Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 54(4) (2016), 929–938 **http://annagricmoshj.com**

Efficacy of some biotic and abiotic treatments in controlling *Alternariasolani* the causal of tomato early blight disease *in vitro* and *in vivo*

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Abstract

Early blight of tomato caused by *Alternariasolani*is one of the most common foliar diseases of tomato. Fivetested *Trichoderma* isolates were most effective in reducing growth of *A. solani*(As-3) than the tested *Bacillus* isolates. The highest reduction% was recorded with *Trichodermaaureoviride*-I(T-1) followed by *Trichodermaaureoviride*-II (T-2), *Bacillussubtilis*-I (B-24) and *Bacillusamyloliquefaciens*-II(B-19). Spraying plants with any of the tested antagonists before inoculation with *A. solani* under greenhouse conditions decreased the early **blight** disease severity (DS) by 15.1 to 45.3% compared with the infected control treatment with the pathogen only which recorded 93.4% of DS%. *Bacillus amyloliquefaciens*-II (B-19)،*T. virens* (T-11)and *T. harzianum*-II (T-13) were the most effective for controlling early blight disease at 21-day post inoculation. All plant oils reduced the growth of *A. solani* isolate (As-3) with superiority of the high concentrations. The highest decreased % in growth of *A. solani* occurred with the 6.0% concentration. Among the five plant oils, cinnamon was the most effective followed by cloves then marjoram, while garlic was the least effective. Moreover, all oils at 3% concentration were moderately effective in controlling the disease. Clove and marjoram oil were the most effective in decreasing the infection at 21-day post inoculation with the pathogen while, garlic oil was the least effective. All tested chemical inducers reduced the growth of *A. solani*(As-3) on PDA plates. The high concentration 400 mM of sodium bicarbonate and potassium hydrogen carbonate was the most effective. Salicylic acid at 3 and 5mM was more effective in reducing the growth of isolate As-3 compared with the same concentrations of ascorbic acid. Spraying plants with the tested chemical inducers against early blight disease caused by *A. solani*(As-3) was moderately effective in controlling the infection. All tested chemical inducers controlled the early blight infection where the recorded DS% was ranged between 30.1 to 45.4% at 21day post inoculation with *A. solani*(As-3). Spraying tomato plants with salicylic acid was the most effective treatment in controlling *A. solani* (As-3) infection compared with the other chemical inducers.

Key words: early blight disease, *Alternariasolani*, mineral salts, organic acids**,** plant oils, Antagonists.

Introduction

Early blight is considering one of the most destructive fungal diseases affecting significantly tomato-plants (*Solanumlycopersicum*L.) and other plants of family Solanaceae, either in conventional or organic cultivations. Early blight of tomato, caused by the necrotrophic fungus *Alternariasolani***(Ellis & Martin, 1882**), 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&MacNab, 1986; Gwary&Nahunnaro, 1998).**As for biological control,**Babu *et al.* (2000)** stated that *Trichodermaharzianum* and *T. viride* were significantly effective in inhibiting the mycelial growth of *A. solani* although it was no significant difference between the effectiveness of *Trichoderma* species in pot culture studies. Also, **Dar *et al.* (2011)** revealed that the fungal antagonists may compete for an ecological niche by consuming available nutrients and by secreting a spectrum of biochemicals effective against various fungal pathogens. These biochemicals may include cell wall degrading enzymes, siderophores chelating irons, and a wide variety of volatile and non-volatile antibiotics. Moreover, **Pane and Zaccardelli (2014)** mentioned that four *Bacillus* species were capable to decrease severity of *Alternaria* disease on tomato. The evident inhibition zone observed in dual culture plates, suggested an antibiosis-like mechanism. Also, **Zghair*et al.* (2014)** mentioned that the bioagents*Trichodermaharzianum* and *Pseudomonas fluorescens* (as seed treatment + two foliar sprays) were effective in reducing the tomato early blight disease intensity.**Chohan *et al.* (2015)** compared between the efficacy of three fungicides (Topsin-M, Bavistin and Ridomil Gold MZ) at 1 g/L, 2 g /L and 3 g/L concentration and two bio-agents (*Trichodermaharzianum* and *T. viride*) against *Alternariasolani in vitro* and under tunnel cultivation. In *in vitro* assay, Topsin-M at 3 g/L concentration was the most effective fungicide among the three tested and compared to control. Comparing of both bioagents, *T. harzianum* was more effective than *T. viride* in inhibiting the growth of *A. solani*. Under tunnel cultivation, early blight

disease of tomato was significantly reduced by foliar applications of Topsin-M and *T. harzianium* at 3 g/L and 10^8 conidia/mL concentrations, respectively comparing with untreated plants.

Concerning the effect of plant oils, **Özcan and Boyraz (2000)** found that *Fusariumoxysporum* f. sp.*phaseoli*, *Macrophominaphaseoli*, *Botrytis cinerea*, *Rhizoctoniasolani*, *Alternariasolani* and *Aspergillusparasiticus* were completely inhibited by both of the concentrations of wild thyme and the 10% level of oregano decoctions in all the incubation periods. **El-Mougy and Abdel-Kader (2007)** observed the highly significant inhibitory effect on radial fungal growth at different concentrations of carnation (*Dianthus caryophyllus*), cinnamon (*Cinnamomumburmannil*), garlic (*Allium sativum*) and thyme (*Thymus vulgaris*). In the greenhouse, carnation and cinnamon spieces showed the highest protecting effect against disease incidence when applied as powder or extracts and gave a similar effect to the fungicide Rhizolex-T in reducing damping-off incidence either at pre- or post-emergence stages of *faba bean* plants. **Ajay(2009)** demonstrated that *Cinnamomumzeylanicum* bark and leaves contain certain fungicidal constituents exhibiting potential antimicrobial activity against *Alternariasolani*.**Lathaet al. (2009)** reported that leaf extract of Zimmu (*Allium cepa* L. *x Allium sativum* L.) demonstrated the highest inhibition of mycelial growth (87%) of *Alternariasolani*and caused the highest reduction of the early blight disease.**Fenget al. (2011)** mentioned that thyme oil possessed great fumigant and contact toxicity against *Alternariaalternata* at different concentrations *in vitro*. Thyme oil at 500 µL/L showed a significant contact inhibition effect on *A. alternata* of cherry tomatoes stored at 25 °C for 3 days.**Maya and Thippanna (2015)** revealed that Rhizome extracts of *Zinger officinalea* recorded maximum mycelial inhibition followed by *M. azardichita*,*P. guajava*,*P. pinnata*,*E. Odoratum*, and *Cassia torawith*which was the least inhibitor of mycelia when evaluated ten locally available aqueous botanical extracts against *Alternariasolani*.

Regarding the effect of chemical inducers, **Janisiewicz and Peterson, (2005)** revealed that spraying of plants with $NaHCO_3$ solution was provided good control of several plant diseases. **El-Gamal *et al.* (2007)** evaluated the ascorbic acid (AA), dichloro-isonicotinic acid (INA), ethylene diamine tetra acetic acid (EDTA) and calcium chloride against late and early blight diseases under greenhouse and field conditions. Under greenhouse conditions, the highest reduction in disease severity was obtained by AA at 2 & 3 g/1, and INA at 50.0 and 100 mM, which reduced late and early blight severity more than 75.0 and 82.1%, followed by EDTA and calcium chloride, respectively. On the other hand, the combined treatments between AA at 2 g/L and INA at 50 or 100 mM reduced the late and early diseases severity between (86.6-90.0%) and (90.0-93.3%), respectively. **El-Mougy and Abdel-Kader (2009)** recorded the suppressive effect of sodium bicarbonate and calcium chloride applied individually or combined with the yeast *Saccharomyces cerevisiae* against *Alternariasolani* of potato *in vitro* and *in vivo*.

This work aimed to evaluate the efficacy of some biotic and abiotic treatments in controlling *Alternariasolani* the causal of tomato early blight disease *in vitro* and *in vivo*.

Materials and Methods

1. Source of early blight pathogen:

Out of 14 *Alternariasolani* isolates which isolated from different localities of seven governorates, *A. solani* isolate(As-3) which isolated from leaves of cultivated tomato plants in Kaha locality (Qalubia governorate) during spring of season 2012 was chosen for the further studies based on its highly pathogenic potentialities in inducing the early blight disease comparing with the others tested isolates of *A. solani*.

2. Source of tested antagonists:

Out of sixteen bacterial and thirteen fungal isolates isolated from tomato phylloplane and tested *in vitro* for their antagonistic activities, five antagonistic isolates of*Trichodermai.e.*, T-1, T-2, T-3, T-11 and T-13 were identified as *Trichodermaaureoviride*-I(T-1)and *Trichodermaaureoviride*-II (T-2), *T. harzianum*-I (T-3), *T. harzianum*-II (T-13) and one isolate *T. virens* (T-11)according to **Samuels *et al.*, (2009).** Whereas, five bacterial isolates among the sixteen tested were found to be the highly antagonistic ones. These isolates were identified as *Bacillusamyloliquefaciens*-I(B-2),*Bacillusamyloliquefaciens*-II(B-19), *B. subtilis*-I (B-24), *B. subtilis*-II (B-15) andone isolate of *Bacillus leavolactius*(B-17)according to Bergey's manual**(Claus and Berkeley, 1986).** These identified fungal and bacterial isolates which having the highly antagonistic potentialities were used in the further studies of biological control *in vitro* and under greenhouse conditions in addition to two bio-formulations *i.e.*, Bio-Arc and Bio-Zeid as well as one fungicide (Bellis) as control. These antagonists were tested for their antagonistic effects against early blight disease by spraying tomato plants with two sprays at 2 and 16 days before inoculation with the causal pathogen *A. solani* (As-3) two days post the last spray.

3. *In vitro* tests:

3.1. Effect of some bioagentson growth of *A. solani* (As-3) isolate:

In this trail, five antagonistic isolates of *Trichodermai.e.*,*Trichodermaaureoviride*-I(T-1)and *Trichodermaaureoviride*-II (T-2), *T. harzianum*-I (T-

3), *T. harzianum*-II (T-13) and one isolate *T. virens* (T-11) in addition to five bacterial isolates i.e., *Bacillusamyloliquefaciens*-I(B-2),*Bacillusamyloliquefaciens*-II(B-19), *B. subtilis*-I (B-24), *B. subtilis*-II (B-15) andone isolate of *Bacillus leavolactius*(B-17) were tested *in vitro* for their antagonistic abilities against *A. solani* (As-3) isolate the causal pathogen of tomato early blight. In this respect, Petri plates (9 cmØ) containing 20 mL PDA were inoculated with an equal disc (5mm) of the bio-agents i.e., *Trichoderma*isolates and *Bacillus subtilis* (by streaking) onto one side of the prepared Petri plates. At the same time, equal discs (5mmØ) of *A. solani* were added separately in the opposite side of the inoculated Petri plates. Three replicates were used for each particular treatment. Petri plates containing the PDA medium and inoculated with the pathogenic fungus only was used as control. All the previous treatments were incubated at 25ºC for seven to ten days. The growth of each treatment was measured when the mycelial growth of the pathogenic fungus in the control completely covered the medium surface, then the reduction percentage was calculated using the following formula according to **Fokemma (1973).**

$$\textbf{Reduction percentage} = \frac{de - di}{de_0} \times 10$$

Where:
de = Mean diameter of growth in control.
di = Mean diameter of growth in treatment

3.2. Effect of some plant oils on growth of *A. solani* (As-3) isolate:

In this experiment, oils extracted from several plants i.e., thymol (*Thymus vulgaris*); marjoram (*Origanummajorana*); clove (*Dianthus curyaphulus*); garlic (*Alliumsativum*) and cinnamon (*Cinnamomumverum*) were used to study their effects on the growth of *A. solani*. Benzene was added to each one of the concentrated plant oils to increase their solubility **Fahiem, (2010)**. Different concentrations *i.e.,* 2, 3, 4, 5 and 6% were made and each one of the prepared concentrations was added to the melted PDA medium before pouring into Petri plates. Oils were dissolved in 2% benzene. Then the poured media were left to solidify. An equal disc (5 mmØ) of *A. solani* was added separately to the center of the Petri plates. Three plates were used for each treatment. Control treatment was in form of inoculated plates without adding any of the tested plant oils, while benzenewas added with 2% to medium. All inoculated plates were incubated at 25°C for 7-10 days then the plates were examined. The percentage of growth reduction of *A. solani* were calculated as mentioned above**.**

3.3. Effect of some mineral salts on growth of *A. solani* (As-3) isolate:

This experiment was carried out to evaluate some mineral salts on the growth of the pathogenic fungus under *in vitro* conditions. In this respect, sodium bicabonateat concentration of 100, 200 and 400 mM, potassium hydrogen carbonate at concentration of 100, 200 and 400 mM, ascorbic acid at concentration of 1, 2, 3 and 5 m.Mol, and salicylic acid at concentration of 1, 2, 3 and 5 m.Mol, were tested to study their effects on growth of *A. solani* (As-3) isolate. Each one of the tested concentrations of each salt was mixed separately with the melted PDA medium before pouring into Petri plates, and then they were left to solidify. The discs (5 mmØ) of *A. solani* were added individually in the center of the poured PDA plates. Each treatment was replicated 3 times. Control treatment was made by inoculating a disc of *A. solani* onto PDA plate without any salt. All treatments were incubated at 20- 22°C for 7-10 days. The diameters of developed colonies were measured using the formula suggested by **Fokemma (1973)** that mentioned above**.**

4. Greenhouse tests (*In vivo*):

Tomato transplants (hybrid Hypeel 303) obtained from the commercial greenhouses in Tersavillage were used in greenhouse studies.

4.1. Effect of some bioagents in controlling tomato early blight under greenhouse conditions:

In this trail, the previously tested antagonistic fungal and bacterial isolates (*Trichoderma*spp and *Bacillus* spp.) *in vitro* trail were tested for their antagonistic potentialities in controlling early blight disease under greenhouse conditions. Additionally, two commercial formula of antagonists i.e., Bio-Zeid and Bio-Arc as well as Bellis fungicide were tested also for this purpose.

-Preparation of bioagentinocula:

The antagonistic bacteria were grown on nutrient broth medium (**Abd-Alla*et al.,* 2007)**. The bacterial isolates were incubated in a rotary shaker at 200 rpm for 24 h at 28±2°C. The bacterial cells were harvested by centrifugation at 6,000 rpm for 10 min, washed twice with 0.05M phosphate buffer at pH 7.0 and resuspended in sterilized distilled water. The concentrations of bacteria were adjusted to $1x10^5$-10^6 cells per milliliter(cfu/mL) using dilution plate assay**Abdel-Kader*et al.,* (2012)**. Meanwhile, the tested antagonistic fungi were grown on PDA mediumandincubated for one week at 25 ± 2°C. Ten mL of sterilized distilled water were added to each plate then, the fungal conidia were harvested by scraping the surface of the colonies with a fine brush and then, filtered through nylon mesh. The collected spore suspension was adjusted to $1x10^4$-10^5 spore per milliliter with the aid of a haemocytometer slide as described by **Abd-Alla*et al.* (2007)**.

- Preparation of *A. solani* (As-3) inoculum:

The pathogen inoculum was prepared by culturing *A. solani* (As-3) isolate onto PDA medium at 25°C

for15 days, then 10 ml of sterile distilled water was added to each plate and colonies were carefully scrapped with a sterile fine brush. The resulting spore suspension of As-3 isolate was adjusted to 5×10^5 cfu /mL).

- **Application of antagonists and early blight pathogen:**

Pottery pots (30 cm. in diameter) were filled with sterilized sandy- clay soil (1:1 v/v, 5Kg/pot), Three seedlings of hybrid Hypeel 303 per pot were transplanted. Pots were kept under greenhouse at temperature at 22-25ºC, relative humidity about 85-90% and irrigated periodically (10 days interval). Each treatment was replicated as 8 pots. Each replicate of transplanted tomato plants one-week old post transplanting was sprayed with 30 ml of the tested antagonist treatments for the first time, then two weeks later, the second spray was applied. Two days post the second foliar spray with the tested antagonists; artificial infection with *A. solani*isolate(As-3) was carried out as foliar spraying of pathogen suspension**Abdel-Kader*et al.*, (2013)**. Control treatments were in form of treated plants with Bellis fungicide pre inoculation with the tested early blight pathogen, Infected plants only with the tested early blight pathogen without spraying of any treatment pre inoculation and un-sprayed plants with any one of the tested treatments and without inoculation of the tested pathogen. Disease severity % (DS%) was calculated based on the percentage of leaf area covered with lesions where the infected plants were rated using the modified scale (0 -10) by **James** (**1971**) as follows: 0: No symptoms,1:1-5% of leaf area covered with lesions, 2:6-15% of leaf area covered with lesions, 3:16-30 % of leaf area covered with lesions,4:31-45% of leaf area covered with lesions,5:46-59% of leaf area covered with lesions, 6:60-69% of leaf area covered with lesions,7:70-79% of leaf area covered with lesions,8:80-89% of leaf area covered with lesions,9:90-99% of leaf area covered with lesions and 10: Plants totally died.

Disease severity (%) was calculated at 7, 14 and 21 days post inoculation with the tested early blight pathogen(As-3 isolate) using the equation suggested by **Townsend** and **Heuberger (1943)** as follows:

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

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.

4.2 Effect of some plant oils in controlling tomato early blight under greenhouse conditions:

In this trail, the five tested plant oilsi.e.,thymol, marjoram, clove, garlic and cinnamon which previously tested *in vitro* were tested again *in vivo* under greenhouse conditions to evaluate their effects in controlling early blight infection by applying them twic eat 3% concentration on tomato plants pre inoculation with the pathogen as mentioned above. Control treatment was un-sprayed plants with any one of the tested plant oils and without inoculation of the tested pathogen. DS% were determined at 7, 14 and 21 days post inoculation with the tested pathogen as mentioned above.

4.3. Effect of some mineral salts in controlling tomato early blight under greenhouse conditions:

In this trail also, the previously four tested chemical inducers*in vitro* in form of two mineral salts *i.e.*, sodium bicarbonate and potassium hydrogen carbonate at three concentrations in addition to, two organic acids *i.e.*, ascorbic acid and salicylic acid were tested again *in vivo* under greenhouse conditions at 100 mM of the two tested mineral salts and at 5 mM of the two tested organic acids to evaluate their effects in controlling early blight infection by applying them twice on tomato plants pre inoculation with the pathogen as mentioned above. Control treatment was in form of treated plants with Bellis fungicide pre inoculation with the tested early blight pathogen.DS% were determined at 7, 14 and 21 days post inoculation with the tested pathogen as mentioned above.

Results and Discussion

1. Effect of some fungal and bacterial antagonists on tomato early blight disease and its pathogen under *in vitro* and *in vivo* conditions:

1.1. On growth of *A. solani*(As-3) *in vitro*:

Data in **Table 1** indicate that all five tested fungal and bacterial isolates as antagonists to *A. solani* (As-3), the causal pathogen of tomato early blight disease reduced the growth of the pathogen to high extent *in vitro*. In this respect, all five tested *Trichoderma* isolates were more effective in reducing the growth of As-3 isolate than the tested Bacillus isolates as antagonists. The highest reduction% was recorded with *Trichodermaaureoviride*-I(T-1) followed by *Trichodermaaureoviride*-II (T-2) and *Trichodermavirens* (T-11) while the least effective *Trichoderma* isolate among the five tested was *Trichodermaharzianum*-I (T-3). On the other hand, the most effective *Bacillus* isolate among the five tested was *Bacillussubtilis*-I (B-24) followed by *Bacillusamyloliquefaciens*-II(B-19) while, the least effective one in reducing the growth of As-3 isolate was *Bacillus subtilis*-II (B-15) followed by *Bacillusamyloliquefaciens*-I(B-2).

Table 1. Antagonistic potentialities of tested antagonists against *A. solani*(As-3) isolate *in vitro*.

Antagonist	**Linear growth (mm)**	**Reduction%**
Trichodermaaureoviride-I(T-1)	24.1	73.22
Trichodermaaureoviride-II (T-2)	24.8	72.44
Trichodermaharzianum-I (T-3)	29.5	67.22
Trichodermavirens (T-11)	25.0	72.22
Trichodermaharzianum-II (T-13)	28.0	68.88
Bacillusamyloliquefaciens-I(B-2)	61.0	32.2
Bacillussubtilis-II (B-15)	61.7	31.4
Bacillus leavolactius(B-17)	60.1	33.2
Bacillusamyloliquefaciens-II(B-19)	59.1	34.3
Bacillussubtilis-I (B-24)	57.5	36.1
Control	90.0	0.0

1.2. On early blight infection under greenhouse conditions (*in vivo*).

In this trail, five antagonistic isolates of *Trichoderma* and five isolates of *Bacillus* in addition to two bio-formulations (Bio-Arc and Bio-Zeid) and one fungicide (Bellis) as control were tested for their antagonistic effects against early blight disease by spraying tomato plants with two sprays at 2 and 16 days before inoculation with the causal pathogen *A. solani* (As-3) two days post the last spray.

Data in **Table 2** indicate that spraying tomato plants with any one of the tested antagonists before inoculation with *A. solani* under greenhouse conditions decreased the early blight disease severity to values ranged between 15.1-45.3% compared with the infected control treatment with the pathogen only which recorded 93.4% of DS% at 21-day post inoculation with the pathogen. In this respect, *Bacillus amyloliquefaciens*-II (B-19)،*T. virens* (T-11)and *T. harzianum*-II (T-13) were the most effective treatments for controlling the early blight disease at 21-day post inoculation where they recorded 15.1, 21.1 and 25.4% of DS% respectively. *T. harzianum*-I (T-3), *T. aureoviride*-I (T-1) and *T. aureoviride*-I (T-2) came in the second rank where they recorded 30.1, 30.2 and 31.1% of disease severity respectively. Meanwhile, *B. leavolactius*(B-17), *B. subtilis*-II (B-15) and *B. amyloliquefaciens*-I(B-2) were the least effective ones in decreasing the early blight disease severity caused by *A. solani* compared with the other tested antagonists under greenhouse conditions which recorded 45.3, 42.2 and 40.8% of disease severity respectively. On the other hand, Bellis fungicide and Bio-Arc formulation treatments reduced the early blight DS% to values similar to those recorded with the first group of antagonists were they recorded 15.3 and 25.1% of disease severity at 21-day post inoculation. Whereas, the recorded DS% with Bio-Zeid treatment was 35.3% which almost similar with the results of the second group of tested antagonists at 21-day post inoculation with the early blight pathogen (As-3). Moreover, the recorded DS% at 21-day with all tested antagonists were higher than those recorded at 14 and 7 days post inoculation with the pathogen. The *in vitro* and *in vivo* results are in agreement with those obtained by **Tran (2010)** who reported that *Trichoderma* species have become popular biological agents to protect crops against plant pathogens all over the world. Researchers have indicated that they can parasitize fungal pathogens and produce antibiotics.Also,**Fontenelle *et al.* (2011)** revealed that treating the soil with 28 *Trichoderma* isolates provided protection in tomato plants from 30.69 to 95.23% against *A. solani* the causal agent of early blight disease at all-time intervals, confirming the ability of the isolates to reduce the severity of these diseases up to 21 days after treatment of tomato plants. **Yazici*et al.* (2011)** confirmed that 23 bacterial isolates were able to inhibit the mycelial growth of *Alternariasolani* by forming inhibition zone ranging from 9.35 to 31.3 mm. The most effective isolate was *Serratiaplymuthica* (IK- 139). In whole plant tests, bacterial isolates of *Paenibacillusmacerans*-GC subgroup A, *Serratiaplymuthica*, *Bacillus coagulans*, *Serratiamarcescens*-GC subgroup A, *Bacillus pumilis*–GC subgroup B and *Pantoeaagglomerans* (1.32) reduced significantly disease severity of early blight when compared with control in tomato. In addition, **Prajapati *et al.* (2014)** found that*Trichodermaviride*, *T. harzianum* and *T. virens* are promising antagonists against *Alternariasolani* under *in vitro* evaluation carried out by dual culture technique for growth inhibition. *Trichoderma* not only overgrew the host fungus *A. solani*, but also it caused mycelialcoiling. Also, **Ramanujam *et al.* (2015)** evaluated the fungal and bacterial antagonists against early blight pathogen of tomato, *Alternariasolani* under *in vitro*, glasshouse and field conditions. Among the isolates tested, *Trichodermaharzianum* (Th-7) showed significant inhibition of *A. solani* under *in vitro* and glasshouse conditions. Seedling dip and foliar applications of *T. harzianum* (Th-7), *T. viride* (Tv-14) and *P. fluorescens* (Pf-1) decreased the early blight disease incidence up to 62% and increased the tomato yield up to 37% over control in field trials.

Table 2. Effect of some tested antagonists on tomato early blight disease severity caused by *A. solani* (As-3) at different incubation periods under greenhouse conditions.

Antagonist	Disease severity % at days			Mean
	7	14	21	
T. aureoviride-I (T-1)	16.0	23.5	30.2	23.2
T. aureoviride-II (T-2)	10.5	20.3	31.1	20.7
T. harzianum-I (T-3)	17.0	27.4	30.1	24.8
T. harzianum-II (T-13)	8.3	15.8	25.4	16.5
T. virens (T-11)	9.9	15.5	21.1	15.5
B. subtilis-I (B-24)	13.5	30.2	37.3	27.0
B. subtilis-II (B-15)	21.1	30.3	42.2	31.2
B. amyloliquefaciens-I (B-2)	17.5	30.7	40.4	29.5
B. amyloliquefaciens-II (B-19)	10.2	15.0	15.1	13.4
B. leavolactius(B-17)	25.5	35.4	45.3	35.4
Bio-Zeid	16.5	20.3	35.3	24.0
Bio-Arc	7.5	15.3	25.1	16.0
Bellis fungicide	10.7	15.1	15.3	13.7
Infected control	68.7	81.8	93.4	81.4
Control	00.0	00.0	5.3	1.8
L.S.D at 5%				

2. Effect of some plant oils on tomato early blight disease and its pathogen under *in vitro* and *in vivo* conditions:

In this trail, five plant oils *i.e.*,Garlic,Cinnamon, Cloves, Marjoram, and Thyme were tested to show their effects on the mycelial growth of *A. solani* isolate (As-3) *in vitro* and early blight infection under greenhouse conditions (*in vivo*).

2.1. On growth of *A. solani*(As-3) *in vitro*:

Different concentrations of the five tested plant oils i.e., 2, 3, 4, 5 and 6% were tested to show their effects on growth of *A. solani*(As-3) isolate. Data in **Table 3** indicate that all tested plant oils reduced the growth of *A. solani*(As-3) isolate with superiority of the high concentrations than the low ones in their effects.

Table 3. Effect of plant oils on the growth of *A. solani* (As-3) *in vitro*.

Plant Oil	Conc. (%)	Linear growth	Reduction%
Cinnamon	2	90.0	0.0
	3	83.6	7.1
	4	77.2	14.3
	5	55.2	38.7
	6	00.0	100.0
Marjoram	2	90.0	0.0
	3	74.0	17.8
	4	67.0	25.5
	5	50.5	43.9
	6	40.0	55.5
Thyme	2	90.0	0.0
	3	86.6	3. 8
	4	80.3	10. 8
	5	76.0	15. 6
	6	62.0	31.1
Garlic	2	90.0	0.0
	3	85.5	5.0
	4	84.7	5.8
	5	81.0	10.0
	6	75.5	16.1
Clove	2	69.0	23.3
	3	56.0	37.78
	4	35.3	60.8
	5	30.2	66.39
	6	22.0	75.55
Control (benzene)		90.0	0.0

In this respect, it was pronounced from results that the most reduction% in growth of *A. solani* was obtained with the concentration 6.0% of all tested plant oils. Among the five tested plant oils, cinnamon was the most effective oil where it reduced the growth of *A. solani* to 100% followed by cloves (75.6%) then marjoram (55.6%), while garlic was the least effective oil (16.1%). The control treatment with benzene had no effect on the mycelial growth reduction of the pathogen.

2.2. On early blight infection under greenhouse conditions (*in vivo*).

Results in **Table 4** illustrated that all tested plant oils at 3% concentration were moderately effective in controlling the early blight infection under greenhouse conditions. On the other hand, clove and marjoram oil were the most effective plant oils in decreasing early blight infection where they recorded 46.2 and 48.6% respectively of DS% at 21-day post inoculation with the pathogen comparing with the others tested. Meanwhile, garlic oil was the least effective one where it recorded 66.2 % of DS. It is clear from the obtained results that in spite of spraying tomato plants with the tested oils before inoculation with early blight pathogen (As-3), the recorded DS% were increased with increasing the incubation periods to be the higher at 21-day than at 14 and 7 days.

Table 4. Effect of some plant oils on tomato early blight disease infection caused by *A. solani* (As-3) at different incubation periods under greenhouse conditions.

Plant oil	Conc. (%)	Disease severity % at days			Mean
		7	14	21	
Cinnamon	3	35.34	44.40	56.12	45.29
Marjoram	3	30.29	36.11	48.57	38.32
Thyme	3	32.91	40.52	55.87	43.10
Garlic	3	41.54	49.73	66.23	52.50
Clove	3	29.51	35.74	46.22	37.16
Control		00.0	00.0	5.3	1.8

The obtained results of the effect of plant oils onto early blight disease *in vitro* and *in vivo* could be interpreting in light the findings of **Amadioha (2000)** who suggested that the mechanisms of disease suppression by plant products have the active principles present in plant extracts may act on the pathogen directly. Also, **Abou-Jawdah *et al.* (2002)** reported that wild marjoram (*Origanumsyriacum*) extract showed the highest and widest range of antimycotic activity against eight phytopathogenic fungi among them *Alternariasolani* where it resulted complete inhibition of mycelial growth. Also, **Wszelaki and Miller (2005)** reported that garlic extracts reduced significantly the early blight disease on tomato. **El-Mougy (2009)** studied the effect of carnation, caraway, thyme oils and Ridomil-MZ fungicide at various concentrations on mycelial growth of *Alternariasolani*. Carnation oil had the strongest inhibitory effect on fungal growth. Slightly less effective were caraway and thyme oils followed by the chemical fungicide. Extended field trials for two cultivation seasons proved that the application of essential oils twice as foliar spray had a superior effect to the fungicide treatment for reducing the early blight incidence comparing with untreated control. In addition, these results are in harmony with those recorded by **Maya and Thippanna (2015).**

3. Effect of some chemical inducers on tomato early blight disease and its pathogen under *in vitro* and *in vivo* conditions:

In this trail, four chemical inducers in form of two mineral salts *i.e.,* sodium bicarbonate and potassium hydrogen carbonate at three concentrations (100, 200 and 400 mM) in addition to, two organic acids *i.e.,* ascorbic acid and salicylic acid at 4 concentrations (1,2, 3 and 5 mM) were tested *in vitro* to show their effects on growth of *A. solani*(As-3) isolate as well as, their effects on early blight disease severity under greenhouse conditions.

3.1. On growth of *A. solani*(As-3) *in vitro*:

Data in **Table 5** indicate that all tested chemical inducers reduced the growth of *A. solani*(As-3) on PDA plates compared with growth on water-treated cultures (control). Also, it was found a positive relation between the high concentration of the tested mineral salts and the recorded reduction % of *A. solani* growth where the high concentration 400 mM of both tested salts was the most effective one than others tested. As for tested organic acids, data indicate that salicylic acid at 5 and 3 mM was more effective in reducing the growth of As-3 isolate comparing with the same concentrations of ascorbic acid.

3.2. On tomato early blight infection under greenhouse conditions.

Results in **Table 6** reveal that spraying tomato plants with the tested chemical inducers (mineral salts or organic acids) against early blight disease caused by *A. solani*(As-3) was moderately effective in controlling the infection. Also, it is clear from the obtained results that all tested chemical inducers controlled the early blight infection where the

recorded DS% were ranged between 30.1 - 45.4% at 21-day post inoculation with *A. solani* (As-3). Spraying tomato plants with salicylic acid was the most effective treatment in controlling *A. solani* (As-3) infection under greenhouse conditions comparing with the other tested chemical inducers. In general, the tested organic acids were more effective than the tested mineral salts however, all tested chemical inducers were less effective than Bellis fungicide in controlling early blight infection. The recorded DS% post inoculation with the pathogen post spraying with each one of the tested chemical inducers was gradually increased with increasing days post inoculation from 7-21-day. The obtained results on the effect of some chemical inducers on early blight pathogen *in vitro* and *in vivo* are in harmony with those obtained by **Fallik *et al.* (1997)** who reported that the inhibitory effect of sodium bicarbonate on microorganisms may be due to reduction of cell turgor that causes a collapse and shrinkage of hyphal and spores. Also, **Spletzer and Enyedi (1999)** mentioned that salicylic acid (SA) is an important signal molecule that plays a critical role in plant defense against pathogen invasion. Data indicate that root feeding 200 μM SA to tomato plants can (i) significantly elevate foliar SA levels, (ii) induce PR-1B gene expression, and (iii) activate SAR that is effective against *Alternariasolani*. Moreover, the recorded results of **El-Gamal*et al.* (2007)** and **El-Mougy and Abdel-Kader (2009)** supported the obtained results in this work.

Table 5. Effect of some chemical inducers on growth of *A. solani* (As-3) under laboratory conditions.

Chemical inducer	Conc. (mM)	Linear growth	Reduction%
		Mineral salts	
Sodium bicabonate	100	23.3	74.1
	200	12.5	86.1
	400	10.0	88.9
Potassium hydrogen carbonate	100	37.0	58.9
	200	23.7	73.7
	400	12.3	86.3
		Organic acids	
Ascorbic acid	1	87.5	2.8
	2	73.5	18.3
	3	49.5	45.0
	5	32.5	63.9
Salicylic acid	1	90.0	0.0
	2	56.6	37.1
	3	28.1	68.8
	5	13.3	85.2
Control		90	0.00

Table 6. Effect of some chemical inducers on the disease severity on tomato plants caused by *A. solani* under greenhouse conditions.

Chemical inducer	Conc. (mM)	Disease severity % at days			Mean
		7	14	21	
Sodium bicabonate	100	16.6	35.3	40.1	30.7
Potassium hydrogen carbonate	100	27.5	40.4	45.4	37.8
Ascorbic acid	5	15.5	27.6	35.8	26.3
Salicylic acid	5	12.8	20.4	30.1	21.1
Bellis fungicide	0.5g/L	10.7	15.1	15.3	13.7

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فاعلية بعض المعاملات الحيوية والغير حيوية فى مقاومة فطر الالترناريا سولانى المسبب لمرض اللفحة المبكرة فى الطماطم تحت ظروف المعمل والصوبة

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الملخص العربى

يعتبرمرض اللفحة المبكرة المتسبب عن فطر *Alternariasolani* واحدا من أمراض المجموع الخضرى الشائعة على الطماطم. وفى هذا الإطار كانت الخمس عزلات المختبرة من *Trichoderma* أكثر فاعلية فى أختزال نمو فطر *A. solani* من عزلات*Bacillus*المختبرة وقد سجلت أعلى نسبة اختزال مع (T–1)*Trichodermaaureoviride*–I و(T–2) *T. aureoviride*–II (B–24), *B. subtilis*–I و(B–19) *B. amyloliquefaciens*–II وعلى الجانب الأخرأدي رش نباتات الطماطم بأي من الكائنات المضادة المختبرة قبل العدوى بفطر *A. solani* تحت ظروف الصوبة إلي خفض شدة الإصابة بمرض اللفحة المبكرة بقيمة تتراوح بين ١٥,١–٤٥,٣% مقارنة بمعاملة الكنترول بالفطر الممرض فقط والتي سجلت شدة إصلبة ٩٣.٤%. وكانت المعاملة ببكتيريا(B–19)*B.amyloliquefaciens*–II، *T. virens* (T–11) و(T–13) *T. harzianum*–II أفضل المعاملات لمقاومة اللفحة المبكرة عند اليوم ٢١ بعد العدوى. أيضا سجلت كل الزيوت المختبرة خفضا في نمو الفطر للعزلة (As–3) *A. solani* بأفضلية لتأثير التركيزات العالية عن التركيزات المنخفضة. وكان أفضل اختزال فى نمو (As–3) *A. solani* تم الحصول عليها بتركيز ٦%من كل الزيوت المختبرة. من بين الزيوت النباتية المختبرة كان زيت القرفة أفضل زيت ثم يليه زيت القرنفل ثم زيت البردقوش. بينما كان زيت الثوم أقل الزيوت فاعلية. إضافة إلى ذلك، كانت الزيوت المختبرة عند تركيز ٣% متوسطة التأثير فى مقاومة الاصابة اللفحة المبكرة تحت ظروف الصوبة. كان زيت القرنفل والبردقوش أفضل الزيوت النباتية فى خفض الاصابة بمرض اللفحة المبكرة عند اليوم ٢١ بعد العدوى بالممرض على الترتيب. بينما كان زيت الثوم أقلهم تأثيرا.على الجانب الأخر كل المستحثات الكميائية اختزلت نمو(As–3) *A.solani* على أطباق بيئة مستخلص البطاطس. التركيز العالى ٤٠٠ملى مول لكلا من بيكربونات الصوديوم وكربونات البوتاسيوم المختبرة كان أكثر فاعلية من غيرها وأيضا كان تركيز ٣ و ٥ ملى مول أكثر فاعلية فى اختزال (As–3) *A.solani* مقارنة بنفس التركيزات لحمض الاسكوربيك، رش النباتات الطماطم بالمستحثات الكميائية المختبرة (أملاح معدنية وأحماض عضوية) ضد مرض اللفحة المبكرة المتسبب عن فطر (As–3) *A. solani* كانت متوسطة التأثير فى مقاومة الإصابة. ومع ذلك كل المستحثات الكميائية المختبرة قاومت ألاصابة باللفحة المبكرة وتراوحت شدة الإصابة التى تم تسجيلها بين ٣٠,١–٤٥,٤ % عند اليوم ٢١ بعد العدوى بفطر

A. solani(As–3).كان رش نباتات الطماطم بحمض السالسيلك أفضل المعاملات فى مقاومة الإصابة بفطر (As–3) *A. solani* تحت ظروف الصوبة مقارنة بالمستحثات الكميائية الاخرى المختبرة .

الكلمات الدالة .

مرض اللفحة المبكرة –الترناريا سولانى*Alternariasolani* – الأملاح المعدنية –الاحماض العضوية –الزيوت النباتية –الكائنات المضادة .