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Efficiency of biotic and abiotic inducers for controlling tomato early blight disease

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ABSTRACT

Four chemical inducers, i.e. oxalic, salicylic acid, cobalt sulphat and dipotassium hydrogen phosphate; eight *Trichoderma* isolates and four bacterial bioagents, *i.e. Bacillus megaterium, B. subtilis, Pseudomonas fluorescens*, *Serratia marcescens,* were tested against early blight diseases of tomato caused by *Alternaria solani. In vitro*, the all tested bioagents and chemical inducers caused significant reduction in the growth of *A. solani. Trichoderma viride*1 caused the highest reduction followed by *T. hamatum* respectively. *P. fluorescens* and *Bacillus subtilis* were the best antagonistic bacteria in reducing growth of *A. solani* while, Salicylic acid at concentrations 10 mM completely inhabited the linear growth of *A. solani*. Under greenhouse conditions, spraying tomato plants with any one of the tested antagonists bioagents before inoculation with *A. solani* decreased the early blight disease severity, as well as increased fresh and dry weight of shoots and roots compared with the infected control plants. *T. viride*1 and *T. hamatum* were the best effective treatments for disease controlling followed by *P. fluorescens* and *B. subtilis*. Tomato plants treated with salicylic acid recorded the lowest disease severity as well as increasing in fresh and dry weight of shoots and roots compared with control treatment. Treated *A. solani* spores suspension with temperature 85°C and 90°C for 10 minutes inhabited spore germination. Moreover, treated tomato plants with *A. solani*spores subjected to temperature 85°C or 90°C for10 minutes reduced disease severity in treated tomato plants comparing with control and increased shoot root fresh and dry weight and Under field conditions, *T. viride*1, *T. hamatum* and *T. lignorum*as fungal bioagents and *P. fluorescens* and *B. subtilis* bacterial bioagents as well as salicylic acid were the best effective treatments for reducing Early blight disease severity and increase the tomato yield comparing with control. All tested chemical inducers and bioagents treatments increased the phenols and flavonoids content in tomato plants. Also, using bioagents and chemical inducers caused considerable increase in the activity of peroxidase, polyphenol oxidase and chitinase enzymes that play an important role in plant defence mechanisms against pathogen infection.

Key words: Tomato, Early blight, *Alternaria*, bioagents, inducing resistance.

Introduction

Tomatoes (*Solanum lycopersicum* L., syn. *Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetables in the world. It occupied the second rank in importance after potato in many countries Prajapati *et al.* (2014). It considers an important cash and industrial crop in many parts of the world (Ayandiji and omidiji, 2011).Early blight disease of tomato caused by the fungus *Alternariasolani*is one of the most common foliar diseases of tomatoes, which damages the leaves, stalks, stems and fruits causing severe destruction of the aerial part and reduction of the size and number of fruits, resulting heavy losses in yield up to 79% (Sherf and MacNab, 1986; Gwary and Nahunnaro, 1998).Best estimates suggest annual expenditure globally on fungicides for control of *Alternaria* spp. is around $32 million in tomatoes and $45 million in potatoes (Kemmitt, 2013). As for biological control, Abdel-Kader *et al*. (2012) evaluated the efficacy of bio-agents, application as foliar spray against early and late blights of tomato diseases incidence in open greenhouse conditions. Application with either *T. harzianum* and *B. subtilis* showed significant reduction in diseases incidence comparing with the other applied bio-agentson tomato. The evident inhibition zone observed in dual culture plates, suggested an antibiosis-like mechanism. Also, Zghair *et al*. (2014) mentioned that the bioagents *Trichoderma harzianum* and *Pseudomonas fluorescens* (as seed

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treatment + two foliar sprays) were effective in reducing the tomato early blight disease intensity. Anuj *et al.* (2015) examined seventeen isolates of *Pseudomonas fluorescens* (Pf) for growth promotion and the induction of systemic resistance against early blight disease of tomato. Microorganisms acting through antibiosis, generally have a wide action spectrum, and thus pathogen inhibition by producing toxic substances is more effective than any other mechanism of action Leelasuphakul *et al.* (2008). Their protective effect involves different mechanisms of action that directly antagonize pathogen growth. *B. subtilis* group is known to produce a variety of bioactive metabolites leading to antibiosis Stein (2005) and Chen *et al.,* (2009b) and able to compete for space and nutrients Nagórska *et al. (*2007). Eid, (2017) indicated that sodium bicarbonate, potassium hydrogen carbonate, ascorbic acid and salicylic acid *in vitro* reduced the growth of *A. solani* on PDA plates compared control. Also, it was found a positive relation between the high concentration of the tested and the reduction of *A. solani*. Salicylic acid was more effective one in reducing the growth of *A. solani*. Under greenhouse conditions, spraying tomato plants with the tested chemical inducers against early blight disease caused by *A. solani*was moderately effective in controlling the infection. EL-Gammal (2005) who showed that spraying faba bean plants with suspension of un-viable heated spores of *B. fabae* spores scored a remarkable depression in chocolate spot disease severity comparing with unsprayed.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). Ramamoorthy *et al*., (2002) they mentioned, the induction of defense enzymes involved in phenylpropanoid pathway accumulation of phenolics and PR-Proteins (Phenylalanine Ammonia-lyase (PAL), Peroxidase (PO) and Polyphenoloxidase (PPO)).

The present research was conducted to evaluate the efficacy of some biotic and abiotic inducersfor controlling tomato early blight disease under greenhouse and fieldconditions in addition to estimate some biochemical response in treated plants.

Materials and Methods

1. Isolation and identification of the causal fungus of tomato early blight disease:

Leaves of tomato plants with early blight typical symptoms were collected from three governorates, *i.e.* El-Behira (El-Nubaryia), El- Qalubia (Moshtohor) and El- Minia (Magagha) during autumn 2013 growing season. Isolation was carried out from the collected samples in the same day of collection as possible or after storage in the refrigerator at 4-6ºC for a few days. The infected tissues were cut into small pieces and were surface sterilized with sodium hypochlorite (0.5%) for 2-3 minutes, then washed for several times with sterilized distilled water. Small pieces from the edges of the sterilized pieces were dried between to sterilized filter paper and transferred directly to the PDA medium in Petri dishes 9 cm and incubated under 12 h light and 12h dark at 25±1°C according to Naik *et al*. (2010). Pure cultures were maintained on PDA slants and stored in a refrigerator at 5-10°C. The purified isolates were identified according to their morphological features using the descriptions of Singh (1982) and Barnett and Hunter (1987).

2. Pathogenicity test and susceptibility of commercial tomato cultivars to early blight disease:

This experiment was carried out under greenhouse conditions in 2014 experiments in a green-house of Plant Pathology Department, Faculty of Agriculture, Benha University, Benha, Egypt.
The inoculum of *Alternaria sp*. was prepared by culturing each of the obtained isolates on PDA medium at 27°C for 15 days. Then 10 ml of sterile distilled water was added to each plate and colonies were carefully scraped with a sterile needle. The resulting conidial suspension from each isolate was adjusted to 5 x 10^6 spores/ml. and used for the inoculation.

Five tomato hybrids namely; super stain B hybrid, super strain B normal, Elisa, Kasel rock and super alteray were obtained from Qaha nursery in El-Qalubia were transplanted in plastic pots (30 cm in diameter) filled with sterilized soil. Three seedlings per pot were transplanted for each hybrids separately. Three replicates were used from each hybrid and inoculated with 30 ml of spore suspension using atomizer. Plants sprayed with the same amount of distilled water were used as

control. After inoculation, to ensure good spore germination, the inoculated plants were kept under polyethylene bags for 48 h to increase the relative humidity (Chen *et al*., 2003). After 48 h, bags were removed and plants were kept under greenhouse conditions. Pots were arranged in a completely randomized design. Two weeks after inoculation, disease severity was recorded following the score chart 0- scale (0 = healthy; 1 = 1-5%; 2 = 6-10%; 3 = 11-25%; 5 = 6-50%, 7 = 51-75%, and 9 = > 76% of the leaf area infected) proposed by Latha *et al.* (2009).

$$\text{Disease severity (\%)} = \frac{\sum (n \times r)}{NR} \times 100$$

Where:

n = Number of infected leaves on the plant.
N= Total number of leaves examined.
r = Numerical rate of infected leaves.
R= Highest numeric rate.

3. Laboratory experiments.

3.1. Effect of antagonistic fungi on growth of A. solani invitro.

Two discs (Ø 5 mm) of 4-day-old of both antagonistic fungi (*T. harzianum (3 isolates), T. viride (2 isolates), Trichoderma hamatum , Trichoderma album* and *Trichoderma lignorum*) were obtained from Plant Pathology Dept., Fac. Agric. Benha Univ. and *A. solani*were inoculated simultaneously each opposite the other 1 cm apart from the plate edge in individual plates (Ø 9 cm) contained 10 ml PDA medium. In control treatment, the plates were inoculated each with 1 discs of mycelial growth of a given isolate of *A. solani*. Three plates were used for each particular treatment. All dishes were incubated at 30±1°C for 10 days (Gomaa, 2001). Percentage of the fungal growth reduction (X) was calculated by using the following formula (4) suggested by (Abd-El-Moity, 1985).

$X = G_1 - G_2 / G_1 \times 100$

Where: X: fungal growth reduction.
G_1: linear growth of the pathogen inoculated alone.
G_2: linear growth of the pathogen inoculated against the antagonistic fungus.

3.2. Effect of antagonistic bacteria on growth of A. solani invitro.

Studying the effect of antagonistic bacteriaisolates (*Pseudomonas fluorescens, Serratia marcensens, Bacillus megaterium and Bacillus subtilis*) were obtained from Plant Pathology Dept., Fac. Agric. Benha Univ. on growth of *A. solani* were conducted as following, individual plates (Ø 9 cm) contained PDA medium were streaked at one side 1cm apart from the plate edge with a given isolate of antagonistic bacteria with a loop full of the antagonistic bacteria (48 hrs- old) grown on liquid nutrient medium(NG) and incubated for 24 hrs at 28° C. Thereafter the same plate was inoculated at the opposite side 1cm apart from the plate edge with Ø 9 mm disc of 4-day-old plain agar culture of the isolate of *A. solani*. Three plates were used for each particular treatment. All plates were incubated at 30±1°C for 5 days. The inhibition zone (in mm) between bacteria and the pathogen was measured according to Maurhofer *et al*. (1995).

3.3. Effect of different chemicals inducers on the linear growth of A. solani invitro:

This study was designed to investigate the inhibitory effect of some chemicals on the linear growth of *A. solaniin vitro*. The used chemicals were tested at 3 concentrations as follow:

A. Oxalic acid and salicylic acid were tested at concentration of 1, 5.0 and 10.0 mM.
B. Dipotassium hydrogen phosphate (K_2HPO_4) at concentrations 150, 200 and 300mM.
C. Cobalt sulphate ($COSO_4$) was tested at concentrations 1, 5 and 10 mg/L.

The amount required for obtaining a known concentration of any chemical was calculated and added aseptically to known amount of warm sterilized Czapek's agar medium and poured before solidification into Petri dishes (Ø 9 ml/plate) then plates were inoculated into the center with equal

discs (Ø 5 cm) obtained from the periphery of 10 days old cultures of *A. solani*. Plates contained media without any chemical inoculated with *A. solani* was served as control treatment. Three plates were used for each particular concentration. All plates were incubated at 30±2°C. The experiment was terminated when mycelial mats covered medium surface in control treatment, all plates were examined and growth reduction was calculated as mentioned**.**

3.4. Effect of exposure A. solanispores suspension to temperature on A. solanispores germination.

The inoculum of *A. solani*. was prepared by culturing on PDA medium at 27°C for 15 days. Then 10 ml of sterile distilled water was added to each plate and colonies were carefully scraped with a sterile needle. The resulting conidial suspension was adjusted to 5 x 10^6 spores/ml. Conidial suspension was exposed to temperature at 60,70,80,85 and 90°C for 10 minutes. 0.1 ml of each spore suspension was placed on sterilized dry clean glass slides. Each slide was placed on a U-shaped glass rod in a moist chamber made up of sterile Petri dish lined with filter paper saturated with sterile distilled water. Petri dishes were incubated at 25±1.5°C for 24 hours before examination. Three replicates were used for each particular treatment. The percentage of germination was based on counts of 300 conidia.

4. Greenhouse experiments:

4.1. Preparation of bio-agent inocula:

Pseudomonas fluorescens, *Serratia marcensens*, *Bacillus megaterium* and *Bacillus subtilis*, were grown on nutrient broth medium for 2 days and then their cell suspensions were adjusted at rate 2.8 x10^8cfu/mL for each one of them. Meanwhile, fungal bioagents *i.e., T. harzianum, T. viride*, *Trichoderma hamatum , Trichoderma album* and *Trichoderma lignorum* were grown on PDA medium for 7 days and then their spore suspensions were adjusted at rate 2.5x10^5 spore/mL of each one of them.

4.2. Effect of treating tomato plants with biological control agents on incidence and severity of early blight disease as well as fresh and dry weight of shoot and root.

This experiment were conducted at the experimental greenhouse of faculty of agriculture, Benha Univ., to evaluate the effect of spraying tomato plants with biological agents on incidence and severity of early blight disease.

Three pots were used as replications for each treatment as well as for the untreated control treatment. Healthy seedlings (30-day-old) of susceptible tomato (cv. Super Strain B) and resistant seedlings of tomato (cv. Castle rock). Plants received one spray at two week intervals with the tested biological agent i.e., *Pseudomonas fluorescens, Serratia marcensens, Bacillus megaterium, Bacillus subtilis* cell suspension*, T. harzianum*, *T. viride*, *Trichoderma hamatum, Trichoderma album* and *Trichoderma lignorum* spore suspension. After 48h plants received one spray of *A. solani*. The disease incidence and severity of early blight was calculated as mentioned before. And also average fresh and dry weight/pot of shoot and root in tomato in cultivars were recorded.

4.2. Effect of treating tomato plants with chemical inducers on incidence and severity of early blight disease as well as fresh and dry weight of shoot and root.

This experiments were conducted as mentioned in (4-2) while plants were sprayed with oxalic acid and salicylic acid at concentration 10.0 mM., K_2HPO_4 at concentration 300mM. and $COSO_4$ at concentrations10 mg/L.

4.4. Effect of treating tomato plants with un-germinated A. solani spores exposed to temperature as cross protection on early blight disease severity.

Under greenhouse healthy seedlings (30-day-old) of susceptible tomato(cv. Super Strain B) and resistant seedlings (30-day-old) of tomato (Castel rock) were treated with un-germinated spores that exposed to temperature at 85 and 90°C for 10 minutes to evaluate their effect in inducing tomato plants resistance. Three pots were used as replications for two different cultivars (cv. Super Strain B and Castle rock) as well as for the untreated control treatment. After 48h tomato plants received one spray of *A. solani*. Percentages of disease incidence and disease severity were assayed as mentioned before.

5. Determination of enzymes activity:

Treated (cv. Super Strain B) leaf samples were ground with 0.2 M TrisHCl buffer (pH 7.8) containing 14 mM*β*-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine enzyme activities (Tuzun *et al.* 1989).

5.1. Determination of Peroxidase (PO):

Peroxidase activity was expressed as the increase in absorbance at 425 nm/gramfresh weight/15 minutes and determined according to the method described by Allam and Hollis, (1972) and Kar and Mishra (1976).

5.2. Determination of Polyphenoloxidase (PPO):

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

5.3. Determination of chitinase

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

6. Determination of phenols and flavonoids activities:

6.1. Determination of total phenols content:

Total phenols was determined using Spectrophotometer (SPECTRONIC 20-D) at 520 nm according to the method of Bary and Thorpe, (1954).

6.2. Determination of total flavonoids content:

The flavonoid content is expressed as milligrams of rutin equivalents per gram of sample (mg RE/g) according to the method of PeixotoSobrinho *et al.* (2008).

7. Activity gel electrophoresis

7.1. Peroxidase.

Peroxidase isozymes activity was performed according the method by Sindhu *et al.* (1984).

7.2. Polyphenol oxidase.

The Polyphenol oxidase isozymes activity was performed according the method by Aydemir (2004).

7.3. 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.

8. Filed experiments

8.1. Effect of spraying with biological control agents on the incidence and severity of the early blight disease in tomato (Cv. Super Strain B).

Two experiments (during March to July 2015 and 2016) were conducted at the experimental farm of faculty of agriculture, Benha Univ., to evaluate the effect of spraying tomato plants with biological agents on incidence and severity of early blight under field conditions.

Field plots (3 x 3.5 m^2) comprised three rows and 4 plants per row arranged in completely randomized block design. Three plots were used as replications for each treatment as well as for the untreated control treatment. Healthy seedlings (30-day-old) of tomato (cv. Super Strain B) were transplanted and received all recommended agricultural practices as irrigation and fertilization. Plants received four sprays at two week intervals with the tested biological agent *i.e*., *Pseudomonas fluorescens, Serratia marcensens, Bacillus megaterium, B.subtilis* cell suspension, *T. harzianum, T. viride, Trichoderma hamatum*, *Trichoderma album* and *Trichoderma lignorum* spore suspension. Disease incidence and severity of early blight was calculated as mentioned before. And also average weight of fruits/plot was recorded.

8.2. Effect of spraying with chemical inducers on incidence and severity of early blight disease in tomato.

Two experiments were conducted as mentioned in (8-1) while plants were sprayed with, oxalic acid and salicylic acid at concentration 10.0 mM., K_2HPO_4at concentration 300mM. and $COSO_4$ at concentrations10 mg/L.

7. Statistical analyses:

Statistical analyses of all the previously designed experiments have been carried out according to the procedures (ANOVA) reported by Snedecor and Cochran (1989). Treatment means were compared by the least significant difference test “L.S.D” at 5% level of probability.

Results

1. Pathogenicity test and susceptibility of commercial tomato cultivars to early blight disease:

Data in Table (1) indicated that all the tested isolates of *A. solani* caused typical early blight symptoms with different degrees of disease incidence and disease severity. Results revealed that *Alternaria solani*1 (AS1) with Super strain B hybrid recorded the highest disease incidence and disease severity 95.00,56.33%,respectively compared with other cultivars. Meanwhile, disease incidence and disease severity in the case of AS1 with Supers train B normal, Kasel rock and Elisarecorded72.30-18.80, 66.00-20.90 and 79.30-25.50%, respectively.However,AS1 with Super Alteray recorded the lowest disease incidence and disease severity.Whereas,AS2 with super stain B hybrid recorded disease incidence and disease severity were 84.30 and 40.00%, respectively.

Generally, AS1 which isolated from El-Behira (El-Nubaryia) was the most virulent isolate and was selected to continue other experiments followed by AS2 which isolated from El- Qalubia (Moshtohor). Whereas, AS3 which isolated from El- Minia (Magagha) was the low virulent isolate. On the other hand, Super Alteray was the most resistance hybrid. Super strain B normal, Elisa and Kasel rock were resistance. While Super stain B hybrid was susceptible hybrid and was selected to continue other experiments.

Table 1: Pathogenicity test and the susceptibility of commercial tomato cultivars to early blight disease:

Cultivars	Isolate1(El-Behira)		Isolate 2 (El- Qalubia)		Isolate 3 (El- Minia)	
	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
Super stain B hybrid	95.00	56.33	84.30	40.00	77.00	30.25
Super strain Bnormal	72.30	18.80	62.60	12.30	51.00	11.00
Super Alteray	60.30	15.50	48.30	8.00	38.70	8.00
Elisa	79.30	25.50	69.30	15.30	68.70	12.00
Castel rock	66.00	20.90	53.30	10.50	58.00	11.80
L.S.D.at 5%	Cultivars2.27		Isolate 2.29		CV. Interaction 6.86	

2. Laboratory experiment

2.1. Effect of antagonistic fungi on growth of A. solaniin vitro.

Data in Table (2) indicated that all the tested antagonistic fungi caused significant reduction in the growth of *A. solani* compared with the control treatment. The antagonistic fungi, *i.e.T.viride*1, *T.hamatum* , *T. lignorum* and *T. album* caused the highest reduction in linear growth of *A. solani*1, by88.52,84.08,81.56and78.52%respectively, followed by, *T. viride*2,*T. harzianum*1, *T.harzianum*2 and *T.harzianum* 3 which reduced the linear growth of the fungus, by77.44, 76.30, 73.71 and 73.33% respectively. Whereas, *harzianum* 3 was the least effective one in this respect.

Table 2: Effect of antagonistic fungi on growth of *A. solaniin vitro.*

Treatment	**Linear growth (mm)**	**% Reduction**
Trichoderma harzianum 1	21.33	76.30
Trichoderma harzianum 2	23.66	73.71
Trichoderma harzianum 3	24.00	73.33
Trichoderma viride 1	10.33	88.52
Trichoderma viride 2	20.30	77.44
Trichoderma hamatum	14.33	84.08
Trichoderma album	19.33	78.52
Trichoderma lignorum	16.60	81.56
Control	90.00	00.00
L.S.D. at 5%	5.06	

2.2. Effect of antagonistic bacteria on growth of A. solani in vitro

Data in Table (3) indicated that all the tested antagonistic bacteria caused significant reduction in the linear growth of *A. solani,* compared with the control treatment. The antagonistic bacteria, *i.e. Pseudomona*s *fluorescens* and *Bacillus subtilis* were the best antagonistic isolates inhibiting the mycelial growth by 35.56 and 32.22%, respectively. However, *Bacillus megaterium* and *Serratia marcensens* reduced the linear growth of *A. solani*, by28.8926.33% respectively compared with the control.

2.3. Effect of some chemical inducers on the growth of A. solani in vitro.

Data in Table (4) indicated that all the tested chemical inducers caused significant reduction in the linear growth of *A. solani* compared with the control treatment. Salicylic acid at concentrations 10 mM completely inhabited the linear growth of *A. solani.* While salicylic acid at concentrations 1.00, and

5.00 mM reduced linear growth by11.11-81.11%respectively followed by oxalic acid at concentrations of 5.00, and 10.00 mM. Meanwhile, Cobalt sulphate ($COSO_4$) at concentrations 1.00, 5.00 and 10.00 mg/L was not affected the linear growth of *A. solani*. Meanwhile, Dipotassium hydrogen phosphate (K_2HPO_4) at concentrations 100, 200 and 300 mM reduced the linear growth by 38.22, 42.66 and 55.22%, respectively. Generally, Salicylic acid was the most effective one in this regard.

Table 3: Effect of antagonistic bacteria on growth of *A. solaniin vitro*.

Antagonistic bacteria	Linear growth (mm)	% Reduction
Pseudomonas fluorescens	58.00	35.56
Bacillus subtilis	61.00	32.22
Bacillus megaterium	64.00	28.89
Serratia marcensens	66.30	26.33
Control	90.00	00.00
L.S.D. at 5%	5.37	

Table 4: Effect of some chemical inducers on the linear growth of *A. solani in vitro:*

Treatment	Concentration	Average of the linear growth (mm)	% Reduction of the linear growth
Salicylic acid	1	80.00	11.11
	5	17.00	81.11
	10	0.00	100.00
Oxalic acid	1	90.00	0.00
	5	72.70	19.22
	10	49.60	44.88
Cobalt sulphate ($COSO_4$)	1	90.00	0.00
	5	90.00	0.00
	10	90.00	0.00
Dipotassium hydrogen phosphate (K_2HPO_4)	150	55.60	38.22
	200	51.60	42.66
	300	40.30	55.22
Control		90.00	0.00
LS.D. at 5% Treatment	3.57 Conc.	4.74 Interaction	10.7

2.4. Effect of exposure A. solanispores suspension to temperature on spores germination.

The results in Table (5) show that treated *A. solani* spores suspension with temperature 85°C and 90°C for 10 minutes inhabited spore germination completely. Treated *A. solani* spores with temperature 60°C and 70°C for 10 minutes spores suspension reduced the percentage of spores germination from 42.50% in control treatment to 17.08 and 11.66% respectively. While *A. solani* spores treated with temperature 80°C for 10 minutes spore suspension reduced the percentage of conidia germination to 8.00%.

Table 5: *Effect of exposure A. solani spores suspension to temperature on spores germination.*

Temperature	**Spore Germination (%)**	**Efficacy %**
60°C	17.08	59.81
70°C	11.66	72.56
80°C	8.00	81.17
85°C	00.00	100.00
90°C	00.00	100.00
Control	42.50	00.00

3. Greenhouse experiments:

3.1. Effect of treating tomato plants with antagonistic fungi on the incidence and severity of early blight disease as well as fresh and dry weight of shoot and root.

Data in Table (6) indicate that all the tested isolates of *Trichoderma spp.* significantly reduced early blight disease incidence and disease severity, as well as increased the fresh and dry weight of shoots and roots. *Trichoderma viride*1, *T. hamatum* and *T. lignorum*were the best effective treatments that reduced disease incidence and disease severity in Castle rock to (15.99 and 3.49%), (17.50 and 4.88%) and (19.55 and 5.45%), respectively compared with control plants. Whereas, plants treated with *T. harzianum* 3 was the least effective in the reduction in disease severity which recorded (26.09 and 9.89 %) respectively. As for Super strain B *T. viride*1 and *T. hamatum* were the highest effective treatments which reduced the disease incidence and disease severity to (51.99 and 19.49%) and (53.50 and 20.88%) respectively compared with control plants which recorded (89.22-71.99%) respectively.

Table 6: Effect of treating tomato plants with antagonistic fungi on the incidence and severity of early blight disease as well as fresh and dry weight of shoot and root.

Treatment	Castle rock						Super strain B hybrid					
	DI	DS	FW (g)		DW (g)		DI%	DS%	FW(g)		DW(g)	
			Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot
T. harzianum 1	23.00	8.77	3.80	3.98	0.77	0.85	67.70	26.87	2.84	2.90	0.74	0. 79
T. harzianum 2	24.33	9.30	3.35	3.85	0.73	0.83	68.33	29.50	2.30	2.82	0.70	0.74
T. harzianm 3	26.09	9.89	3.11	3.37	0.70	0.77	69.29	31.80	2.14	2.33	0.66	0.70
T. viride 1	15.99	3.49	5.01	6.09	0.96	0.98	51.99	19.49	4.05	5.07	0.95	0.97
T. viride 2	21.60	7.60	3.97	4.00	0.79	0.88	61.60	25.30	2.95	3.00	0.78	0.81
T. hamatum	17.50	4.88	4.89	5.09	0.95	0.96	53.50	20.88	3.80	4.02	0.94	0.95
T. album	19.80	6.87	4.00	4.77	0.82	0.89	58.66	24.67	3.02	3.70	0.80	0.88
T. lignorum	18.55	5.45	4.51	4.88	0.92	0.94	54.55	21.45	3.53	3.80	0.90	0.93
Control	53.56	18.23	1.30	1.88	0.30	0.39	89.22	71.99	1.19	1.33	0.12	0.15
L.S.D. at 5%	1.81	2.11	0.72	0.46	0.21	0.16	3.12	1.12	1.01	1.17	0.25	0.20

3.2. Effect of treating tomato plants with antagonistic bacteria on incidence and severity of early blight disease as well as fresh and dry weight of shoot and root.

Data in Table (7) indicated that the efficacy of treated tomato plants with the tested antagonistic bacteria was reflected on disease severity and disease incidence under greenhouse condition as well as increased fresh and dry weight of shoots and roots in Castle rock and Super strain B hybrid. Tomato plants treated with *Pseudomonas fluorescens* and *Bacillus subtilis* recorded the lowest disease severity 10.44-37.40 and 11.12-39.10%), respectively. Whereas, in the case of treated tomato plants with *Serratia marcensens* and *Bacillus megaterium* the disease severity were recorded (13.22-42.20 and 12.80-40.80 %) respectively comparing with control plants where disease incidence and disease severity recorded (53.56-18.23%) and (89.22-71.99%) respectively in Castle rock and Super strain B hybrid. The highest increase in shoot and root fresh and dry weight were recorded in case of treatment with *Pseudomonas fluorescens* and *Bacillus subtilis* in Castle rock.

Table 7: Effect of treating tomato plants with antagonistic bacteria on the incidence and severity of early blight disease as well as fresh and dry weight of shoot and root.

Treatment	Castle rock						Super strain B hybrid					
	DI	DS	FW(g)		DW(g)		DI%	DS%	FW(g)		DW(g)	
			Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot
Ps. fluorescens	27.00	10.44	3.02	3.21	0.65	0.72	54.00	25.40	2.09	2.28	0.62	0. 68
B. subtilis	29.22	11.12	2.98	3.00	0.60	0.69	63.00	27.10	2.00	2.22	0.58	0.63
B. megaterium	30.88	12.80	2.55	2.89	0.55	0.64	64.78	28.80	1.90	2.18	0.50	0.60
S. marcensens	32.25	13.22	2.45	2.77	0.49	0.60	66.00	30.20	1.77	2.12	0.45	0.55
Control	53.56	18.23	1.30	1.88	0.30	0.39	89.22	71.99	1.19	1.33	0.12	0.15
L.S.D. at 5%	5.07	3.73	0.88	1.19	0.11	0.13	8.10	2.26	0.65	0.68	0.36	0.15

DI: disease incidence**, DS:** disease severity**, FW:** Fresh weight**, DW:** Dry weight

3.3. Effect of treating tomato plants with chemical inducers on incidence and severity of early blight disease as well as fresh and dry weight of shoot and root.

Data in Table (8) indicated that, treating tomato plants with tested chemical inducers reduced the disease severity and disease incidence as well as its increased the fresh and dry weight of shoots and roots in Castle rock and Super strain B hybrid under greenhouse condition compared with control treatment. Tomato plants treated with dipotassium hydrogen phosphate (K_2HPO_4) and Salicylic acid were recorded the lowest disease severity (1.72 and 14.44%) in Castle rock and (14.11- 36.19) in Super strain B hybrid respectively. Meanwhile the disease incidence being (7.20 - 33.66 and 26.55-70.22 %), respectively compared with control treatment. Meanwhile, tomato plants treated with oxalic acid and cobalt sulphate ($COSO_4$) were recorded the highest disease severity (15.22-16.20 and 37.11 - 40.22%) respectively. Whereas, the disease incidence were (36.09 - 40.22 and 72.00 - 75.92%), respectively comparing with control plants where disease incidence and disease severity recorded (53.56 - 89.22%) and (18.23 - 71.99%) respectively in Castle rock and Super strain B hybrid.

Table 8: Effect of treating tomato plants with chemical inducers on incidence and severity of early blight disease as well as fresh and dry weight of shoot and root.

Treatment	Castle rock						Super strain B hybrid					
	DI	DS	FW (g)		DW (g)		DI%	DS%	FW (g)		DW (g)	
			Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot
Salicylic acid	7.20	1.72	5.57	6.60	0.97	0.99	26.55	14.44	4.55	5.66	0.96	0. 98
K_2HPO_4	33.66	14.11	1.88	2.43	0.30	0.45	70.22	36.19	1.55	1.68	0.29	0. 35
Oxalic acid	36.09	15.22	1.67	2.35	0.25	0.40	72.00	37.11	1.41	1.60	0.20	0.23
$COSO_4$	40.22	16.20	1.44	1.93	0.37	0.45	75.92	40.22	1.29	1.49	0.15	0.19
Control	53.56	18.23	1.30	1.88	0.30	0.39	89.22	71.99	1.19	1.33	0.12	0.15
L.S.D. at 5%	5.56	1.77	1.42	2.53	0.17	0.11	5.74	5.10	1.47	1.33	0.37	0.16

DI: disease incidence**, DS:** disease severity**, FW:** Fresh weight**, DW:** Dry weight

3.4. Effect of treating tomato plants with un-germinated A. solani spores as cross protection on early blight disease severity.

Results in Table (9) reveal that, treating tomato plants with *A. solani* spores after treated with the temperature 85°C or 90°C for10minuts (un-germinated spores) were reducing the disease severity to 10.09 and 9.00% compared with 18.23%in Castle rock untreated control respectively. Meanwhile in Super strain B hybrid were17.00and 16.09%, respectively comparing with 71.99% in control plants where disease severity recorded. Moreover, treated tomato plants with spores subjected to temperature 85°C or 90°C for10 minutes were increased shoot and root fresh and dry weight in Castle rock.

Table 9: Effect of treating tomato plants with un-germinated *A. solani*spores as cross protection on the early blight disease severity.

Treatment	Castle rock						Super strain B hybrid					
	DI	DS	FW(g)		DW(g)		DI%	DS%	FW(g)		DW(g)	
			Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot
85°C	18.99	10.09	2.48	3.10	0.58	0.67	47.71	17.00	1.89	2.00	0.53	0.56
90°C	16.75	9.00	2.99	3.18	0.61	0.70	45.78	16.09	2.02	2.24	0.59	0.64
Control	53.56	18.23	1.30	1.88	0.30	0.39	89.22	71.99	1.19	1.33	0.12	0.15
L.S.D. at 5%	4.49	4.24	1.32	2.14	0.19	0.18	9.84	6.29	0.96	1.18	0.61	0.10

DI: disease incidence**, DS:** disease severity**, FW:** Fresh weight**, DW:** Dry weight

4. Determination of enzymes activity.

4.1.Effect of treating tomato plants with some antagonistic fungi on the activity of peroxidase (PO), polyphenoleoxidase (PPO) and Chitinase enzymes.

Data in Table (10) show that all tested antagonistic fungi increased Peroxidase (PO), Polyphenol oxidase (PPO) and Chitinase activity. The antagonistic fungi *i.e. Trichoderma viride*1*, T. hamatum, T. lignorum, T. album* increased the activities of PO and PPO which recorded compared

with control treatment. Whereas, *T. harzianum* 3was the least activities of PO, (PPO) and Chitinase activity compared with other treatments.

4.2. Effect of treating tomato plants with some antagonistic bacteria on the activity of peroxidase (PO), polyphenoleoxidase (PPO) and Chitinase enzymes.

Data in Table (11) indicated that all tested antagonistic bacteria increased the activities of Peroxidase (PO), Polyphenoloxidas (PPO) and Chitinase compared with control treatment.

Pseudomonas fluorescens and *Bacillus subtilis* induced the highest increase of PO, (PPO) and Chitinase activity compared with control treatment.

Meanwhile, *Serratia marcensens* was the least effective in this respect compared with other treatments.

Table 10: Effect of treating tomato plants with some antagonistic fungi on activity of peroxidase (PO), polyphenoleoxidase (PPO) and Chitinaseenzymes.

Treatments	PO	PPO	Chitinase	Efficacy		
				PO	PPO	Chitinase
T. harzianum 1	35.00	36.00	40.00	600.00	414.29	900.00
T. harzianum 2	30.00	32.00	39.00	500.00	357.14	875.00
T. harzianum 3	25.00	27.00	35.00	400.00	285.71	775.00
T. viride 1	45.00	50.00	60.00	800.00	614.29	1400.00
*T. viride*2	37.00	38.00	45.00	640.00	442.86	1025.00
T. hamatum	42.00	44.00	55.00	740.00	528.57	1275.00
T. album	38.00	39.00	50.00	60.00	457.14	1150.00
T. lignorum	40.00	41.00	53.00	700.00	485.71	1225.00
Control	5.00	7.00	4.00	0.00	0.00	0.00

Table 11: Effect of treating tomato plants with some antagonistic bacteria on the activity of peroxidase (PO), polyphenoleoxidase (PPO) and Chitinase enzymes.

Treatments	PO	PPO	Chitinase	Efficacy		
				PO	PPO	Chitinase
Ps.fluorescens	20.00	22.00	33.00	300	214.29	725.00
B.subtilis	19.00	20.00	30.00	280.00	186.71	650.00
B.megaterium	17.00	18.00	28.00	240.00	157.14	600.00
S.marcensens	15.00	16.00	25.00	200.00	128.57	525.00
Control	5.00	7.00	4.00	0.00	0.00	0.00

4.3. Effect of treating tomato plants with some chemical inducers on activity of peroxidase (PO), polyphenoleoxidase (PPO) and Chitinase enzymes.

Data in Table (12) showed that all the tested chemical inducers were increasing the activities of Peroxidase (PO), Polyphenoloxidas (PPO) and Chitinase compared with control treatment.

Salicylic acid followed bydipotassium hydrogen phosphate (K_2HPO_4) induced the highest increase of PO, (PPO) and Chitinase activity compared with other treatments.

Whereas, Cobalt sulphate ($COSO_4$) was the least effective treatment in this respect compared with other treatments.

Table 12: Effect of treating tomato plants with some chemical inducers on activity of peroxidase (PO), polyphenoleoxidase (PPO) and Chitinase enzymes.

Treatments	PO	PPO	Chitinase	Efficacy		
				PO	PPO	Chitinase
Salicylic acid	50.00	53.00	65.00	900.00	657.14	1525.00
K_2HPO_4	10.00	12.00	18.00	100.00	71.43	350.00
Oxalic acid	8.00	10.00	13.00	60.00	42.86	225.00
COSO4	7.00	9.00	9.00	40.00	28.57	125.00
Control	5.00	7.00	4.00	0.00	0.00	0.00

5. Activity gel electrophoresis

5.1. Peroxidase.

Native gel electrophoretic separation of enzyme extract from tomato plants treated with *A. solani* un-germinated spores*, Pseudomonas fluorescens, T. virdi1* and SA and inoculated with *A. solani*. Super strain B tomato plants treated with *T. virdi1* and SA from showed different PO patterns and induced the intensity of PO isozymes.

As for Castle rock, all treatments showed induction of different isoforms of PO as compared with control plants. Levels of PO isozymes intensity was clearly induced in Castle rock (resistant hybrid) more than Super strain B (susceptible one).

5.2 Polyphenol oxidase.

The isoform pattern of PPO in tomato plants treated with *A. solani*un-germinated spores*, Pseudomonas fluorescens, T. virdi1* and SA and inoculated with *A. solani* spores was studied. All treatments led to an induction of isoforms. The increased intensity of the induced PPO was found in Salicylic acid with Super strain B compared with other treatments and control. Also, all treatments showed a greater intensity of the PPO isoform with Castle rock compared with control.

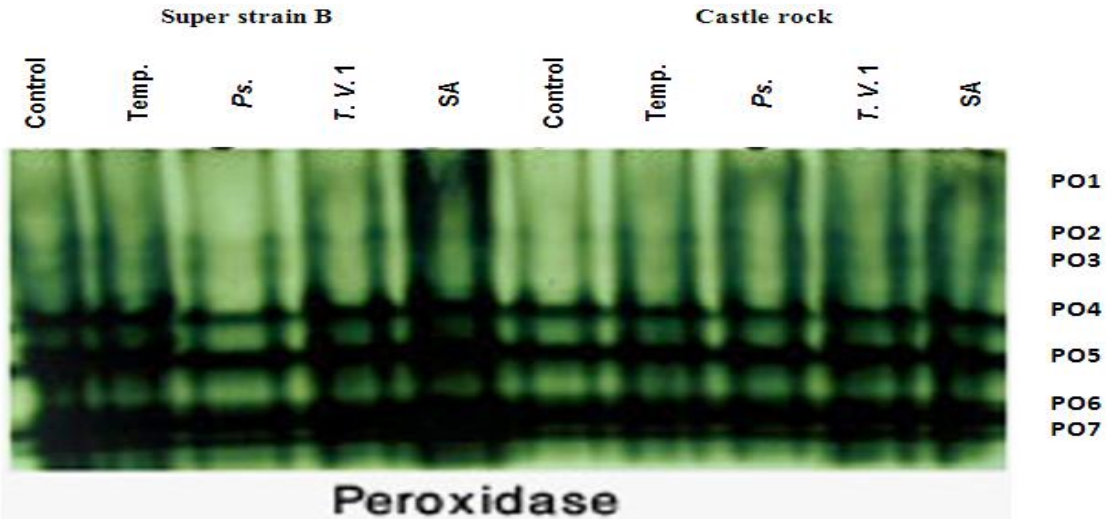

Fig.1: Peroxidase isoenzymes in tomato leaves treated with selected treatments.

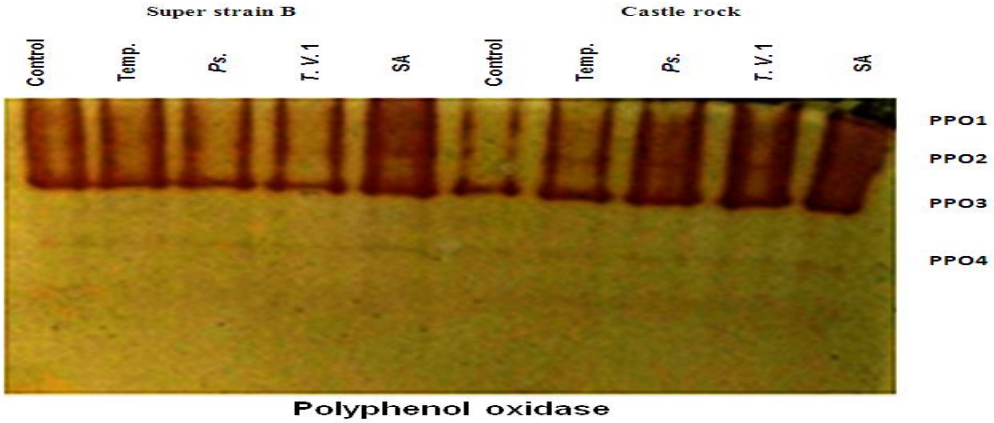

Fig. 2: Polyphenol oxidase isoenzymes in tomato leaves treated with selected treatments.

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

Concerning the results of SDS (PAGE) presented in Table (13) and demonstrated in Fig. 3 show that 15 protein bands with molecular weights ranging from 255.822 to 11.726 kDa are contained

in tomato plants while protein band with molecular weight 255.822 was appeared in Castle rock plant which treated with *A. solani* spores exposed to temperature 85°Cun-germinated spores), *Pseudomonas fluorescens, Trichodermaviride1*andSalicylic acid mean while, this band was absent in all treatments with Super strain B and in untreated control Castle rock. Moreover, protein band with molecular weight 194.741 was disappeared in untreated control of Super strain B and plants treated with *A. solani* spores exposed to temperature.

Table (13): Molecular weights of fractionated protein profiles of tomato leaves treated with selected treatments**.**

Band No	M.W KDa	Super strain B					Castle rock				
		Control	Temp.	*Ps.*	*T.V.* 1	SA	Control	Temp.	*Ps.*	*T.V.* 1	SA
1	**255.822**	-	-	-	-	-	-	+	+	+	+
2	**226.741**	+	+	+	+	+	+	+	+	+	+
3	**194.46**	-	-	+	+	+	+	+	+	+	+
4	**143.555**	+	+	+	+	+	+	+	+	+	+
5	**124.021**	+	+	+	+	+	+	+	+	+	+
6	**81.445**	+	+	+	+	+	+	+	+	+	+
7	**60.787**	+	+	+	+	+	+	+	+	+	+
8	**46.207**	+	+	+	+	+	+	+	+	+	+
9	**36.432**	+	+	+	+	+	+	+	+	+	+
10	**32.055**	+	+	+	+	+	+	+	+	+	+
11	**27.191**	+	+	+	+	+	+	+	+	+	+
12	**24.366**	+	+	+	+	+	+	+	+	+	+
13	**19.212**	+	+	+	+	+	+	+	+	+	+
14	**14.339**	+	+	+	+	+	+	+	+	+	+
15	**11.726**	+	+	+	+	+	+	+	+	+	+
Total		**13**	**13**	**14**	**14**	**14**	**14**	**15**	**15**	**15**	**15**

+= bands appeared - = bands disappeared

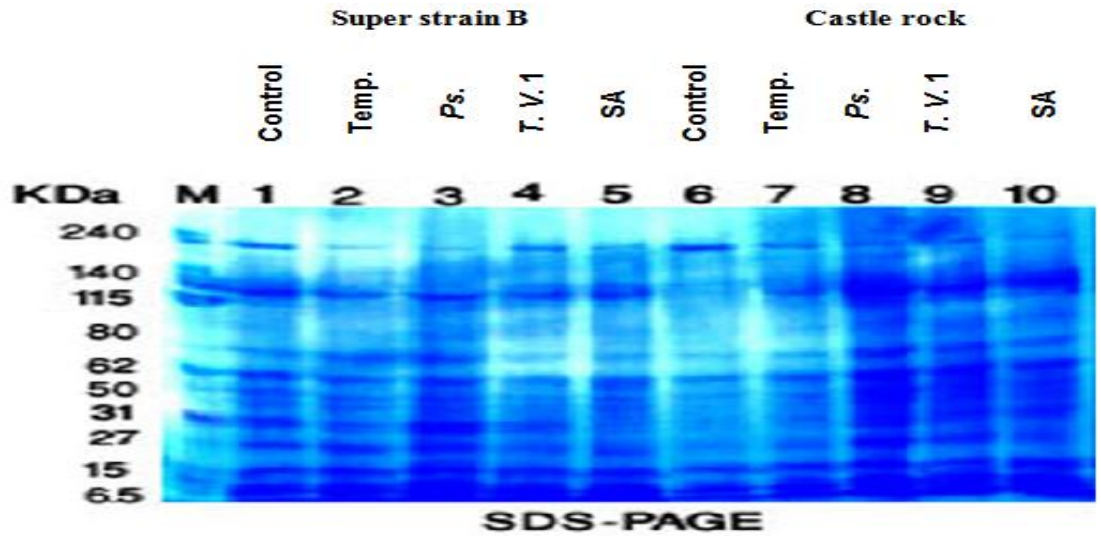

Fig. 3: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS- PAGE) analysis of total protein extracted from tomato leaves treated with selected treatments.

6. Chemical analysis:

6.1. Effect of treating with antagonistic fungi on the total flavonoids and phenols of tomato plants (Super strain B).

Data in Table (14) indicated that all the tested antagonistic fungi were increased the total flavonoids and phenols compared with control. The highest effective treatment on flavonoidsand phenols was *Trichoderma viride* 1followed by *T. hamatum.*
Meanwhile, *T. harzianum*3 was the least effective to induction flavonoids compared with other treatment.

Table 14: Effect of treating with antagonistic fungi on the total flavonoids and phenols of tomato plants (Super strain B) under field conditions.

Treatments	Total flavonoids	Total Phenols	Efficacy	
			Total flavonoids	Total Phenols
T. harzianum 1	8.40	7.66	159.26	157.05
T. harzianum 2	8.20	7.00	153.09	134.90
T. harzianum 3	7.11	6.55	119.51	119.80
T. viride 1	32.14	12.51	891.98	319.79
*T. viride*2	9.18	8.22	183,33	175.84
T. hamatum	17.42	11.94	437.65	300.67
T. album	9.77	8.77	201.54	194.29
T. lignorum	9.86	10.18	204.32	241.61
Control	3.24	2.98	0.0	0.00

6.2. Effect of treating with antagonistic bacteria on total flavonoids and phenols of tomato plants (Super strain B).

Data in Table (15) reveal that treating tomato plants with tested antagonistic bacteria affected greatly on total flavonoids and phenols compared with control. The highest increase in the total flavonoids was recorded in tomato plants treated with *Pseudomonas fluorescens* followed by *Bacillus megaterium*. Meanwhile, *Pseudomonas fluorescens* followed by *Bacillus megaterium* were the best effective treatments on total phenols where the efficacy recorded 116.11 and 101.01% respectively . On the other hand, the least increase of total flavonoids and phenols was recorded with *Serratia marcensens*.

Table 15: Effect of treating with antagonistic bacteria on the total flavonoids and phenols of tomato plants (Super strain B) under field conditions.

Treatments	Total flavonoids	Total Phenols	Efficacy	
			Total flavonoids	Total Phenols
Ps. fluorescens	6.014	6.44	85.62	116.11
B. subtilis	5.768	5.99	78.02	101.01
B. megaterium	5.96	5.44	83.95	82.55
S. marcensens	5.65	4.95	74.38	66.11
Control	3.24	2.98	0.0	0.00

6.3. Effect of treating with chemical inducers on total flavonoids and phenols of tomato plants (Super strain B).

Data in Table (16) indicate that spraying tomato plants with tested chemical inducers increased the total flavonoids and phenols. The highest increase in the total flavonoids and phenols was recorded in case of treated tomato plants with Salicylic acid 956.17 and 387.92% respectively. On the other hand the increase of total flavonoids and phenols recorded with Dipotassium hydrogen phosphate (K_2HPO_4) 24.38 and 56.38 % respectively. Meanwhile, the least increase of total

flavonoids and total phenols was recorded with Cobalt sulphate ($COSO_4$) 10.62 and 19.13%, respectively compared with control treatment.

Table 16: Effect of treating with chemical inducers on the total flavonoids and phenols of tomato plants (Super strain B) under field conditions.

Treatments	Total flavonoids	Total Phenols	Efficacy	
			Total flavonoids	Total Phenols
Salicylic acid	34.22	14.54	956.17	387.92
K_2HPO_4	4.03	4.66	24.38	56.38
Oxalic acid	3.86	3.75	19.26	25.84
$COSO_4$	3.58	3.55	10.62	19.13
Control	3.24	2.98	0.0	0.00

7. Field experiments.

7.1. Effect of treating tomato plants (Super strain B) with antagonistic fungi on the disease incidence and yield weight (during 2015 and 2016).

Data in Table (17) indicate that the tested antagonistic fungi were significantly reduced the disease incidence and disease severity. *Trichodermaviride*1, *T. hamatum* and *T. lignorum* were the best effective treatments and reducing disease severity during two seasons by (79.36 and 78.15%), (77.31 and 75.81%) and (73.72 and 72.29%) respectively. Whereas, plants treated with *T. harzianum* 3 recorded the least reduction in disease severity during the two seasons which recorded (32.18 and 60.41 %) respectively. Control plants recorded the highest disease incidence and disease severity which were 97.00-98.50% and 70.20-68.2% respectively during (2015 and 2016).

As for fruit yield, plants sprayed with *T. viride* 1, *T. hamatum* and *T. lignorum* produced the highest fruit yield which recorded 175.00-173.00, 170.00-174.00 and 168.00-165.00 kg / plot for respectively. Whereas, plants sprayed with *T.harzianum*3 produced the lowest fruit yield 148.00-150.00 kg / plot, respectively compared with control treatment which recorded 98.70-97.80 kg/plot, respectively.

7.2. Effect of treating tomato plants (Super strain B) with the antagonistic bacteria on the disease incidence and yield weight (during 2015 and 2016).

Data in Table (18) indicated that, the efficacy of treated tomato plants with the tested antagonistic bacteria was reflected on disease severity and disease incidence under field condition. Tomato plants treated with *Pseudomonas fluorescens* and *Bacillus subtilis* recorded the lowest disease severity 33.00-33.40 and35.10-34.50%, respectively. Whereas, treated tomato plants with *Serratia marcensens* and *Bacillus megaterium* the disease severity were recorded 38.20-38.60 and 36.80-36.44 %, respectively during (2015 and 2016). Moreover *Pseudomonas fluorescens* and *Bacillus subtilis* produced the highest fruit yield being 133.00-134.00 and 129.00-130.00 kg/plot respectively. Meanwhile, plants sprayed with *Bacillus megaterium* and *Serratia marcensens* produced the lowest fruit yield, being 129.00-130.00 and 123.00-125.00 kg / plot, (during 2015 and 2016) respectively compared with the control treatment which recorded 98.70-97.80 kg /plot, respectively(during 2015 and 2016).

7.3. Effect of treating tomato plants with the chemical inducers on the disease incidence and yield weight (during 2015 and 2016).

Data in Table (19) showed that, treating tomato plants with tested chemical inducers reduced the disease severity and disease incidence under field condition compared with control treatment.. Tomato plants treated with dipotassium hydrogen phosphate (K_2HPO_4) and salicylic acidrecorded the lowest disease severity 42.10 - 41.80 and 10.26 - 9.90% respectively. Meanwhile disease incidence being 86.20 - 85.80 and 22.50-21.70 % respectively compared with control treatment. Meanwhile,

Table 17: Effect of treating tomato plants (Super strain B) with antagonistic fungi on the disease incidence and yield weight (during 2015 and 2016).

Treatments	2015			2016			Efficacy					
							2015			2016		
	Disease incidence (%).	Diseases severity (%)	Yield/weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/weight kg/plot
T. harzianum 1	63.00	23.70	159.00	62.50	23.90	166.00	35.05	66.24	61.09	36.55	64.96	69.73
T. harzianum 2	65.00	26.30	154.00	65.60	26.00	152.00	32.99	62.54	49.95	33.40	61.88	53.37
*T. harzianm*3	67.00	26.80	148.00	66.80	27.00	150.00	30.93	61.82	56.03	32.18	60.41	55.42
T. viride 1	47.00	14.49	175.00	46.50	14.90	173.00	51.55	79.36	77.30	52.79	78.15	76.89
T. viride 2	58.00	22.60	163.00	59.60	22.50	164.00	40.21	67.81	65.15	39.49	67.01	67.69
T. hamatum	49.50	15.93	170.00	51.00	16.50	174.00	48.97	77.31	72.24	48.22	75.81	77.91
T. album	55.00	21.87	165.00	56.00	21.50	161.00	43.30	68.85	67.17	43.15	68.48	64.62
T. lignorum	51.00	18.45	168.00	52.00	18.90	165.00	47.42	73.72	70.21	47.21	72.29	68.71
Control	97.00	70.20	98.7.00	98.50	68.20	97.8.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0.05		1.03	4.74		0.68	5.91						

Table 18: Effect of treating tomato plants with the antagonistic bacteria on the disease incidence and yield weight (during 2015 and 2016).

Treatments	2015			2016			Efficacy					
							2015			2016		
	Disease incidence (%).	Diseases severity (%)	Yield/weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/weight kg/plot
Ps. fluorescens	70.00	33.40	133.00	70.60	33.00	134.00	27.84	52.42	34.75	28.32	51.61	35.99
B. subtilis	79.00	35.10	129.00	78.50	34.50	130.00	18.56	50.00	30.70	20.30	49.41	32.92
B. megaterium	80.70	36.80	126.00	80.50	36.44	128.00	16.80	47.58	27.66	18.27	46.57	30.88
S. marcensens	82.00	38.20	123.00	81.70	38.60	125.00	15.46	45.58	24.62	17.06	43.40	27.81
Control	97.00	70.20	98.70	98.50	68.20	97.80	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0.05		1.49	5.35		0.36	5.66						

Table 19: Effect of treating tomato plants with chemical inducers on the disease incidence and yield weight (during 2015 and 2016).

Treatments	2015			2016			Efficacy					
							2015			2016		
	Disease incidence (%).	Diseases severity (%)	Yield/ weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/ weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/ weight kg/plot	Disease incidence (%).	Diseases severity (%)	Yield/ weight kg/plot
Salicylic acid	22.50	10.30	220.00	21.70	9.90	215.00	76.80	85.38	122.90	77.97	85.48	119.84
K_2HPO_4	86.20	42.10	118.00	85.80	41.80	117.00	11.13	40.03	19.55	12.89	38.71	19.63
Oxalic acid	88.00	43.10	115.00	88.20	43.50	116.00	9.28	38.60	16.51	10.46	36.22	18.61
$COSO_4$	91.90	46.20	112.00	92.00	45.90	114.00	5.26	34.19	13.48	6.60	32.70	16.56
Control	97.00	70.20	98.70	98.50	68.20	97.80	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0. 05%		1.50	4.21		0.64	3.20						

tomato plants treated with cobalt sulphate ($COSO_4$) andoxalic acidrecorded the highest disease severity 46.20- 45.90 and 43.10-43.50 % respectively. Whereas, disease incidence were 91.90- 92.00 % and 88.00- 88.20 %, respectively **(**during 2015 and 2016).

Concerning the fruit yield, plants sprayed with salicylic acid and dipotassium hydrogen phosphate **(**K_2HPO_4**)** produced the highest fruit yield 220.00-215.00 and 118.00-117.00 kg / plot respectively. Meanwhile, plants sprayed with oxalic acid and cobalt sulphate **(**$COSO_4$**)** produced the low fruit yield which recorded 115.00-116.00 and 112.00-114.00 kg/plot, respectively compared with controltreatment which recorded the lowest yield 98.70-97.80 kg/plot (during 2015 and 2016**)**.

Discussion

Early blight disease of tomato caused by the fungus *Alternaria solani*is one of the most common foliar diseases of tomato plants, which damages the leaves, stalks, stems and fruits causing severe destruction of the aerial part and reduction of size and number of fruits, resulting heavy losses in yield up to 79% (Sherf & MacNab, 1986; Gwary & Nahunnaro, 1998).

Concerning evaluation pathogenicity of *A. solani* isolates with five tomato hybrids, *i.e.* Super Alteray, Super strain B normal, Elisa, Kasel rock and Super stain B hybrid for their reactions to *A. solani* isolates. Generally, Super Alteray was the most resistance hybrid. Super strain B normal, Elisa and Kasel rock were resistance. Whereas, Super stain B hybrid was susceptible hybrid. Moreover, AS1 which isolated from El-Behira (El-Nubaryia) was the most virulent isolate followed by AS2 which isolated from El- Qalubia (Moshtohor). Whereas, AS3 which isolated from El- Minia (Magagha) was the low virulent isolate. These results could be discussed in light the findings of Patel *et al.,* (2011), Alsafadi *et al.,* (2012), Poly & Srikanta (2013) and Singh *et al.,* (2017).Eid, (2017) supported the obtained results where he confirmed the pathogenicity of 14 *Alternaria solani* isolates under greenhouse conditions with great variations in virulence.

In vitro, all the tested antagonistic fungi and bacteria caused significant reduction in the linear growth of *A. solani. Trichoderma viride*1 was the best in inhibiting the mycelial growth of *A. solani* followed by *Trichoderma hamatum*. While regards to antagonistic bacteria *Pesudomona*s *fluorescens* and *Bacillus subtilis* were the best antagonistic bacteria in reducing growth of *A. solani*. Also, spraying tomato plants with any one of the tested antagonists before inoculation with *A. solani* under greenhouse conditions decreased the early blight disease severity, as well as increased fresh and dry weight of shoots and roots compared with the infected control treatment. Moreover, under field conditions, *Trichoderma viride*1, *Trichoderma hamatum, Trichoderma lignorum, Pseudomona*s *fluorescens* and *Bacillus subtilis* were the best effective treatments and reducing disease severity up to 72.29% and its cause increased of the tomato yield up to 68.71% over control. Whereas, *Serratia marcensens* the least effective ones. These results are in harmony with the previously mentioned results of Babu *et al.* (2000), Wickramaarachchi (2005) and Fontenelle*et al*. (2011). Microorganisms acting through antibiosis, generally have a wide action spectrum, and thus pathogen inhibition by producing toxic substances is more effective than any other mechanism of action Leelasuphakul *et al.,* (2008). Their protective effect involves different mechanisms of action that directly antagonize pathogen growth. *B. subtilis* group is known to produce a variety of bioactive metabolites leading to antibiosis Stein, (2005) and Chen *et al.,* (2009b) and able to compete for space and nutrients Nagórska *et al.,(*2007).

All the tested chemical inducers caused significant reduction in the linear growth of *A. solani*. Salicylic acid was the most effective one and at the concentrations 10 mM completely inhabited the linear growth of *A. solani.* Generally, the effect of chemical inducers increased by increasing concentrations. Under greenhouse condition treating tomato plants with tested chemical inducers reduced the disease severity and disease incidence as well as increased the fresh and dry weight of shoots and roots compared with control treatment. Tomato plants treated whit Salicylic acid recorded the lowest disease severity. Meanwhile, tomato plants treated with Cobalt sulphate ($COSO_4$) recorded the highest disease severity. Moreover, under field conditions, all tested chemical inducers controlled the early blight disease. Also, spraying tomato plants with salicylic acid was the best treatment in controlling *A. solani* infection and in increasing the tomato yield comparing with the other chemical inducers. The obtained results, it could be interpreting in light the findings of Spletzer and Enyedi

(1999) who mentioned that salicylic acid (SA) is an important signal molecule that plays a critical role in plant defense against pathogen invasion. Eid, (2017) indicated that sodium bicarbonate, potassium hydrogen carbonate, ascorbic acid and salicylic acid *in vitro* reduced the growth of *A. solani*on PDA plates compared control. Under greenhouse conditions, spraying tomato plants with the tested chemical inducers against early blight disease caused by *A. solani*was moderately effective in controlling the infection.

Treated *A. solani* spores suspension with temperature 85°C and 90°C for 10 minutes inhabited spore germination. Moreover, treated tomato plants with *A. solani* spores subjected to temperature 85°C or 90°C for 10 minutes reduced disease severity in treated tomato plants and increased shoot and root fresh and dry weight comparing with control. the results could be interpreting in light the findings of Ahmed, (2005) who reported that treating cucumber with powdery mildew spores killed by UV, temperature, chloroform reduced powdery mildew disease severity by 69.84, 62.25 and 54.73% less than control and increased the number and weight of fruits/plant by UV (25.02 and 25.00%) and temperature (18.75 and 19.88%) compared to control. EL-Gammal (2005) who showed that spraying faba bean plants with suspension of un-viable heated spores of *B. fabae* spores scored a remarkable depression in chocolate spot disease severity comparing with unsprayed one which being 2.07 and 8.87%, respectively after 7 days from inoculation.

All tested antagonistic fungi and bacteria increased Peroxidase (PO) and Polyphenol oxidase (PPO) and Chitinase activity. *Trichoderma viride* 1followed by *Trichoderma hamatum*, *Pseudomona*s *fluorescens* and *Bacillus subtilis* were induced the highest increase of PO, PPO and Chitinase activity. Meanwhile, *Serratia marcensens* was the least effective one.

Concerning chemical inducers, Salicylic acid followed by Dipotassium hydrogen phosphate (K_2HPO_4) induced the highest increase of PO, PPO and Chitinase activity compared with control treatment respectively. Whereas, Cobalt sulphate ($COSO_4$) was the least effective treatment compared with other treatments.It can be concluded that treatments induced activity of PO, PPO and Chitinase associated with reducing early blight disease intensity in tomato plants. The obtained results could be discussed in light the findings of A higher enzymatic activity of PO, PPO and Chitinase 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). Ramamoorthy *et al*., (2002) they mentioned that, the induction of defense enzymes involved in phenylpropanoid pathway accumulation of phenolics and PR-Proteins (PhenylalanineAmmonia-lyase (PAL), Peroxidase (PO) and Polyphenoloxidase (PPO)) might have contributed to restriction of invasion of *Fusarium oxyporum* f.sp. *lycopersici* tomato roots. Liao *et al.,* (2003) found that, treating tomato leaves with chitosan before inoculation with *A. solani* was reduced the rates of leaf infection and disease index. Chitosan treatments increased the activities of peroxidase. Polyphenoloxidase [catechol oxidase], phenylalanine ammonia-lyase, chitinase and β-1,3-glucanase in the leaves but at various degrees depending on the cultivar.

All the tested antagonistic fungi and bacteria as well as chemical inducers increased total flavonoids, total phenols and free phenols compared with control. The tested fungal isolates were found to be more effective than bacterial isolates. *Trichoderma viride1,Pseudomonas fluorescens* and Salicylic acid, and Dipotassium hydrogen phosphate (K_2HPO_4)was the highest effective treatments and expressed the highest increase in flavonoidsand total phenolsin tomato plants. Rajik*et al.,* (2012) found that pretreatment with bio-agents *Trichoderma harzianum,* (Kan.), *T. harzianum* (Del.), *T. harzianum*(Pant),*Trichoderma viride* (Kan.), *T. viride* (Del.), *T. viride* (Pant), *Aspergillus niger* AN-27 (Kan.) *Chaetosphaeridium globosum* (Del.) and *Pseudomonas fluorescens* (Del.) provided induced resistance in plant against *F. o. f.sp. lycopersici*. The induction of resistance was associated with certain biochemical changes in tomato leaves. A high content of phenols which are the indication of first stage of defense mechanism was also recorded in treated plant with maximum in *T. harzianum* (Kan.). Ahmed (2015) recorded that significant reduction in the chocolate spot disease (*Botrytis fabae*) incidence (%) and severity (%) in faba bean plants treated with *Bacillus subtilis* and *Trichoderma album*. treated faba bean plants with biotioc inducers increased chlorophyll, phenols and flavonoids, content in treated faba bean plants at 0, 3 and 5 days post inoculation of faba bean plants with *B. fabae* spores.

Native gel electrophoretic separation of enzyme extract from tomato plants treated with some treatments and inoculated with *A. solani*. Super strain B tomato plants treated with *T. virdi1* and SA

showed different PO and PPO patterns and induced the intensity of isozymes. As for Castle rock , all treatments showed induction of different isoforms of PO and PPO compared with control plants. Levels of PO and PPO isozymes intensity was clearly induced in Castle rock (resistant hybrid) more than Super strain B (susceptible one).

Concerning the results of SDS (PAGE) showed that 15 protein bands with molecular weights ranging from 255.822 to 11.726 kDa are contained in tomato plants while protein band with molecular weight 255.822 was disappeared in all treatment with Super strain B and in untreated control Castle rock. Moreover, protein band with molecular weight 194.741 was disappeared in untreated control of Super strain B and plants treated with *A. solani* spores exposed to temperature. Band with molecular weight 255.822 was appeared in Castle rock plants treated with *A. solani* spores exposed to temperature*, Pseudomonas fluorescens Trichoderma viride* and salicylic acid. Moreover, these treatments induced the production of proteins associated with reducing early blight disease intensity in tomato plants. The obtained results in the current study supported by the finding of many authors, Patel *et al.,* (2011) used alternaric acid and fungal culture filtrate as an elicitor in NDT-96 (tolerant) and GP-5 (susceptible) tomato varieties in order to study and compare their abilities to induce defense-related enzymes, viz., catalase, peroxidase, beta -1,3 glucanase, phenylalanine-ammonia-lyase (PAL), chitinase and polyphenol-oxidase (PPO) along with total phenols, and total soluble proteins. NDT-96 showed a rapid induction of all these pathogenesis-related enzymes except catalase along with total phenols as compared to GP-5 with both the treatments. Differential expression of total soluble proteins revealed higher protein content in NDT-96 as compared with GP-5. A 49.48 kDa protein was observed to be absent in GP-5. In addition, 25 microsatellite markers (SSR) were screened for polymorphisms among the above mentioned two tomato varieties. Of these, SSR 286 revealed a significant polymorphic band of 108 bp in NDT-96. Al-Ani and Shaker (2013) determined the role of salicylic acid in inducing resistance genes in tomato to control early blight disease caused by *Alternaria solani*. The gel electrophoresis profile of the proteins of tomato plants sprayed with salicylic acid extracted, showed the presence of two novel protein bands of 44 and 23 KDa in size, while such bands were absent in the unsprayed tomato plants. It can be concluded from this study that salicylic acid induced the production of proteins associated with reducing early blight disease intensity in tomato plants.

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