

# Effect of Abscisic Acid, Salicylic Acid, Potassium Silicate, and *Trichoderma harzianum* As Biocontrol Agent to Induce the Tomato Resistance Against Early Blight Disease Caused by *Alternaria solani*

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## ABSTRACT

In the present study and under greenhouse conditions, tomato plants cv. Albium, grown in potted soil inoculated with the early blight fungus *A. solani*, were treated with abscisic acid (ABA), salicylic acid (SA), and potassium silicate (PS) abiotic inducers, singly, and in combination with the biotic inducer *Trichoderma harzianum*. The different tested inducers significantly decreased the intensity (severity) of early blight disease on tomato, as well as some growth characteristics, photosynthetic capacity, defense-related enzymes and expression analysis of the PR1, PR3 and PR5 genes using Real Time-q PCR. PS+ *Trichoderma* showed the lowest disease intensity, followed by ABA+ *Trichoderma*, and *Trichoderma* treatment had the highest disease intensity when compared to untreated inoculated control. PS + *Trichoderma*, ABA+ *Trichoderma*, and SA+ *Trichoderma* were also the most effective for the best growth characteristics (fresh shoot, dry shoot, fresh root, dry root weight, and total fruits weight/plant). Photosynthetic pigment content was significantly increased with all treatments by chemical inducer and *T. harzianum* compared with *A. solani* treatment. PS + *Trichoderma* was the most effective and significantly enhanced chlorophyll "A" and chlorophyll "B" content comparing with the *A. solani* treatment, while, the highest level of carotene content was observed with alt+ABA+ *Trichoderma* treatment at 2 days post inoculation. Peroxidase (POD), polyphenol oxidase (PPO) and Phenylalanine Ammonia-Lyase (PAL) enzymes activity and PR1, PR2 and PR5 gene expressions were significantly increased in all treatments with chemical inducer comparing with the treatment by *A. solani*. In addition to the treatment by chemical inducer combined with *Trichoderma* were the most effective compared with chemical inducer alone. The maximum POD, PAL activities and PR1 gene expression were observed with alt+PS + *Trichoderma* followed by alt+ABA+ *Trichoderma* while, PR2, PR5 gene expressions and POD activity reached the highest level with alt+PS+ *Trichoderma* followed by alt+SA+ *Trichoderma* and alt+ABA+ *Trichoderma*. *Trichoderma* was of the highest rate of the number of cfu in treatment with alt+PS+ *Trichoderma* with cfu was  $5.9 \times 10^7$ , while the number of cfu in treatment with alt+ABA + *Trichoderma* was  $3.77 \times 10^7$  and the treatment with alt+SA + *Trichoderma* was  $3.68 \times 10^7$ .

**Key words:** Tomato, Early blight, *Alternaria solani*, *Trichoderma harzianum*, Chemical inducer, enzymes, gene expressions.

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widespread and popular vegetables in the world. It ranks second in the economic importance after potato in many countries (Prajapati *et al.*, 2014). Tomato early blight caused by the fungus *Alternaria solani* is economically important tomato disease in several parts of the world (Ali *et al.*, 2016). Infection of tomato plants by the early blight fungus can lead to great losses in crop yield due to damaging leaves and fruits (Chaerani *et al.*, 2007 and EL-Tanany *et al.*, 2018). Use of fungicides is considered the shortest way to obtain efficient results for disease management. However applications of fungicides have hazard effect on human and animal health. Therefore, more balanced, cost effective and eco-friendly approaches were searched and by farmers. Several investigators indicated that certain biotic and abiotic inducers have potentiality to control plant diseases (Anand *et al.*, 2009a and Simonetti *et al.*, 2012). Biological control using antimicrobials, resistance promoters and growth promoters offered good alternatives in controlling plant pathogens (Chandrashekara *et al.*, 2012 and EL-Tanany *et al.*, 2018). *Trichoderma* spp use as biocontrol agents due to their ability to antagonize the pathogens of plant (Harman, 2006), to induce plant defense responses against pathogens, with beneficial effects on plant growth and development (Harman *et al.*, 2004). Recently, researchers have turned to activate the systemic induced resistance (SIR) of plants using some chemical inducers, such salicylic acid, as this substance enhance the same physiological and biochemical changes in plants as do systemic active biological resistance (El-Shennawy and Abd El-All, 2018). Also, potassium silicate is used as a silicon source and contains small amounts of potassium that can improve environmental stress tolerance and increase crop productivity (Tarabih, *et al.*, 2014, Talebi *et al.*, 2015 and Abou-El-Hassan *et al.*, 2020). Abscisic acid

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effectively reduced early blight (*Alternaria solani*) severity in tomato plants (Song *et al.*, 2011). On the other hand, during host-pathogen interaction abscisic acid is involved in signal transduction pathways to activate the defense genes against plant cell membrane damage during host-pathogen interaction (Adie *et al.*, 2007; Lee and Luan, 2012; Nassar and Adss, 2016). EL-Tanany *et al.*, 2018, Khalil and Abdelghany, 2021 and Prakash *et al.*, 2021. found that, treating tomato plants with abscisic acid, salicylic acid and *Trichoderma* before inoculation with *A. solani* increased the activities of the plant defense-related enzymes such phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase and reduced the rates of leaf infection and disease index. The gene of PR1 expression was upregulated in leaves were treated by salicylic acid and abscisic acid at one day after of inoculation with *alternaria solani*, which confirms the role of salicylic acid and abscisic acid in systemic acquired resistance (Prakash *et al.*, 2021). the Arabidopsis were high resistant to infection with *Golovinomyces cichoracearum* than control plants when treated with Si, and corresponded with higher expressions of PR1, PR2 and PR5 defense genes (Vivancos *et al.*, 2015). The aim of this study determination the effect of abscisic acid, salicylic acid, potassium silicate, and *Trichoderma harzianum* as Biocontrol agent to induce the tomato resistance against early blight disease caused by *Alternaria solani*.

## MATERIALS AND METHODS

### 1. Plant materials:

Tomato seedlings cv. Albiun were surface sterilized for 5 minutes in 1% sodium hypochlorite (from Chlorax® 15 percent) and then washed three times with sterilized water. Seedlings were planted in autoclave-sterilized peat moss- in 30-cm-diameter plastic pots (one seedling per pot). Fertilizers and irrigation were applied according to the normal greenhouse production program for tomato.

### 2. *Alternaria solani* inoculum preparation and inoculation:

Tomato plants showing the early blight symptoms were collected from commercial tomato fields in El-Behera Governorate, Egypt and the causal fungus . *A. solani* isolate was isolated from early blight lesions on leaf sections. The isolate was kept at 20°C for 7 days on PDA. Cultures were grown on PDA at 23-25 °C for 6 days under near UV light (310-400 nm) with 16h to induce sporulation. Conidia were collected on plates that had been washed in sterile water. On a hemocytometer, the spore suspension concentration was adjusted to 10<sup>6</sup> spore/ ml<sup>-1</sup>. Spraying 45-day-old tomato plants, cv. albiun, with the spore suspension was conducted independently (Bokshi et al., 2003). The

plants were then covered in clear plastic bags for 24 hours to promote humidity (> 95%) and enable fungal infection and kept under. Standard greenhouse condition at a temperature of 26 °C (Bokshi *et al.*, 2003).

### 2. Chemical inducers:

Absesic acid (ABA> 98 % purity), Potassium silicate (PS> 98 % purity), and salicylic acid (SA> 98 % purity), were obtained from Sigma-Aldrich, P2126 were purchased from local supplier and the shown concentrations were prepared in sterile distilled water were 10 µM, 5 g/l, 2 µM, respectively. Chemical inducers were sprayed before one week of inoculation

### 3. *Trichoderma* inoculum:

Cultures of *Trichoderma harzianum* was obtained from plant pathology department, faculty of agriculture, Damanhour university, kept on were kept on PDA media and stored at 4°C for later use. One week before *Alternaria solani* inoculation, the soil was treated by applying 50 mL of *Trichoderma* from chosen tomato fields at a concentration of 108cfu/ ml.

### 4. The treatments:

Four replicate pots, each with single tomato plant cv. albiun were prepared for each treatment and the conducted treatments were as follows: Uninfected untreated plants (control), untreated inoculated control (infection), *Alternaria solani*+*Trichoderma harzianum* (alt+*Trichoderma*), *Alternaria solani* + abscisic acid (alt+ABA), *Alternaria solani* +ABA+ *T. harzianum* (alt+ABA+*Trichoderma*), *Alternaria solani* +Potassium silicate (alt+PS), *Alternaria solani* +Potassium silicate +*T. harzianum* (alt+PS+ *Trichoderma*), *Alternaria solani* + salicylic acid (alt+SA) and *Alternaria solani* + SA+ *T. harzianum* (alt+SA+ *Trichoderma*).

### 5. Disease assessment:

After 15 days of inoculation, the infected plants were examined and rated using Pandey and Pandey (2002) scale with 0-5 numerical values as follows: 0 = Disease free, 1 = 1-10 % infected leaf area, 2= 11-25% infected leaf area, 3 = 26-50% infected leaf area, 4 = 51-75 % infected leaf area, 5 = > 76 % infected leaf area. Then, disease intensity (severity). was calculated according to Rodríguez et al. (2007) using the following equation:

$$DS \% = \{ \sum (N \times V) / (N \times S) \} \times 100$$

Where, N = Number of leaves in each category, V = Numerical value of infected leaves, and S = Maximum numerical value/grade

### 6. Determination of the defense-related enzymes activity:

The defense-related enzymes; peroxidase (POD), phenylalanine ammonia lyase (PAL), and polyphenol

oxidase (PPO) activities were evaluated in plants after 0, 1, 2, 4, 8, 15 days of *A. solani* inoculation

#### a- Peroxidase (POD) activity:

One gram of tomato leaflets taken from the top six leaves was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4 °C. At 10,000 rpm the homogenate was centrifuged for 15 min at 4 °C. The activity of POD enzyme was estimate in supernatant. The reaction was contained 1.5 ml of pyrogallol (0.05 M), 0.5 ml of H<sub>2</sub>O<sub>2</sub> (1%) and 0.5 ml of enzyme. The reaction was incubated at 28 ± 2 °C. The reaction's absorbance was measured every 20 s interval for 3 mins at 420 nm.

(Jenway UV/VIS spectrophotometer, Model 6305, Bibby Scientific Limited, Staffordshire, UK). a boiled enzyme was prepared to use a control. The POD activity was expressed as a change in absorbance of the reaction mixture min<sup>-1</sup> g<sup>-1</sup> of fresh tissue (Hammerschmidt and Kuc, 1982).

#### b- Polyphenol oxidase (PPO) activity:

One gram of tomato leaflets taken from the top six leaves was homogenized in A 2 ml sodium phosphate buffer (0.1 M) (pH 6.5). The homogenate was centrifuged (Universal 32R, Hettich Centrifuge model D-78532, Germany) at 10,000 rpm for 15 minutes at 4 °C, and the supernatant was utilised as a crude enzyme. Polyphenol oxidase activity was determined using a mixture of 1.5 ml phosphate buffer, 200 l enzyme, and 200 l catechol (0.01 M). After 2 minutes at room temperature, the absorbance of the reaction mixture was measured at 495 nm spectrophotometer (Jenway UV/VIS, Model 6305, Bibby Scientific Limited, Staffordshire, UK). Changes in absorbance were measured every 30 seconds for 2 minutes, and the activity was expressed as a change in absorbance min<sup>-1</sup> g<sup>-1</sup> of fresh tissue. (Mayer *et al.*, 1965).

#### c- Phenylalanine Ammonia-Lyase (PAL) Activity:

According to Dickerson and colleagues (1984), A 2-mercaptoethanol (1.4 mM) and insoluble polyvinylpyrrolidone (PVP) in 3 ml of ice-cold sodium borate buffer (0.1 M) pH 7.0 (0.1 g) A total of 1 gram of leaf was homogenized. The extract was filtered through cheese cloth at 4 °C, and the filtrate was centrifuged for 15 minutes at 15,000 g. (Universal 32R, Hettich Centrifuge model D-78532, Germany). The supernatant was used as crude enzyme. A 0.4 ml of enzyme extract was incubated with 0.5 ml of borate buffer (0.1 M) pH 8.8 and 0.5 ml of L-phenylalanine (12 mM) for 30 minutes at 30 °C The rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm was used to determine the phenylalanine ammonia-lyase activity by spectrophotometer (Bibby Scientific Limited, Staffordshire, UK, Jenway UV/VIS, Model

6305). The enzyme's activity was calculated in mol trans-cinnamic acid min<sup>-1</sup>g<sup>-1</sup> fresh weight (Dickerson and colleagues, 1984).

#### 7. Trichoderma density:

according to Hirte (1969) the dilution plate count technique was applied to determinate the populations of *Trichoderma* in soil after five weeks of plant inoculation plants with *Trichoderma* in the previous experiment. Soil suspension, dilution and aliquot for *Trichoderma* counts to estimate the adequate soil suspension, dilution and aliquots to be added to the culture medium, soil samples were weighed (10 g), transferred to 100 mL of distilled and sterile water, and mixed in a rotary shaking machine (260 rpm) for homogenization during 30 min. The soil suspension was diluted from 10<sup>-1</sup> to 10<sup>-6</sup>, and an aliquot (50, 100, 200, 400, 800 and 1000 µL) from each dilution was transferred to the appropriate culture medium. Two Petri dishes per sample, containing 10 mL of each culture medium, were incubated 7 days at 25 °C, with 8 h of light. The number of colons forming units per gram of dry soil (CFU) was calculated after this period.

#### 8. Assessment of tomato growth parameter:

At the end of the conducted experiment, seven weeks after inoculation plants were uprooted and the following growth parameters were recorded:

□ Shoot fresh weight (g), Shoot dry weight (g), Root fresh weight (g), Root dry weight (g), Total fruit weight/plant (g).

#### 9. Determination of photosynthetic pigments:

Fresh leaflet samples from the top six leaves were gathered from all tomato treatments to determine photosynthetic pigments according to Wintermans and De Mots (1965). In a pestle and mortar, half-gram fresh leaves were pulverized and extracted in 15 ml of 80% acetone (1:100 w/v) and 0.5 g calcium carbonate. The mixture was filtered using a glass funnel, and the residue was washed with a little amount of acetone until it reached 25 ml. For chlorophyll A, chlorophyll B, and carotene, the optical density (O.D) of a constant volume of filtrate was estimated at wave lengths of 662 nm, 644 nm, and 440 nm, respectively. Three times the experiment was carried out. The following equations were used:

$$\text{Chl.A} = 9.784 E_{.662} - 0.99 E_{.644} = \text{mg/L}, \text{Chl.B} = 21.426 E_{.644} - 4.65 E_{.662} = \text{mg/L}$$

$$\text{Carotene} = 4.695 E_{.440} - 0.268 (\text{Chl.A} - \text{Chl.B}) = \text{mg/L}, \text{where, E.} = \text{Optical density at the wavelength indicated.}$$

### 10. Expression analysis of the PR1, PR3 and PR5 genes using Real Time-q PCR:

Total RNA was isolated from leaf tissues sampled after 0, 1, 3, 5, 10, 15 and 20 dpi post inoculation using BioTeke Corporation RNA Isolation kit according to the manufacturer's protocol. cDNA was synthesized from total RNA using dNTPS, poly T primer, and reverse transcriptase enzyme (Fermentas, USA) according to the standard protocol. QRT-PCR was conducted for PR1, PR3 and PR5 genes. The actin gene of tomato used as reference gene (Molinari and Leonetti., 2019)

The following primers were used in the RT-PCR reaction: PR3 (Molinari and Leonetti., 2019), F-5'-AACTATGGGCCATGTGGAAGA -3', R-5'-GGCTTTGGGGATTGAGGAG -3', PR5 (Molinari and Leonetti., 2019), F-5'-GCAACAACGTCCATACACC -3', R-5'-AGACTCCACCACAATCACC -3', PPO (Goel *et al.*, 2017), F-5'-CATGCTCTTGATGAGGCGTA -3', R-5'-CCATCTATGGAACGGGAAGA -3', Actin (Molinari and Leonetti., 2019), F-5'-GATACCTGCAGCTTCCATACACC -3', R 5'-GCTTTGCCGCATGCCATTCT -3'. The Real-Time PCR Reaction (25 µl) contained 12.5 µl of 2× Quantitech SYBR® Green RT Mix (Fermentas, USA), 1 µl of 10 pmol/µl of each primer, 1µl of template cDNA (50 ng), and 9.5 µl of RNase free water. The PCR reaction was conducted according Chin *et al.* (2000): initial denaturation for 10 min at 95 °C followed by 40 cycles(denaturation for 15 s at 95°C annealing for 30 s at 60° C and extension for 30 s at 72°C and a final extension for 10 min at 72°C. Gene expression relative quantification was determined by the  $2^{-\Delta\Delta Ct}$  method (Livak and Schmittgen, 2001). The expression levels of the target genes were normalized relative to Actin gene and relative expression of untreated control plants at each time were set as 1.

### Statistical analysis

Data collected from the conducted experiments were analyzed by two-way analysis of variance (treatments and times) using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Treatments mean were separated by Duncan's multiple range test at 5% probability. Tukey's HSD test was used to determine the significant differences between means at a probability level of  $\leq 0.05$ .

## RESULTS

### 1. Effect of biotic and biotic inducers on early blight disease severity on tomato:

Data in Table (1) show the early blight disease intensity (PDI), *i.e.* severity, on tomato plants 15 days after inoculation; chemical inducers treatments were used alone or in combination with the *T. Harzianum* has shown to be effective in protecting against *A. solani*. When compared to infection by *A. solani*, the treatment with potassium silicate + *Trichoderma* was the most efficient, followed by ABA+ *Trichoderma* in establishing systemic resistance against the early blight.

### 2. Effect of biotic and abiotic inducers on enhancing defense related enzymes activity in tomato against *A. solani* under greenhouse conditions

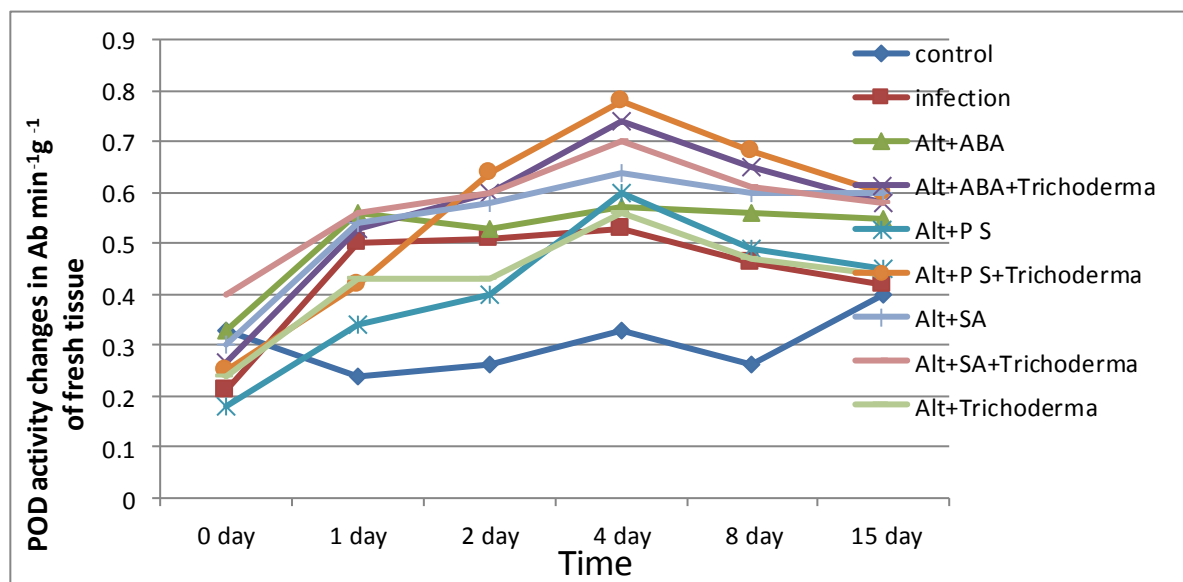
The phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and peroxidase enzyme (POD) activity were determined in the tomato cv. albiun. at 0, 1, 3, 6, 9, and 15 days after inoculation. The tomato plants were treated with ABA, PS, SA, and *Trichoderma* in combination for one week after inoculation.

POD enzyme activity was significantly increased in all chemical inducer treatments in tomato leaves when compared to the treatment by *A. solani* alone, and control as shown in fig (1).

**Table 1. Effect of certain biotic and abiotic inducers on early blight disease intensity (severity) on tomato cv. albiun, 15 days after inoculation with *Alternaria solani* in pot experiment under greenhouse conditions**

Treatments	Disease severity ( % )
<i>Alternaria solani</i> (inoculated untreated control)	34.71 ± 2.09 <sup>a</sup>
<i>Alternaria solani</i> + <i>T. harzianum</i>	29.35 ± 2.03 <sup>b</sup>
<i>Alternaria solani</i> + ABA	20.36 ± 2.03 <sup>c</sup>
<i>Alternaria solani</i> + ABA + <i>T. harzianum</i>	17.27 ± 0.81 <sup>e</sup>
<i>Alternaria solani</i> + Potassium silicate	18.02 ± 1.12 <sup>d</sup>
<i>Alternaria solani</i> + Potassium silicate + <i>T. harzianum</i>	13.31 ± 0.90 <sup>g</sup>
<i>Alternaria solani</i> + SA	20.03 ± 2.01 <sup>c</sup>
<i>Alternaria solani</i> + SA + <i>T. harzianum</i>	15.35 ± 0.86 <sup>f</sup>

\*All data are averages of triplicate measurements ± standard deviation, means followed by the different letter(s) are significantly different at  $p = 0.05$



**Figure 1. Effect of the treatment with ABA, PS, SA and *Trichoderma* on POD enzyme activity of tomato leaves infected with *A. solani***

In addition, the treatment by chemical inducer combined with *Trichoderma* was the most effective when compared to chemical inducer alone. Alt+P S + *Trichoderma* had the highest POD activity, followed by alt+ABA+ *Trichoderma* and alt+SA+ *Trichoderma*. POD activity rose over time, peaked at 4 days after inoculation, and then began to diminish. On the other hand, the treatment with SA alone, resulted in the highest POD activity, followed by (ABA) and finally PS.

Meanwhile, Data illustrated in figure (2) revealed that PPO activity rose with time, peaked at 4 days after inoculation in all treatments, and then declined. The chemical inducers considerably boosted enzyme activity in all treatments, while the treatment by chemical inducer combined with *Trichoderma* were most effective compared with chemical inducer alone. Alt+P S + *Trichoderma* was the most effective, followed by alt+SA+ *Trichoderma* and alt+ABA+ *Trichoderma*, all of which increased PPO enzyme activity significantly as compared to the control. The treatment with SA alone resulted in the maximum POD activity, followed by PS. *Trichoderma* therapy, on the other hand, was the least successful when compared to other treatments and when compared to infected plants.

Concerning PAL, data in figure (3) showed that PAL enzyme activity significantly increased in all treatment by chemical inducer on tomato, while the treatment by chemical inducer combined with *Trichoderma* were most effective compared with chemical inducer alone. Alt+P S + *Trichoderma* was highest activity followed by alt+ABA+ *Trichoderma* and alt+SA+ *Trichoderma*, while the treatment of PS individually was the most effective on increase of PAL activity followed by SA individually. Data also revealed that, the high level of PAL activity was observed from 4 days in the most treatments.

### 3. *Trichoderma* density:

Results in Table (2) showed the number of *Trichoderma harzianum* cfu under the effect of chemical inducer on tomato cultivar that infected with *A. solani*. The number of *Trichoderma* cfu has differed significantly during the period of the tomato cultivar growth. This indicated the success of soil sterilization process and eliminate all the microorganisms. While chemical inducers were applied have increased the number of *Trichoderma*. *Trichoderma* was the highest rate of the number of cfu in treatment with alt+PS+ *Trichoderma* cfu was  $5.9 \times 10^7$ , while the number of cfu in treatment with alt+ABA + *Trichoderma* was  $3.77 \times 10^7$  and the treatment with alt+SA + *Trichoderma* was  $3.68 \times 10^7$ .

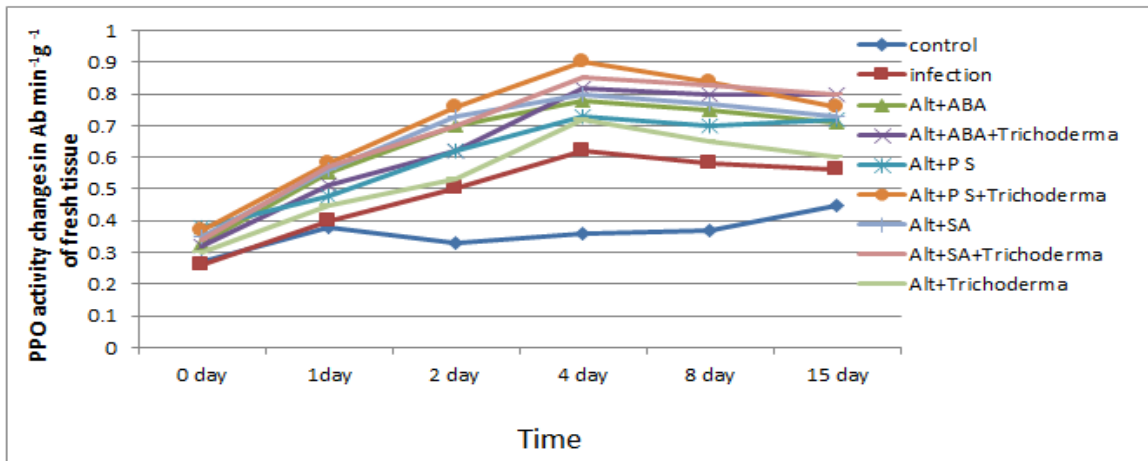


Figure 2: Effect of the treatment with ABA, PS, SA and Trichoderma on PPO enzyme activity of tomato infected with *A. solani*

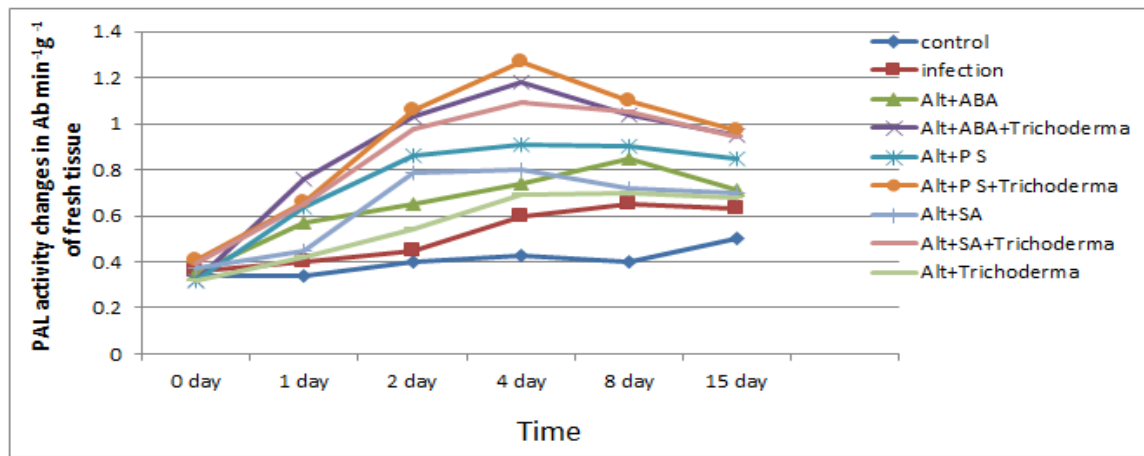


Figure 3: Effect of the treatment with ABA, PS, SA and Trichoderma on PAL enzyme activity of tomato infected with *A. solani*

Table 2. Quantification of *Trichoderma harzianum* population density (cfu) in soil infested with *A. solani* and cultivated with tomato cv.albium

Treatments	<i>Trichoderma</i> density (cfu)
Alt+ <i>Trichoderma</i>	3.64 x10 <sup>7c</sup>
Alt+ABA + <i>Trichoderma</i>	3.77 x10 <sup>7b</sup>
Alt+PS+ <i>Trichoderma</i>	5.9 x10 <sup>7a</sup>
Alt+SA+ <i>Trichoderma</i>	3.68 x10 <sup>7c</sup>

Values are average of three replicates. values followed by the different letter(s) are significantly different at p = 0.05

4. Effect of biotic and abiotic inducers on tomato growth characteristics in tomato grown in potted soil infested with *A. solani*:

Data in Table (3) showed the effect of chemical inducer and *Trichoderma* on tomato growth

characteristics (fresh shoot, dry shoot, fresh root, dry root and total fruits weight / plant) in tomato cultivar infected by *A solani*. There were significant differences between all treatment in the tested growth characteristics The treatment with alt+P S + *Trichoderma*, alt+ABA+ *Trichoderma* followed by

alt+SA+ *Trichoderma* were the most effective and showed the highest growth characteristics values with average of (191.93g, 24.63g, 22.07g, 4g and 69.8g) and (180.26g, 21.43g, 20.73g, 3.20g and 64.78g) for the previous characteristics, respectively compared with the other treatments. However, there was no significant differences between alt+ABA+ *Trichoderma* and alt+SA+ *Trichoderma* in the fresh shoot, dry shoot and fruits weight.

### 5. Effect of biotic and abiotic inducers on photosynthetic pigments in tomato grown in potted soil infested with *A. solani*:

The effect of chemical inducer and *Trichoderma harzianum* on photosynthetic pigments (chlorophyll "A," chlorophyll "B," and carotene) in tomato cultivar was infected by *A. solani* isolate, is as shown in figs. (4, 5, and 6). When compared to *A. solani* treatment alone, photosynthetic content was dramatically increased with all chemical inducer and *T. harzianum* treatments. When compared to the *A. solani* treatment alone, alt+PS + *Trichoderma* was the most effective, dramatically increasing (chlorophyll "A" and chlorophyll "B") content. The highest level of chlorophyll "A" and chlorophyll "B" content was observed at 4 days, followed by a drop. At two days after inoculation, the ABA+ *Trichoderma* treatment had the highest level of carotene concentration. While the PS and ABA treatments were the most successful, when compared to the SA and *Trichoderma* treatments, they greatly

increased the chlorophyll "A," chlorophyll "B," and carotene levels.

### 6. Quantification of the PR1, PR3 and PR5 gene expression with biotic and abiotic inducers treatment to tomato grown in potted soil infested with *A. solani*.

The expression of PR1 gene was induced by all applied treatments with different relative expression levels among treatments compared with the basal expression level of the infected plant alone. Tomato albium cultivar was used to determine gene expression of PR1, PR3 and PR5. Defense PR genes were evaluated in tomato 0, 1, 3, 6, 9 and 15 d post inoculations by *A. solani*. Tomato cultivar was treated with ABA, PS, SA individually and in combination with *Trichoderma* as a biocontrol agent for two weeks after inoculation.

Data illustrated in Fig. (7) showed that PR1 gene expression was increased in all treatments with a chemical inducer of tomato cultivar compared the infection by *A. solani* alone, while the treatment with chemical inducer combined with *Trichoderma* were of the highest gene expression compared with chemical inducer alone. Alt+P S + *Trichoderma* showed high gene expression followed by alt+ABA+*Trichoderma* and alt+SA+ *Trichoderma*, while the treatment with alt+PS was highest PR1 gene expression followed by alt+ SA in the treatment with chemical inducer alone. Data also revealed that, the highest increase of PR1 gene expression was observed at 8 days.

**Table 3. Effect of biotic and abiotic inducers on tomato growth characteristics of tomato albium. cv grown in potted soil infested with *A. solani***

treatments	Shoot weight plant(g)		Root weight plant(g)		Fruit fresh weight(g)
	fresh	Dry	Fresh	Dry	
Control(non-Infected untreated control)	210.72±1.61	20.72±1.35	20±0.36	3.7±0.20	74.77±2.21
A. solani	140.4±4.90	18.17±0.25	20.83±0.29	3.48±0.13	44.52±1.1
Alt+ <i>Trichoderma</i>	160.03±1.90	19.33±.04	18.40±0.33	3.22±0.13	53.48±2.48
Alt+ABA	170.73±0.32	21.27 ±0.40	18.97±0.68	2.89±0.13	55.84±1.38
Alt+ABA+ <i>Trichoderma</i>	179.6±2.25	21.33 ±0.37	21.1±0.8	3.88±0.10	63.6±0.38
Alt+PS	170.17±0.70	22.33±0.37	18.27±0.21	3.3±0.26	59.73±0.73
Alt+PS+ <i>Trichoderma</i>	191.93±2.75	24.63±2.10	22.07±0.11	4±0.17	69.8±0.52
Alt+SA	161.03±1.41	20.54±0.59	18.50±0.63	3.06±0.13	55.48±2.48
Alt+SA+ <i>Trichoderma</i>	180.26±4.04	21.43±2.02	20.73±1.61	3.20±0.16	64.78±1.34
LSD <sub>0.05</sub>	4.052	0.3502	0.220	0.102	2.09

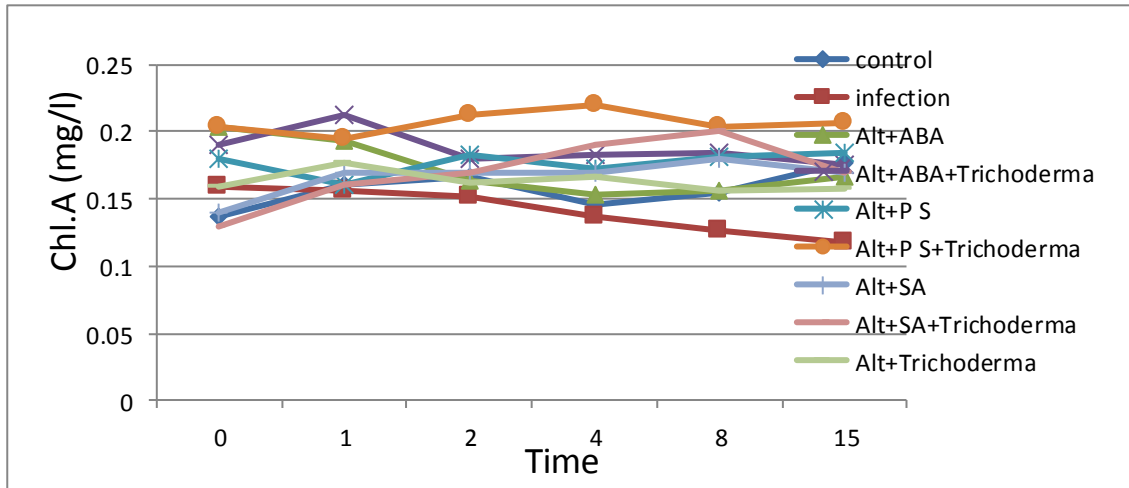


Figure 4. Effect of chemical inducers and *Trichoderma* on chlorophyll A in tomato cultivar infection with *A. solani*

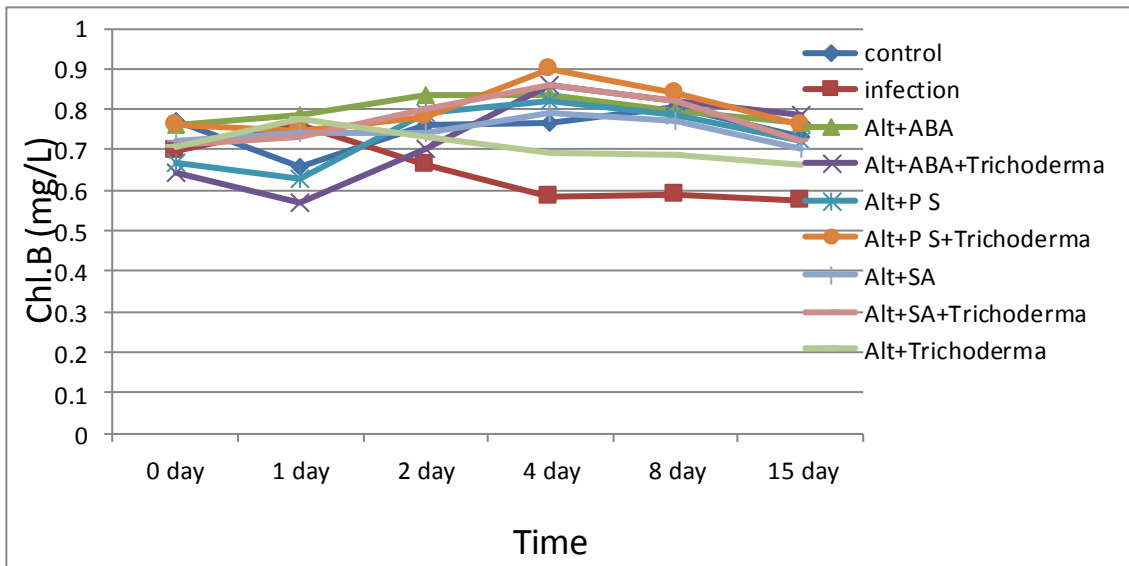
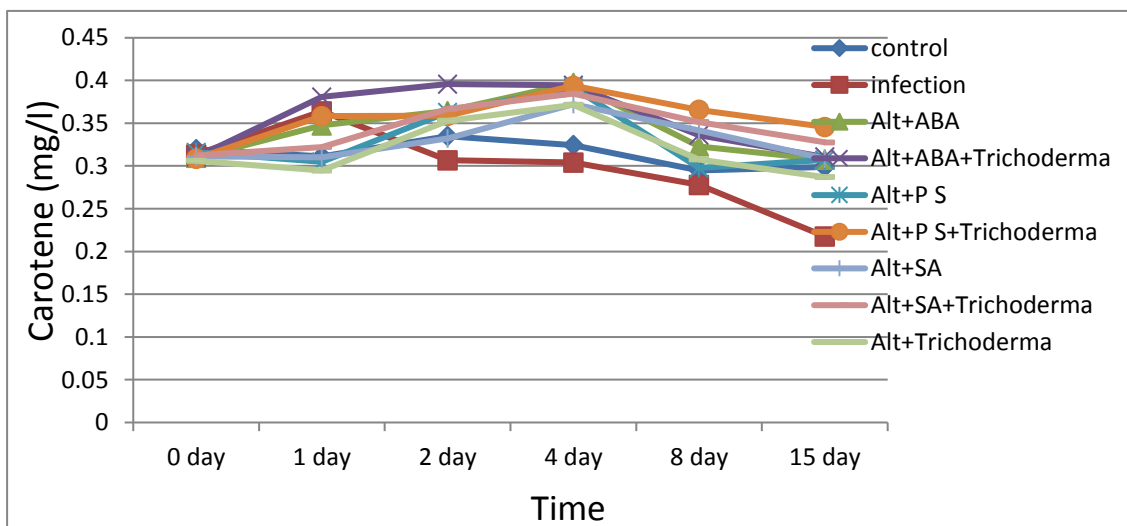
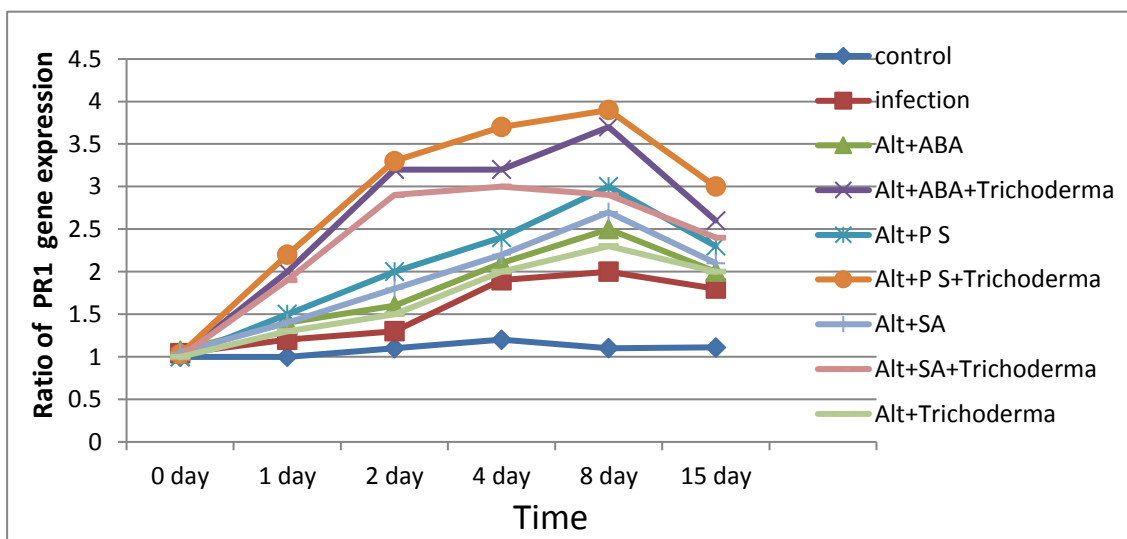


Figure 5. Effect of chemical inducer and *Trichoderma* on chlorophyll B in tomato cultivar infection with *A. solani*





**Figure 6.** Effect of chemical inducer and *Trichoderma* carotene contents in tomato cultivar infection with *A. solani*



**Figure 7.** Effect of the treatment with ABA, PS, SA and in combination with *Trichoderma* on the PR1 gene expression of tomato grown in potted soil infested with *A. solani*

On the other hand, Fig. (8) showed that PR3 gene expression was increased in all treatments with the chemical inducer of tomato cultivar compared the infection by *A. solani* alone, while the treatment with chemical inducer combined with *Trichoderma* showed high gene expression compared with chemical inducer alone. Alt+P S +*Trichoderma* was of the highest gene expression followed by alt+SA+*Trichoderma* and alt+ABA+*Trichoderma*, while the treatment with alt+ABA alone showed high PR3 gene expression

followed by alt+PS in the treatment with chemical inducer alone. Data also revealed that, a high increase of PR3 gene expression was observed at 8 days.

Also, Fig. (9) showed that PR5 gene expression was increased in all treatments with the chemical inducer of tomato cultivar compared the infection by *A. solani* alone, while the treatment with chemical inducer combined with *Trichoderma* were high gene expression compared with chemical inducer alone.

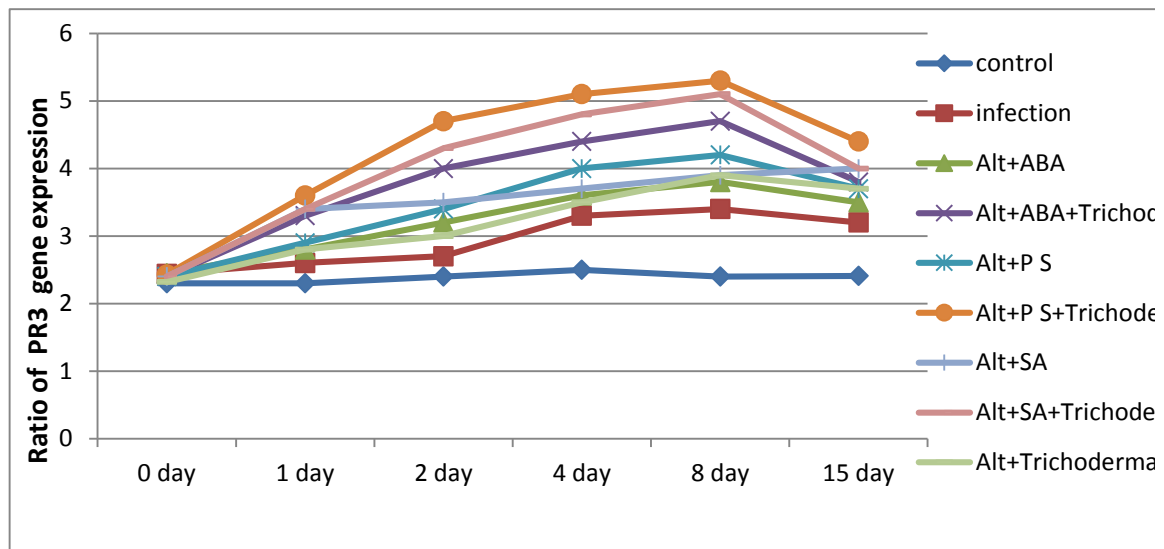


Figure 8. Effect of the treatment with ABA, PS, SA and combination with Trichoderma on the PR3 gene expression of tomato grown in potted soil infested with *A. solani*

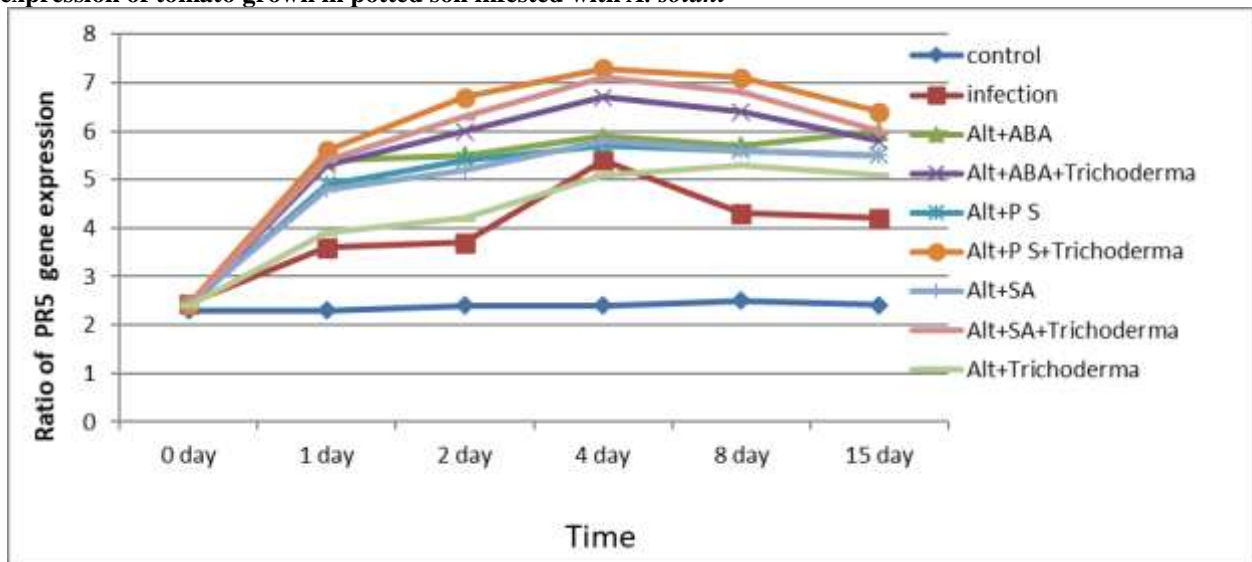


Figure 9. Effect of the treatment with ABA, PS, SA and combination with Trichoderma on the PR5 gene expression of tomato grown in potted soil infested with *A. solani*

Alt+P S +Trichoderma was high gene expression followed by alt+SA+Trichoderma and alt+ABA+Trichoderma, while the treatment with alt+PS was high PR1 gene expression followed by alt+SA in the treatment with chemical inducer alone. Data also revealed that, a high increase of PR5 gene expression was observed at 4 days. treatment with ABA alone was high PR3 gene expression followed by PS in the treatment with chemical inducer alone. Data also revealed that, a high increase of PR3 gene expression was observed at 8 days.

## DISCUSSION

Early blight disease of tomato caused by *A. solani* is one of the most serious foliar diseases causing large yield losses especially in plastic houses (Jagadeesh and Jagadeesh, 2009 and EL-Tanany *et al.*, 2018). Fungicides successfully managed the disease; however, fungicides have many hazardous effects on the environment and humans. Plant resistance inducers are among the most important alternative methods for controlling plant diseases that are safe and rapidly biodegradable (Anand *et al.*, 2009a, Ragab *et al.*, 2009 and Simonetti *et al.*, 2012). On the basis of the results

obtained in the present study inducer treatments when used individually or in combination with the *Trichoderma* showed significant protective effects against *A. solani* in pot experiment under greenhouse conditions. The treatment with Potassium silicate (PS)+ *Trichoderma* was the most effective, followed by ABA+*Trichoderma* in decreasing PDI of early blight on tomato by inducing systemic resistance against the Early Blight compared with infection with *A. solani* alone and control. These findings were in harmony with reports published by EL-Tanany *et al.* (2018), Abou-El-Hassan *et al.* (2020) and Prakash *et al.* (2020). Potassium silicate could increase the concentration of antifungal compounds such as increase the concentration of phenolic compounds which leads to decrease disease incidence mechanisms (Van Bockhaven *et al.*, 2013 and Sakr, 2016). Also, Silicon has offered protection against fungal diseases by strengthening cell walls which make it difficult to penetrate and colonize the plant by pathogens where, the effectiveness of silicon is due to the physical barrier formed by the deposition of silicon under the cuticle (Keller *et al.*, 2015). Exogenous ABA, on the other hand, could improve disease resistance against *A. solani* infection in tomatoes by activating defense genes and increasing defense-related enzyme activity (Song *et al.*, 2011, EL-Argawy *et al.*, 2017 and Prakash *et al.*, 2021).

*Trichoderma* fungal species are known to be effective in reducing pathogenic fungi by generating metabolites derived from cyclopentenone, which have an antagonistic effect on the pathogen (George *et al.*, 1977 and Strunz *et al.*, 1977). Several investigators have noted that fungi belonging to the genus *Trichoderma* have antifungal properties, which may be attributed to stimulation of anti-pathogen chemicals or the ability to wrap around the pathogen and create aspirin on its surface. In addition, most antagonistic fungi produce a variety of enzymes that break down the pathogen's cell wall, as well as medications (Inbar *et al.*, 1998; Chet *et al.*, 1998). *Trichoderma* spp use as biocontrol agents due to their ability to regulated the expression level of some PR-protein genes i.e., PR-1 and PR-5 under infected by *Alternaria solani* (Selim, 2015) and improve the efficiency of photosynthetic and respiratory activity, by reprogramming plant gene expression (Mastouri *et al.* (2010)

The POD, PPO, and PAL activities significantly increased in all treatments with chemical inducer and *Trichoderma* of tomato fruits when compared to the treatment with untreated inoculated control (infected) and healthy plant (control). In addition, the treatment with chemical inducer combined with *Trichoderma* was the most effective when compared to chemical inducer and *Trichoderma*. The maximum POD and PAL

activities were observed with P S + *Trichoderma* followed by ABA+ *Trichoderma* and SA+ *Trichoderma*). Biotic inducers are known to have eliciting activities leading to a variety of defense reactions in host plants in response to microbial infection, including the defense related enzymes and accumulation of phenolic compounds as well as specific flavonoids (Abd El-Rahman *et al.*, 2012; Hussein *et al.*, 2018 and Sarhan *et al.*, 2018). Silicon-enhanced biochemical resistance is associated with increasing the activity of defense-related enzymes, such as polyphenoloxidase, glucanase, peroxidase, and phenylalanine ammonia-lyase (PAL) (Fauteux *et al.*, 2005; Datnoff *et al.*, 2007; Van *et al.*, 2013). However, P S + *Trichoderma* was the most effective followed by SA+ *Trichoderma* and ABA+ *Trichoderma* and they significantly enhanced PPO enzyme activity comparing with the control. On the other hand, the treatments with SA, PS and ABA alone were the highest in POD, PPO and PAL activities followed by ABA, PS and ABA, respectively. The activities of POD and PPO were reached the highest level at 4 days, then declined. Whereas, *Trichoderma* treatment was the least effective in this respect compared with other treatments comparing with the infected plants. These findings are in harmony with reports published by EL-Tanany *et al.*, (2018), Khalil and Abdelghany (2021) and Prakash *et al.* (2021) as they found that, treating tomato plants with ABA, SA and *Trichoderma* before inoculation with *A. solani* increased the activities of peroxidase, Polyphenol oxidase [catechol oxidase] and phenylalanine ammonia-lyase and reduced the rates of leaf infection and disease index. Salicylic acid was endogenous signal for the activation of certain plant defense responses, including pathogenesis related gene expression, oxidative defensive enzymes, polyphenol oxidase (PPO), Catalase (CAT) superoxide dismutase,  $\beta$ -1, 3 glucanase, peroxidase (POD) and phenylalanine ammonia lyase (PAL) and enhanced resistance to many pathogens such as *A. solani*, *Botrytis cinerea*, *Clavibacter michiganensis* subsp *michiganensis*, *Ralstonia solanacearum* and *Meloidogyne javanica* (Li and Zou 2017 and Wang *et al.* 2013). ABA treatment to tomato plants reduces early blight disease severity by triggering defense genes and defense related compounds viz., POD, PPO and PAL (Song *et al.*, 2011, Wang *et al.*, 2013 and Nassar and Adss, 2016). Our results indicate the positive relationship between the decrease of disease severity of early blight and increased activity of polyphenol oxidase, peroxidase and PAL enzymes, which is related to induction of induced systemic resistance activity in tomato plants against *A. solani*.

On the other hand, data showed that there were significant differences between all treatments in growth characteristics (fresh shoot, dry shoot, fresh root, dry

root weight and total fruits weight / plant) under infection with *A. solani*. The obtained results are in accordance with those obtained by El-Shennawy and Abd El-All (2018), Khalil and Abdelghany (2021). The treatment with P S + Trichoderma, ABA+ Trichoderma and SA+ Trichoderma were the most effective they have the highest growth characteristics compared with the other treatments. No significant differences were detected between ABA+ Trichoderma and SA+ Trichoderma in the fresh shoot, dry shoot and fruits weight. This is in harmony with (Abay and Sheleme, 2011, and Abou-El-Hassan *et al.*, 2020) as they reported that the potassium silicate application increases growth parameters of the potato plants. Trichoderma spp. as a biocontrol agents, they are known to reduce plant pathogenic assault and boost plant growth and yield by increasing growth hormones and the beneficial microbiome of the plant (Dubey *et al.*, 2007) Improving the growth of plants that were treated with potassium silicate can be attributed to the role of potassium in many metabolic processes and acts as a catalyst or activator of certain enzymes into the plant, thus improving growth parameters of plants (Dubey *et al.* 2007; Khatabi *et al.* 2012). Tomato plants treated with antioxidants (salicylic acid, ascorbic acid, and boric acid) increased growth traits, plant height, leaf area fresh and dry weight, according to El-Shennawy, Abd El All. (2018) and El-Garhy *et al.* (2020).

The combination of potassium silicate and *Trichoderma* was the most beneficial, greatly increasing (chlorophyll "A" and "B") content when compared to control. At 4 days, the levels of chlorophyll "A" and "B" were at their peak, then began to fall. At 2 days post-inoculation, the ABS+ Trichoderma therapy had the highest level of carotene concentration. This could be owing to potassium's active engagement in a range of physiological activities, such as photosynthesis, protein synthesis, and plant tissue water status maintenance (Ebert, 2009; Marschner, 2012). Also, silicon has a role in improving the chain of photosynthesis and preventing the deterioration of chlorophyll, where the action of silica bodies as windows allowed the light transmission to the mesophyll area (Epstein, 1999; Pilon *et al.*, 2014). In this respect, Salim *et al.* (2014), Abd El-Gawad *et al.* (2017) and Abou-El-Hassan *et al.* (2020) demonstrated that foliar application of potassium silicate improved the vegetative growth parameters of potato plants. Mady (2009) and El-Shennawy and Abd El-All. (2018) reported that, application of salicylic acid increased photosynthetic pigments, total carbohydrates and crude protein concentrations in leaves of treated plants as compared with those of untreated ones on tomato plants. Improved photosynthetic capabilities of different plant species induced by various endophytic strains of *Trichoderma* due to the increase of photosynthetic

pigments or gene expressions regulating chlorophyll biosynthesis, light-harvesting complex proteins were documented by Harman *et al.* (2021). Results also indicated that the chemical inducers applied have increased the number of *Trichoderma* in soil. *Trichoderma* was the highest rate of the number of cfu in treatment with PS+ *Trichoderma* cfu was  $5.9 \times 10^7$ , while the number of cfu in treatment with ABA + *Trichoderma* was  $3.77 \times 10^7$  and the treatment with SA + *Trichoderma* was  $3.68 \times 10^7$ .

Genetically, PR1, PR2 and PR5 gene expressions increased in all treatments with chemical inducer of tomato cultivar compared the infection by *A. solani* alone, while the treatment with chemical inducer combined with *Trichoderma* were highest gene expressions compared with chemical inducer alone. P S + *Trichoderma* showed the highest PR1 gene expression followed by ABA+ *Trichoderma* and SA+ *Trichoderma* at 8 di post inoculation, while PR2 and PR5 gene expressions reached the highest level with P S + *Trichoderma* followed by SA+ *Trichoderma* and ABA+ *Trichoderma* at 8 di post inoculation. The highest PR1 and PR5 gene expressions were found in the treatment with PS followed by SA alone. The PR3 gene expression was the highest in the treatment with ABA followed by PS alone. These findings matched those of Selim (2015). These findings suggested that inducing systemic defense mechanisms using mutualistic *Trichoderma* isolates like T10 could be one of the ways to combat *Alternaria solani* infection. The gene of PR1 expression was upregulated in leaves were treated by salicylic acid and abscisic acid at one day after of inoculation with *alternaria solani*, which confirms the role of SA and ABA in systemic acquired resistance (Prakash *et al.*, 2021). These findings are in agreement with our results. Meanwhile, Vivancos *et al.* (2015) found that the *Arabidopsis* were high resistant to infection with *Golovinomyces cichoracearum* than control plants when treated with Si, and corresponded with higher expressions of PR1, PR2 and PR5 defense genes.

## CONCLUSION

The present study confirmed that the treatment with chemical inducers combined with the bio agent *Trichoderma harzianum* have the potential to decrease disease severity of tomato early blight and to improved growth characters and photosynthetic pigments through different mechanisms of action including stimulation of defense-related enzymes and pathogen related proteins such as PR1, PR3 and PR5 gene can be involved in management of early blight disease.

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## الملخص العربي

### تأثير حمض الأبسيسيك وحمض الساليسيليك وسيليكات البوتاسيوم والتريكودرما كعامل مكافحة حيوى لاستحثاث المقاومة في الطماطم ضد مرض اللفحة المبكرة الناجم عن *Alternaria solani*

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بمعاملة *A. solani* زيادة نشاط إنزيمات POD و PPO و PAL والتعبيرات الجينية PR1 و PR2 و PR5 بشكل كبير في جميع المعاملات مقارنة بالمعاملة بواسطة *A. solani* والكنترول ، بالإضافة إلى المعاملة بالمحفز الكيميائي جنباً إلى جنب مع *Trichoderma* كانت أعلى مقارنة مع المعاملة بالمحفز الكيميائي. وحده. لوحظ اعلى نشاط للانزيمات من POD وأنشطة PAL وتعبير جين PR1 كان في المعاملة ب + alt+PS *Trichoderma* متبوعاً بـ *Trichoderma* + alt+ABA بينما وصلت التعبيرات الجينية PR2 و PR5 ونشاط POO إلى أعلى مستوى بالمعاملة *Trichoderma* + alt+PS + ثليها + alt+SA *Trichoderma* و *Trichoderma* + alt+ABA كان *Trichoderma* كان اعلى كثافة لعدد cfu للتريكودرما عند المعاملة بـ *Trichoderma* PS الكلمات الدالة: طماطم، لفحة، الترناريا، التريكودرما، مستحثات، انزيمات، التعبير الجيني.

في هذه الدراسة أردنا أن نرى كيف أثرت ثلاثة محفزات كيميائية هي حمض الأبسيسيك وسيليكات البوتاسيوم وحمض الساليسيليك مختلطة مع التريكودرما على شدة مرض اللفحة المبكرة في الطماطم ، وكذلك بعض خصائص النمو والقدرة على التمثيل الضوئي والإنزيمات المتعلقة بالدفاع وتحليل التعبير الجيني لجينات PR1 و PR3 و PR5 ، كذلك تأثيرها على كثافة التريكوديرما. وكانت النتائج: المعاملة *Trichoderma* + alt+PS هو الأقل شدة للمرض ، يليه *Trichoderma* + alt+ABA ، وكان معاملة *Trichoderma* أعلى كثافة للمرض مقارنة بمعاملة *A. solani*. وكانت alt+P *Trichoderma* + alt+SA و *Trichoderma* + alt+ABA + *Trichoderma* هي الأكثر فاعلية و أفضل في خصائص النمو. وزاد محتوى التمثيل الضوئي معنوياً مع كل المعاملات بالمحفز الكيميائي و *T. harzianum* مقارنة بمعاملة *A. solani*. كان *Trichoderma* + alt+PS هو الأكثر فاعلية حيث عزز بشكل كبير محتوى الكلوروفيل مقارنة