products reduced growth of *Xanthomonas vesicatoria* and plant disease symptoms following challenge and are related to induction of pathogenesis-related (PR) genes (Ward *et al.*, 1991).

Pretreatment with acibenzolar-*S*-methyl (ASM) also significantly enhanced the disease resistance of pepper plants against the bacterial pathogen *Xanthomonas campestris* pv. *vesicatoria* (Romero *et al.*, 2001; Buonauro *et al.*, 2002; Obrdovic and Jones 2004 and Madhusudhan *et al.*, 2008). Similar results were obtained by Itako *et al.*, (2012), who reported a reduction in the disease incidence and increased production of peroxidase, polyphenoloxidase, and β -1,3-glucanase on leaves of tomato sprayed with ASM and pyraclostrobin.

A foliar spray of phosphate salt significantly reduced disease severity in pepper plants infected with *Xanthomonas vesicatoria*, the causal agents of bacterial spot disease of pepper, (Abbasi *et al.*, 2002). ASM, pyraclostrobin, and pyraclostrobin+ metiram controlled bacterial spot, and increased the production of peroxidase, polyphenoloxidase and β -1,3-glucanase in the leaves. Many studies have pointed out the antimicrobial effectiveness of certain phenolic classes, such as coumaric and caffeic acid, flavonoids, coumarins, tannic acid and catechol (Amborabe *et al.*, 2002; Baidez *et al.*, 2006; Mansour, 2005 and Kocacaliskan *et al.*, 2006).

The obtained results also clearly illustrated that, PO, PPO PAL, chitinase and β -1,3-glucanase activities increased as a result of spraying pepper plants with tested phenolic compounds compared to untreated control. As well as, the profiling of peroxidase isoenzymes in treated pepper plants. Moreover, concerning the results of SDS (PAGE) of protein showed that 15 protein bands with molecular weights ranging from 122 to 25 kDa are contained in pepper plants. New protein bands expressed as a result of treating pepper plants with phenolic inducers. Four new bands found between 25 and 56 Kda and a fifth band at 84 Kda. New protein bands expressed as a result of spraying phenolic compounds. The obtained results confirmed that new protein bands with low molecular weight had a progressive relationship on reducing disease severity of bacterial spot. Treatment faba bean plants with citric, benzoic and salicylic acids significantly reduced chocolate spot disease severity and

new proteins in pre-treated plants were induced (Hassan *et al.*, 2006). A higher enzymatic activity of PAL, PO and PPO induces an additional production and accumulation of phenolics (Anand *et al.*, 2009), which might hinder the pathogen to spread from the infected cells into the healthy ones, and thus the infection can be inhibited or restricted (Gogoi *et al.*, 2001). The toxic phenolic compounds in plant cells acting through: (1) the structure of bond form with cell wall components of plant tissues (Mahadevan and Sridhar, 1986), (2) enhance host resistant by stimulating host defense mechanisms (Subba Rao *et al.*, 1988), (3) prevent the extent of fungal growth in plant tissues (Soni *et al.*, 1992) and (4) penetrate the microorganisms and cause considerable damage to the cell metabolisms (Kalaichelvan and Elangovan, 1995). According to Cutt and Klessig (1992), β -1,3-glucanases cause the release of glycosidic fragments not only from the pathogen itself but also from the walls of the plant cells, and these glycosidic fragments can elicit the host defense. Some plant chitinases also have lysozyme activity and can therefore hydrolyse bacterial cell walls (Boller *et al.*, 1983 and Heitz *et al.*, 1994).

The enzymatic activities of several PR proteins have been identified and include β -1,3-glucanases (PR-2) and chitinases (PR-3), which possess direct antimicrobial activity by degrading microbial cell wall components (Van Loon, 1997).

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تقييم فعالية بعض المواد الفينولية في مقاومة مرض التبغ البكتيري والتغيرات البيوكيميائية المصاحبة في نباتات الفلفل تحت ظروف الصوبة جمال عاشور أحمد مدرس أمراض النبات- كلية الزراعة بمشهر – جامعة بنها

أدت معاملة نباتات الفلفل بالمواد الفينولية المختبرة (البيروجالول، الكاتيكول، الكافيك، التانك و السيناميك) إلى خفض معنوي في مرض التبغ البكتيري في الفلفل. وكانت معاملة نباتات الفلفل بالمواد الفينولية قبل العدوي ببيكتيريا *Xanthomonas vesicatoria* أكثر فاعلية في خفض نسبة حدوث وشدة المرض عن المعاملة بالمواد الفينولية والعدوي بالبيكتيريا *Xanthomonas vesicatoria* في نفس الوقت. وبالنسبة لمعاملة النباتات قبل يومين من العدوي كان البيروجالول أكثر المعاملات فعالية وخفض نسبة وشدة حدوث المرض بنسبة 85.71 - 91.99 % على الترتيب وتبعه الكاتيكول والسيناميك والذان خفضا نسبة الإصابة بنسبة 80,96 و 76,19 % على الترتيب وخفضا شدة حدوث المرض بنسبة 89,66 - 86,67 % على الترتيب. وبالنسبة للتغيرات البيوكيميائية أظهرت النتائج المتحصل عليها ويوضح زيادة نشاط أنزيمات البيروكسيداز والبولي فنيل أوكسيداز والفنيل ألانين أمونيالايز والشيتينيز و البيتا 1 و3 جلوكانيز في نباتات الفلفل المعاملة بالمواد الفينولية مقارنة بالكنترول الغير معاملة. بالإضافة إلى ان كل المعاملات أدت إلى تحفيز تكوين مشابهات أنزيم البيروكسيداز في النباتات المعاملة وأشارت النتائج ويوضح إلى تكوين اثنان من مشابهات أنزيم البيروكسيداز الجديدة وكانت خاصة بالبيروجالول2 والبيروجالول3 بالإضافة إلى تكوين مشابه واحد جديد بالنسبة للبيروجالول 1. أظهرت نتائج التفريد الكهربائي للبروتين تكوين 15 حزمة بروتين بأوزان جزيئية مختلفة تتراوح بين 122 و 25 كيلو دالتون في نباتات الفلفل وتكونت 5 حزم بروتينية جديدة نتيجة معاملة نباتات الفلفل بالمواد الفينولية. ووجد أن أربع حزم بروتينية جديدة تقع بين 56 و 25 كيلو دالتون بينما الحزمة الخامسة كانت بوزن 84 كيلو دالتون.