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CONTROLLING OF TOMATO EARLY BLIGHT DISEASE USING SOME OF BIOTIC AND A BIOTIC AGENTS

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) early blight caused by *Alternaria solani* is one of the most important economic diseases, which causing considerable loss in their yield and quality. *Trichoderma viride*, *Bacillus subtilus* and actinomycetes isolate A were the most effective bioagents on decreasing linear growth of *A. solani*. All bioagents tested reduced disease incidence using detached leaf and/or intact leaf techniques, when treated 2 and 7 days before inoculation with the pathogen. *T. harizanum* was the most effective isolate on reducing percentage of disease protection 2 and 7 days before inoculation (96.57 and 89.84) followed by *T. viride* (92.65 and 83.77%) then *B. subtilus* (83.34 and 66.89) using detached leaf. The same trend was obtained in case intact leaf, *T. harizanum* was the most effective isolate on reducing disease severity, percentage of disease and increase percentage of protection 2 and 7 days before inoculation (16.44 and 58.62 and 38.28 at two days and 22.26, 62.43 and 35.17 afeter 7 days) followed by B*. subtilus* then *T. viride.* Generally bioagents tested were most effective using detached leaf than intact leaf.

In vitro experiments, chitosan and salicylic acid (SA) 4% were the effective concentrations on reducing growth of *A. solani* (84.44 and 67.77 %) and reducing spores germination (55.31 and 81.56 %). All the tested concentrations of chitosan and SA as inducer of resistance in tomato plants, which reduced number and diameter of lesion as well as percentage of disease and increase percentage of protection 7 days after treatment using detached and intact leaf techniques. Both water and ethanol extracts of garlic were the most effective on inhibition the mycelial growth, spore germination and disease parameters using detached and intact leaf techniques, followed by ginger extract.

Keywords: Bio-control, induced resistance, plant extracts, early blight, *Alternaria solani,* bio-control induced resistance, tomato.

INTRODUCTION

Early blight of tomato caused by the necrotrophic fungus *Alternaria solani* (Ellis & Martin) Jones & Grout, is one of the most common diseases of tomatoes. The disease can occur over a wide range of climatic conditions, but it is most iprominent in areas with dew, rainfull and high relative humidity. On tomato, infection of the plants can result in a complete loss of the crop as yields are reduced by damaging foliage and fruits (Chaerani *et al*. 2007).

In the recent years, there are an environmental and economical concerns regarding agricultural chemicals have resulted in searching of alternative disease control agents that minimize fungicides use without increasing disease losses *i.e.* bio-agents (Atia, 2005, Taghian, Shadia, *et.al.* 2008 and Esh *et al*., 2010), essential plant extracts and systemic inducers of resistance, which were, safe for men and environmental, has long lasting activity and effective against wide rang of pathogen (Kessman *et. al.*, 1994 and Van Loon *et.al*. 1997).

Several investigators used plant surface flora as a bioagent against several plant pathogenic fungi and bacteria (Jindal *et al.,* 1988, Aly *et al*., 2002 and Esh *et al*., 2010). Different microorganisms were select as an bioagents against *A. solani, i.e. Cladosporium herbarum, Aspergillus* spp., *Penicillium* spp., *Acremonium strictum*, *A. alternate*, *Ulocladium botrytis* and *Scopulariopsis brevicaulis* (Ahmed and Saleh, 1991), *T. harzianum* (El-Farnawany, 2006 and Ahmed, Amal, 2009), *Pseudomonas* sp.(Casida and Lukezic (1993), *Bacillus megaterium*, *B. brevis* and *B. subtilis* var *globigii* (Liu and Wu 1997) and S*treptomyces pulcher* (El-Abyad *et al.,* 1993).

The modern plant protection system has involved the new means that act not directly on the pathogen, but activate the natural defence mechanisms of the plant and enhance the same physiological and biochemical changes in plants as does the biological systemic activated resistance (Surviliene *et al.* 2003). Systemic acquired resistance (SAR) can be induced in various plants by different biotic agent (VanLoon *et al.,* 1997 and Aly *et al.,* 2002) and by chemical inducers such as Dl-ß-aminobutyric acid (Suli *et al.*, 2002; Atia *et al.*, 2003, Atia, 2005), bion (Atia, 2000), chitosan (Benhamou *et al.* 1998 and Atia, *et al*., 2005) and salicylic acid (Atia, *et al.,* 2003 and Liao, *et al*., 2003).

Salicylic acid (SA) is an importants singnal molecule that plays a critical role in plant defence against pathogen invasion (Chaturvedi and Shah, 2007). Liao *et al.* (2003) found that, resistance in tomato plants to early blight caused by *A. solani* was induced by chitosan (1 mg/ml) at the four leaf stage of seedlings and activate its defense mechanisms. Atia (2005), found that, melon plants treated with Dl-ß-amino-n-butyric acid (BABA) (10 mM) 7 days before inoculation with *A. cucumerina* resulted in significant disease protection locally and systemically. Treating tomato plants with chitosan (1mg ml^{-1}) 7 days before inoculation with *Phytophthora infestans* resulted in significant disease protection (Atia *el al.*, 2005).

Essential plant extract and oils has been used to control several plant diseases on various crops (Hafez, 2008 and Fawzi *et al*., 2009). Ashrafuzzaman *et al*., (1990) mentioned that plant extracts of medicinal species play an important role in controlling the early blight pathogen *in vitro* and *vivo*. Generally, garlic extract was the best extract used tomato early blight disease. Suwitchayanon and Kunasakdakul, (2009) found that, clove extract was very effective in controlling *A. cucumerina* on melon.

Thus, this work aimed to investigate the antagonistic activities of different microorganisms, induced resistance by the selected chemical inducers, anti-fungal activity of some plant extracts on mycelia growth, spores germination and infection with *Alternaria solani* the causal of early blight disease of tomato under greenhouse conditions.

MATERIALS AND METHODS

Sourse of isolate*: A. solani* isolate used during this investigation was isolated and its pathogeneicity was confirmed (Ahmed, Amal, *et al*., 2009), detached and/or intact leaf techniques were used during this study (Atia *et al*. 2005 and Reni *et al*. 2007).

Sourse of bioagents: Tomato leaf surface microflora was isolated, purified using dilliution method. Bacteria were isolated using King's B medium (King, *et al.* 1945). Actinomycetes were isolated on Jensens agar medium (Jensen, 1930). Identification of fungal bacteria and actinomycetes were identified according to their shape, pigmentation and culture characteristics based on Berge's Manual of Determinative Bacteriology 9th ed. (Holt *et al.,* 1994). *Trichoderma* spp., *Bacillus subtilus* and *Pseudomonas flouescense* bioagents tested were obtained from Pl. Path. Laboratory, Agric. Bot. & Pl. Path. Dept., Fac. Agric., Zagazig Univ.

Tomato plants: tomato Gs cv. seedlingsand/or plants were transplanted in plastic pots (30 cm. in diameter) filled with a soil mixture, pots were kept under greenhouse conditions at Agric. Bot. & Pl. Path. Dept. Fac. Agric. Zagazig Univ. Plants at 4-5 weeks old, with 5-6 full true leaves were used

Inoculum preparation: inoculum of *A. solani* isolate was prepared from cultures grown on PDA medium for 7 days at 28±2°C. Mycelial mats were washed several times with sterilized distilled water, then blended with water for 3 min. Then fungal suspension concentration was adjusted microscopically with aid of the hemocytometer technique to10^5 cfu/ml (Brame and Flood 1983).

Biological control

In vitro assay

The interaction between different isolated microorganisms *i.e*. bacteria (*Bacillus subtilus*, *Pseudomonas flouescense*, A and B isolates); actinomycetes (A and B isolates) and fungi (*Trichoderma* spp.) and *A. solani* growth isolate was tested. Petri dishes (9 cm in diameter) containing PDA medium were inoculated in the center with disk (5 mm in diameter) taken from the edges of 7 days old *A. solani*. Inoculation with the tested isolated bacteria and actinomycetes were done by streaking on the surface of the media at the distance of 1.5 cm from the edge of the plates with aid of dual culture method. While in case of fungi, plates were inoculated with agar disks (5 mm in diameter) of the tested fungi at the distance of 1.5 cm from the edge of the plates. Plates inoculated with *A. solani* alone were used as a control. Then plates were incubated at 28 ± 2°C. Three plates were used for each treatment. When the plates of control were covered with the mycelial growth of *A. solani* (7-10 days later) the mean diameter of the mycelial growth of different treatments was measured. The percentage of growth was calculated using the following formula: A/B X 100.

A = the mean diameter of the growth in the treatment.

B = the mean diameter of the growth in the control.

***In vivo* assay:**

Detached leaf technique:

Effect of the most antagonistic fungi, bacteria and actinomycetes on the infection with *A. solani* using detached leaf technique was carred out. Fungal isolates (*Trichoderma* spp.) were grown on 200 ml of sterilized potato dextrose broth medium in 500 ml Erlenmeyer flasks on a rotary shaker (100 rpm) for 7 days at 28±2°C. (Aly *et al.*, 2002). The liquid culture were mixed in a blender and adjusted to contain 10^6 cfu/ml. Bacterial and actinomycetes

isolates were grown on King's B liquid medium (King *et al*., 1945). Flasks (500 ml) each containing 100 ml of King's B medium were inoculated with a lop full of 24 h old of bacteria and/or actinomycetes cultures. Flasks were incubated at 28°C on rotary shaker (100 rpm) for 24 h in case of bacterial (Aly *et al*., 2002) and 9 days in case of actinomycetes (Lotfy, Maisa, 1996).

Tomato plants were sprayed with 30ml/ plant of each tested organism suspension alone, control treatment was sprayed with autoclaved potato broth dextrose and or King's B media. Then all treated plants were covered with plastic box under greenhouse conditions. After 2 and 7 days detached leaves were transferred into 15 cm. ∅ Petri dishes with moisten filter papers. Three Petri-dishes were used as a replicates for each concentration. The lower surface of treated leaflets was inoculated with 6 drops (30 µl)/leaflet of 10^5 cfu of *A. solani* isolate. Inoculated leaflets were incubated at 23°C and the disease incidence was calculated after 7-10 days. Number and diameter of necrotic lesions (mm) as well as blighted area (mm^2)/ leaflet were determined. Percentage of protection was calculated as follows:

Percentage of protection = 100- A/B

A= Percentage of disease in treated (100× blighted area in treated/ blighted area in the untreated (control).

B= percentage of disease in untreated (control) mentioned before.

Intact leaves:

Effect of the antagonistic microorganisms on early blight disease was also studied under greenhouse conditions on intact leaves. The inoculum of the tested organisms was prepared as mentioned before. Tomato plants (4-5 weeks old) were sprayed with 30ml/plant of each tested organism suspension alone, and the control treatment was done. Three replicates for each particular treatment were used. The sprayed plants were covered with plastic box under greenhouse conditions. After 2 and 7 days after spraying, plants (intact leaves) were sprayed with 30 ml/plant of 10^5 cfu/ml of *A. solani*. Inoculated plants were incubated under plastic box under greenhouse conditions for 48 days. Infection with *A. solani* and disease assessment was determined using 1-9 scale (Pandey *et al*. 2003).

Induced resistance:

These experiments aimed to study the effect of some inducers on enhance disese resistance in tomato plants to early blight pathogen *A. solani* as well as dirct antifungal activity on linear growth and spore germination under *in vitro* conditions were also tested.

***In vitro* assay:**

Effect of different concentrations of chitosan and salicylic acid on linear growth of *A. solani*:

Different concentrations (0, 1, 2 and 4g/l) of chitosan and salicylic acid **(**SA) were tested on the linear growth of *A. solani* using PDA medium. Purified chitosan solution (Sigma Chemical Company) was prepared using methods similar to those of El-Ghaouth *et al*. (1994) and Atia *et al.*, (2005) and pH was adjusted to 6.5 with 0.5 NaOH (Benhamou *et al*., 1998). One drop (10 µl) of each chitosan solution was placed onto the surface of PDA in each of five Petri dishes. A mycelial disc was then placed facing downwards

onto the chitosan droplet. Sterile water and pH was adjusted to 6.5 with HCl (0.25 N) solution were used as a control (to check whether it had any effect on pathogen growth or not).

Different SA concentrations (0.1, 0.2, 0.5, 1, 2 and 4 g/l) were incorporated in 100 ml PDA media and poured in 9 cm Petri dishes. A 5 mm diameter agar disc containing fungal mycelium growth was transferred to the test medium. Three plates for each concentration were used, and control one was also done. Then plates were incubated at 28±2°C. Colony diameters were measured when the surface of control in Petri dishes was covered with the fungal growth.

Effect of different concentrations chitosan and salicylic acid on spore germination of *A. solani*:

The effect of different concentrations of chitosan and SA was studied using the slide technique described by Nair and Ellingboe (1962). A drop of each inducer concentration alone was deposited on dried clean glass slide as a film. A drop of spore suspension of *A. solani* 10^5 cfu/ml was spread over this film. Control treatment was prepared using sterilized distilled water. Three replicates were used for each concentration. Slides were placed on u-shap glass rod in Petri-dish under moisten conditions and incubated for 24h at 28±2°C. Spores germination (%) was calculated.

***In vivo* assay:**

Effect of different chitosan and salicylic acid concentrations on disease incidence using detached leaf technique:

Different concentrations of chitosan (1, 2 and 4 g/l) and SA (0.1, 0.2, 0.5, 1, 2 and 4 g/l) were tested against early blight on tomato Gs cv. detached leaves. The tested concentrations were sprayed on the upper and lower surface of leaves. Then, sprayed tomato leaves were detached 7 days after treatment. Detached leaves were removed immediately before inoculation and then placed in Petri-dishes (15 cm∅) containing filter paper saturated with sterilized water. Detached leaves were inoculated with 6 drops (30 µl)/leaflet *A. solani* and/or water as a control. Three replicates were used for each concentration. Inoculation and disease incidence were done as mentioned before.

Intact leaves:

Effect of different concentrations of chitosan and SA on early blight disease incidence under greenhouse conditions:

Different concentrations of chitosan (1, 2 and 4%) and SA (0.4, 0.6, 0.8, 1.0mM) were tested against tomato early blight using detached leaves of Gs cv. Tomato plants at 4-5 weeks old were sprayed with the inducer concentrations. After 7 days treated plants were inoculated with spore suspension of *A. solani* 10^5 cfu/ml. Inoulation with *A. solani* and disease assessment were carried out as mentioned before.

Plant extracts:

This experiment was conducted to study the effect of eight plant extracts illustrated in Table (1) with different methods of extraction (cold water and ethanol) on growth, spore germination of *A. solani*, as well as on infection

with late blight pathogen using detached leaf technique and/or intact leaves under greenhouse conditions.

Preparations of plant extracts:

Plant materials were collected separately, and then washed thoroughly with distilled water then transferred and left to dry at room temperature. Solvents (water and ethanol) were added at 1:1 (V/W). The juice of frizzed cloves of garlic was obtained by crushing them in blender for five min., and then filtrated through two layers of cheesecloth.

Fifty grams of green leaves of sweet basil, banyan tree and henna as well as herb of thyme, sweet marjoram, and rhizome of ginger were crushed with 50 ml of distilled water in a blender for 5 minutes. The filtered extract was centrifuged at 3000 rpm, for 15 minutes and sterilized using Zeits filter. The sterilized crude extract was considered as representative to 100% concentration, and serial dilutions (i.e.100, 50, 20, 10, 5 and 2%) were prepared using sterilized distilled water.

Table (1): List of tested plant extracts.

English name	Scientific name	Family	The plant parts used
Garlic	*Allium sativum* L.	Liliaceae	Cloves
Banyan tree	*Ficus nitida* L.	Moraceae	Leaves
Henna	*Lowsonia inermis* L.	Lythraceae	leaves
Thyme	*Thymus vulgaris* L.	Labiatae	Herb
Sweet basil	*Ocimum basilicum* L.	Labiatae	Leaves
Sweet majoram	*Origanum majorana* L.	Labiatae	Herb
Ginger	*Zingiber officnalis* L.	ingiberaceae	Rhizome

For extraction by ethanol, fifty grams of green leaves, herbs and rhizome were crushed with 50 ml of ethanol 95% in a blender for 5 minutes and then stored in refrigerator for 24 h. filtration; centrifugation and sterilization were done as mentioned before. Supernatant was collected in 250 ml flask and incubated at 60°C in water bath to allow solvents evaporation till flasks contents become powder film, then flasks removed and dried residues were re-dissolved in 50ml of distilled water. The crude extract was considered as representative to 100% concentration, and serial dilutions (from 100 to 5%) were made using sterile distilled water.

Effect of different concentrations of plant extracts on the linear growth of *A. solani* under *in vitro* conditions:

Different concentrations of each extract (0 concentration used as a control, 2, 5, 10, 20, 50 and 100% of the crude extracts) 5 ml from each of the concentrations was dispensed into 9 cm diameter Petri dishes after which 20 ml of melted PDA medium were poured into the plate, then shaken together and allowed to solidify. Three plates for each concentration were used and inoculated at the center with equal discs (5 mm in diameter) taken from 7 days old culture of *A. solani*. Then plates were incubated at 28±2°C until mycelial growth of pathogen covered the surface of medium in control treatment (Qasem and Abu-Blan, 1996**)**. The antifungal activity of plant extract was calculated and measured as percentage reduction of growth of pathogen comparing with control using this formula:

Linear growth reduction (%):

$$= \frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \times 100$$

Effect of plant extracts on spore germination of *A. solani*:

The most effective plant extracts on mycelia growth of *A. solani* were tested on spores germination. Plant extracts were prepared as mentioned before, Antifungal activity of plant extracts on spores germination of *A. solani* was tested using the slide technique method reported by Nair and Ellingaboe (1962) and El-Nagger (1997).

Effect of plant extracts on disease incidence using detached leaf technique:

Tomato leaves (leaf number 2 and 3) were sprayed with different concentrations (10, 20, 50, 100%) of garlic and ginger, the most effective plant extracts on mycelia growth and spores germination of *A. solani*. One day after treatment, leaves of sprayed tomato plants were detached and transferred into Petri-dishes (15cm in diameter) containing filter paper saturated with 10 ml. of sterilized water. Inoculation, incubation and disease parameter were done as mentioned before.

In addition, tomato detached leaves were inoculated with a mixture of the aforementioned concentration and fungal spore suspension (1:1 v/v) to test the direct effect on the disease incidence at the same time.

Effect of plant extracts on disease incidence using intact leaves:

Tomato Gs cv. 4-5 weeks old seedlings were sprayed with the plant extracts. After 24 h. plants were sprayed with *A. solani* as mentioned above. Plants sprayed with distilled water served as a control. Sprayed plants were covered with plastic box under greenhouse conditions. Incubation and disease parameter were done as mentioned before.

Statistical analysis:

Data were statistically analyzed by analysis of variance according to Snedecor and Cochron, (1982) using SPSS system version 8, (1997).

RESULTS AND DISCUSSION

All tested fungal isolates were effective on reducing growth of *A. solani* the causal organism of early blight disease. Results also indicated that, *Trichoderma viride* was the most effective which completely (100%) reduced *A. solani* mycelial growth, followed by *T. harizianum* (88.32%), actionmycetes A (70.56%) and *Bacillus subtilus* (63.33%) followed by bacteria A (56.04), bacteria B (52.10%) and bacteria A (56.04%) and finally *Pseudomonas florcense* (42.22%), Table (2). *T. viride* and *T. harizanum* were the most effective species resulting a sharply reduction in the pathogenic fungal growth comparatively with the others teste microorganisms. This high potentiality in antagonism might be due to *T. viride* acts through the lack of substrate effect on mycelium dray weight, also with different pectin substrates (Metha *et al*., 1975). *Trichoderma* spp. are prolific producers of extra cellular proteins, and are best known for their ability to produce enzymes. (Harman and Kubicek 1998). Hyphal interaction between the bio-agent *T. harzianum* and *A. solani*

occurred by different means such as growing in contact, complete colonization and coiling around the hyphae of the pathogen, direct penetration and formation of appressorium-like structures. The interaction between *T. harzianum* and conidia of *A. solani* resulted in malformations and changes in spore shape, growing in contact and attaching spores and formation of node-like structure between two successive conidia (El-Farnawany 2006).

Table (2): Effect of different bio-agents on growth reduction (%) of *Alternaria solani*.

Bio-agents	Growth reduction (%)
Actionmycetes (*Streptomyces* sp.) A	70.56
Actinomycetes (*Streptomyces* sp.) B	40.37
Bacillus subtilus	63.33
Bacteria (*Bacillus* sp.) A	56.04
Bacteria (*Bacillus* sp.) B	52.10
Pseudomonas florcense	42.22
Trichoderma viride	100.00
Trichoderma harizianum	88.32
Control	0.00
L.S.D at 5%	3.12

B. subtilius isolates were used against several diseases *i.e*. CLS of sugar beet (Taghian, Shadia *et al.* 2008 and Esh *et al.*, 2011); cucurbit powdery mildew (Gilardi, *et al.,* 2008) and gray mould of strawberries (Ju, *et al*., 2007). Several bacterial genera have been successfully used for the biological control (Aly *et al.,* 2002 and, Yusran, *et al*., 2008). On the other hand, actenomycetes are known to produce one or more antibacterial, anti-fungal, antiviral, anti-protozoal, (Holt *et. al.* 1994). *Streptomyces longisporus*, isolated from field soil, showed *in vitro* antagonism against *A. solani*. It produced antifungal activity compounds, such as guanidyl, nigericin and geldanamycin. It also, produced hydrolytic enzymes *i.e.* chitinase and β-1, 3-gluconase (Trejo *et al*. 1998).

Data in Table (3) indicate that, *T. harizanum* inculcated 2 days before pathogen application was the most effective one on reducing mean number of lesion, diameter of lesion (mm), infected area (mm^2) and protection (%), which gave 2.25, 1.96mm, 9.09mm^2 and 96.57% respectively, followed by *T. viride* (3.75, 2.53mm, 19.00 mm^2 and 92.65% then *B. subtilus* (4, 3.72mm, 43.64 mm^2 and 83.34%), respectively, compaed to the control. The same trends were obtained one week before inoculation, (Table, 3). Wherase, *T. harizanum* was the most effective one (3.33, 2.9mm, 21.86mm^2 and 89.84%) followed by *T. viride* (4, 3.33mm, 34.92mm^2 and 83.77% respectively), then *B. subtilus* (5, 4.26mm, 71.23 mm^2 and 66.89% respectively), compaed to the control (6, 6.77, 216.99 and 0).

T. harizanum was the most effective bioagent in controlling tomato early blight which reduced disease severity and percentage of infection (16.44 % and 58.62%) followed by *B. subtilius* (20.56 % and 64.44%) then *T. viride* (27.12% and 68.91%) two days before inoculation with the pathogen. While, actinomycets-A was the lowest effective one (35.53% and 76.00%),

compaed to the control. Results e also indicat that, *T. harizanum* was the most effective one causing a reduction in disease severity (70.18%) and reduction percentage of infection (38.28%), followed by *B. subtilius* (62.65 % and 31.53%) and *T. viride* (50.75% and 26.8 %). While, Actinomycets-A was the lowest effective one (35.51% and 19.25%), compaed to the control.

Table (3): Effect of different bio-agents on tomato early blight disease incidence using detached leaf technique two and seven days after spraying bio-agents.

The tested bio-agents	Time	Number of lesions	Diameter of lesion (mm)	Infected area (mm^2)	Protection (%)
Actinomycets A	2 days	4.20	4.08	56.72	78.40
Bacillus subtilus		4.00	3.72	43.64	83.34
Trichoderma viride		3.75	2.53	19.00	92.65
Trichoderma harizanum		2.25	1.96	9.09	96.57
Control		6.00	7.06	264.94	0.00
Mean		**4.04**	**3.87**	**78.68**	**70.19**
L.S.D at 5%		0.34	0.39	27.33	2.65
Bacillus subtilus	7 days	5.00	4.26	71.23	66.89
Trichoderma viride		4.00	3.33	34.92	83.77
Trichoderma harizanum		3.33	2.90	21.86	89.84
Control		6.00	6.77	216.99	0.00
Mean		**4.58**	**3.32**	**86.25**	**60.13**
L.S.D at 5%		0.37	0.70	21.55	3.64

T. harizanum was the most effective one in controlling the disease severity and percentage of infection (22.26% and 62.43 %) followed by *B. subtilius* (28.25 and 69.70 %), then, *T. viride* (35.56% and 73.85%) Table, 4. Results also indicate that, *T. harizanum* was the most effective on reducing disease severity (61.84%) and percentage the infection (35.17%), followed by *B. subtilius* (51.52 % and 27.62 %), then, *T. viride* (39.03% and 23.32%), compared to the control. Under greenhouse conditions *T. harizanum* controlled tomato early blight 2, and 7 days after application.

Table (4): Effect of different bioagent application on tomato early blight disease incidence under greenhouse conditions, two days spraying bioagent on intact leaf.

The tested bioagents	Time	Disease severity	Disease severity (reduction)	Percentage of infection	Percentage infection (reduction)
Actinomycetes A		35.53	35.51	76.00	19.25
Bacillus subtillus		20.56	62.65	64.44	31.53
Trichoderma viride		27.12	50.75	68.91	26.8
Trichoderma harizanum	2 days	16.44	70.18	58.62	38.28
Control		55.2	0.00	94.14	0.00
Mean		**30.97**	**43.82**	**72.42**	**23.17**
L.S.D at 5%		2.78	2.53	2.25	2.24
Control		58.42	0.00	96.32	0.00
Trichoderma viride		35.56	39.03	73.85	23.32
Trichoderma harizanum	7 days	22.26	61.84	62.43	35.17
Bacillus subtillus		28.25	51.52	69.70	27.62
Mean		**36.12**	**39.1**	**75.58**	**21.52**
L.S.D at 5%		2.64	2.56	1.55	1.06

This effect might be due to induce anti-fungal or layatic compounds (Yahia *et al*., 1990) in its culture or mycoparasitism (Handlesman and Park 1989). *T. harizanum* attached host and can coil around it and forming appressoria on the host surface. Attachment is mediated by the binding of carbohydrates in the *Trichoderma* cell wall to lectins on target fungus (Inbar *et al*., 1998). Once in contact, *Trichoderma* spp. produced several fungitoxic cell-wall degrading enzymes (Chet *et al*., 1998), and probably also peptaibol antibiotics **(**Schirmböck *et al*., 1994). The combined activities of these compounds resulted in parasitism of target fungus, and dissolution its cell walls. At the sites of the appressoria, holes can be produced in the target fungus, and direct entry of *Trichoderma* hyphae into the lumen of the target fungus occurs. There are at least 20-30 known genes, proteins and other metabolites that are directly involved in this interaction, which is typical of the complex systems that are used by these fungi in their interactions with other organisms.

Data in Table (5) show that, all tested concentrations of chitosan and SA reduced the linear growth of *A. solani* on agar plates compared to the control. Concentration 4 g/l of both tested inducers was the most effective (84.44 and 67.77 %), followed by 2 g/l (78.8 and 56.84), then 1g/l (73.7 and 44.44). Chitosan was the most effective than SA on reducing mycelial growth. The reduction effect was increased with increasing concentrations of both inducers. The same trends were obtained in case of spore germination (Table, 5). Concentration 4g/l of chitosan and SA reveald the hightest reduction percentage of spore germination (55.31 and 81.56) followed by 2g/l (42.09 and 76.18), then 1g/l (28.39 and 65.61) of chitosan and SA respectively. it is also clear that, SA was most effective than chitosan on reducing spore germination of *A solani*. Increasing concentration of both chitosan and SA resulted in reducing spore germination percentage.

Table (5): Effect of different concentrations of salicylic acid and chitosan on growth reduction and spores germination (%) of *Alternaria solani*.

Chemical inducers	Concentration (g/l)	Linear Growth (reduction%)	Linear Spores germination (reduction %)
Chitosan	**0.0**	0.00	0.00
	1.0	73.70	28.39
	2.0	78.80	42.09
	4.0	84.44	55.31
	Mean	**59.26**	**31.459**
Salicylic acid	**0.0**	0.00	0.00
	0.1	13.68	25.56
	0.2	21.08	35.40
	0.5	34.42	56.26
	1.0	44.44	65.61
	2.0	56.84	76.18
	4.0	67.77	81.56
	Mean	**24.35**	**37.00**
L.S.D at 5%		3.55	2.67

The inducer concentrations tested decreased number of lesion; diameter of lesion, blighted area and protection (%) of tomato leaves Gs cv. (Table, 6). It is also clear that, increasing the concentrations of chitosan and/or SA lead to decrease the disease incidence and increase protection (%). The highest concentration (4 g/l) of chitosan and SA was the most effective on reducing disease number of lesion (3.25 and 2) and diameter of lesion (3.48 and 1.26), infected area (31.29 and 2.5) and protection% (88.56 and 99.09 %) respectively. Followed by 2g/l [(4.5, and 2), (4.66, and 1.94), (76.84 and 9.09) and (71.99 and 96.68) of chitosan and SA respectively], then 1 % compared to control one (6, 7.63, 274.44 and 0 %). Data also indicated that SA was most effective than chitosan on tomato early blight disease.

Table (6): Effect of application different concentrations of selected chemical inducers on tomato early blight disease incidence using detached leaf technique.

Chemical inducers	Concentration (g/l)	Number of lesions	Diameter of lesion (mm)	Infected area (mm^2)	Protection (%)
	0.0	6.00	7.63	274.44	0.00
	1.0	5.33	5.16	112.69	58.91
Chitosan	2.0	4.50	4.66	76.84	71.99
	4.0	3.25	3.48	31.29	88.56
	Mean	**4.77**	**5.23**	**123.82**	**54.87**
	0.0	6.00	7.63	274.44	0.00
	0.5	4.16	3.83	48.27	82.40
Salicylic acid	1.0	3.50	2.60	18.76	93.16
	2.0	3.00	1.94	9.09	96.68
	4 .0	2.00	1.26	2.5	99.09
	Mean	**3.73**	**3.45**	**70.61**	**74.27**
L.S.D at 5%		0.55	0.37	15.77	19.00

Data present in Table (7) reveal that, all tested concentrations of chitosan and SA induced diseased resistance and decreased disease severity and percentage of disease incidence. Also, disease severity and percentage of disease incidence decreased with increasing concentrations of chitosan and SA. Data also indicated that, the highest concentrations (4g/l) of chitosan and SA were the most effective one in inducing disease resistance, reducing disease severity and percentage of infection (30.32 and 69.64 in case of chitosan and 11.03 and 49.99 in case of SA respectively), then 2g/l. The lowest effective in inducing resistance to tomato early blight disease compared to the control (56.52 and 95.75). Simillar results were obtained by (Atia *et. al.*, 2003 and 2005, Liao, *et. al.* 2003).

The inhibition effect of tested inducers (chitosan and SA) on the growth of the causal organism of tomato early blight disease and disease assessment were agreement with the results obtained by Bahaskara *et al*., (2000) and Atia *et al.*, (2003 and 2005). The anti-fungal activities due to 2-hydroxy-5-nitrobenzylideneamino and 5-chloro-2-hydroxybenzylideneamino group. As antifungal groups, the nitro and chloro groups are used in many fungicides such as pentachloronitrobenzene (PCNB) and chlorothalonil. But

these fungicides have pronounced toxicities and their residues in the environment have been developing as serious problems. When these groups are grafted onto chitosan, they should be released slowly and might meet the requirements of environmental safety (Zhanyong *et al*. 2006).

Table (7): Effect of application different concentrations of selected chemical inducers on early blight disease incidence using intact leaves.

Chemical inducers	Concentration (g/l)	Disease severity	Reduction of disease severity	Percentage of infection	Reduction of percentage infection
Chitosan	0.0	56.52	0.00	95.75	0.00
	1.0	47.39	16.15	81.03	15.36
	2.0	39.83	29.49	75.56	21.08
	4.0	30.32	46.34	69.64	27.28
	Mean	**43.52**	**23.00**	**80.50**	**15.93**
	0.0	56.52	0.00	95.75	0.00
	0.5	34.14	39.88	69.72	27.17
Salicylic acid	1.0	26.83	52.44	64.35	32.77
	2 .0	18.79	66.74	55.72	41.79
	4.0	11.03	80.48	49.99	47.78
	Mean	**29.46**	**47.91**	**67.11**	**29.90**
L.S.D at 5%		2.55	2.85	6.35	1.51

Chitosan treatments increased the activities of peroxidase, polyphenol oxidase (catechol) oxidase, phenylalanine ammonia-lyase, chitinase and beta-1, 3-glucanase in the leaves but at various degrees depending on the cultivar (Liao *et al*. 2003 and Atia *et al.,* 2005). The mode of action of chitosan is to stimulate natural defence response systems in treated plants. Poly-D-glucosamine binds to fungal receptor sites, mimicking an attack by fungal spores. This in turn results in signals being sent to the nuclei of the plant and triggering signals which elicit multiple genetic and biological responses, including the production of phytoalexins (anti-microbial compounds produced in plants, aimed at inhibiting infections (Peniston and Johnson. 1980).

SA played an important role in plant defense. Its rol in plant disease resistance is well documented for dicotyledonous plants, were it is required for basal resistance against pathogens as well as for the inducible defense mechanism of SAR, which confers resistance against a broad-spectrum of pathogens. The activation of SAR is associated with the heightened level of expression of the pathogenesis-related proteins (PRs), some of which possess antimicrobial activity (Atia *et al.*, 2005 and Chaturvedi and Shah 2007). SA is an important endogenous molecule involved in plant defense. The link between SA production and SAR has been well established (Klessig and Malamy 1994). Transgenic plants expressing the salicylate dehydrogenase (nahG) gene, which converts SA into inactive catechol, do not establish SAR (Gaffney *et al*. 1993). Furthermore, there is a correlation between an increase in SA levels and plant gene expression. PR-proteins show up a few hours after the SA level begins to rise (Yalpani *et al.* 1993).

Exogenous SA can induce simultaneous PR expression and resistance to pathogens, even in the absence of pathogenic organisms (Ward *et al*. 1991).

Table (8): Effect different water and ethanol plant extracts on mycelia growth reduction *Alternaria solani*.

Plant extracts	Extraction method	Concentration %							
		0	2	5	10	20	50	100	Mean
Garlic		0.00	10.30	32.22	55.18	74.07	100	100	53.11
Banyan tree		0.00	9.99	22.59	27.38	32.22	38.20	53.70	26.29
Henna		0.00	9.25	14.0	18.1	32.5	41.80	48.40	23.43
Sweet basil	Water	32.59	29.60	28.45	26.99	23.70	22.22	16.99	25.79
Sweet majoram	extract.	0.00	2.22	3.70	8.13	9.62	16.6	28.8	9.86
Thyme		0.00	0.00	0.00	1.48	10.74	13.33	25.16	7.24
Ginger		0.00	5.55	31.85	53.70	61.48	70.00	78.51	43.01
L.S.D at 5%		0.79	23.64	2.36	2.54	3.40	23.46	2.37	
Garlic	Ethanol extract	0.00	29.95	62.22	100	100	100	100	69.69
ginger		0.00	25.18	52.95	62.94	73.23	84.81	94.78	55.92
L.S.D at 5%		0.05	8.30	5.48	3.48	3.87	3.89	4.41	

All tested concentrations (0, 2, 5, 10, 20, 50 and 100%) of plant extracts decreased linear growth of *A. solani* compared with the control. Increasing concentration of any plant extract decreased the linear growth of the pathogen. Water extraction of garlic was the most effective on decreasing mycelial growth of *A. solani* (53.11%) followed by ginger (43.01%) while thyme was the lowest effective (7.24%). On the other hand, sweet basil extracts increased growth of *Alternaria solani* (Table, 8). Both water and ethanol extracts of garlic were the most effective on inhibition spore germination of *A. solani* (62.01 and 72.20%) followed by ginger extracted (45.59 and 50.79%). Increasing concentration decreased the percentage of germinated spores (Table, 9). These results were in agreement with those obtained by (Thiribhuvanamala *et al.* 2001). Several reports mentioned that the plant extracts play an important role in controlling the early blight pathogen *in vitro* and *vivo* (Ashrafuzzaman *et al*., 1990, Prasad and Naik 2003 and Satya *et at*., 2005).

Table (9): Effect different water and ethanol plant extracts on reduction of *Alternaria solani* spore germination.

Plant extracts	Spore germination (%)						
	0	5	10	20	50	100	Mean
Garlic	0.00	57.96	66.80	71.32	87.08	100	62.01
ginger	0.00	42.14	48.98	55.95	63.15	73.46	45.59
L.S.D at 5%	1.45	4.89	7.98	6.35	6.31	4.83	
Garlic	0.00	66.78	79.62	86.81	100	100	72.20
ginger	0.00	43.87	50.73	60.49	69.88	79.80	50.79
L.S.D at 5%	1.25	4.68	4.39	4.28	5.09	3.39	

Using detached leaf technique, all plant extracts tested decreased number and diameter of lesion, infected area and increased protection (%) of treated tomato leaves. Increasing concentrations of plant extract resulted in

decreasing the mean number of lesions, diameter of lesion as well as infected area. Garlic extract was the most effective when used at the same time of inoculation on reducing mean number of lesion (1.21), diameter of lesion (1.21mm), infected area (34.36 mm^2) and protection % (79.98%) followed by ginger (1.99; 1.90 mm; 37.04mm^2 and 78.46%). In addition garlic was the most effective as spray application one day before inoculation with *A. solani* on reducing number of lesion (1.97); diameter of lesion (1.56mm), infected area (35.15 mm^2) and % of protection (79.53%) followed by ginger extract (3.24; 3.07 mm; 54.76 mm^2 and 68.11%)**.** Also, data indicated that, used plant extracts at the same time of inoculation with *A. solani* was better than when used one day before inoculation (Table, 10).

Table (10): Effect of plant extracts concentrations on tomato early blight disease incidence using detached leaf technique at different application times.

Plant extracts	Concentration (%)	Number of lesions		Diameter of lesion (mm)		Infected area (mm^2)		% of protection	
		At the same time	One day later	At the same time	One day later	At the same time	One day later	At the same time	One day later
Garlic	0	6	6	6.04	6.04	171.8	171.8	0	0
	10	0.083	2.65	0.013	1.33	0.01	3.80	99.9	97.78
	20	0	1.16	0	0.41	0	0.19	100	99.89
	50	0	0.083	0	0.026	0	0.0003	100	99.99
	100	0	0	0	0	0	0	100	100
	Mean	1.21	1.97	1.21	1.56	34.36	35.15	79.98	79.53
Ginger	0	6	6	6.04	6.04	171.8	171.8	0	0
	10	2.66	5.25	2.34	5.33	13.00	116.96	92.59	31.9
	20	0.5	4.16	1.02	4.00	0.42	52.44	99.74	69.43
	50	0.83	2.25	0.14	3.27	0.009	19.54	99.99	88.60
	100	0	1.41	0	1.37	0	2.08	100	98.78
	Mean	1.99	3.81	1.90	4.00	37.04	72.56	78.46	57.74
L.S.D at 5%		0.59	0.39	0.29	0.48	2.83	5.83	0.79	3.24

Under detached leaf technique, all tested plant extracts decreased disease severity and percentage of infection. Disease severity decreased with the increasing plant extract concentrations. Garlic was the most effective on decreased disease severity (24.19%) and percentage of infection (60.83%), followed by ginger (36.88% and 77.02%) Table, 11. Garlic reduced the disease severity and percentage of infection (55.63% and 35.94%), followed by ginger (32.33% and 18.90%), respectively. The inhibitory effect of the tested extracts might be due to natural bioactive materials presented in these extracts (Khalil, 2001).

Generally, garlic extract was the most effective extract used against the pathogen of tomato early blight disease. This might be due to garlic extract through presence of antimicrobial compound that were described mainly as allicin (allyl-2-propenethiosulfinare) which is generated from alliin (S-allylcystein-S-oxide) after injury of the garlic tissue is a rather labile compound and is further transformed into a variety of substance. The volatile

organic compounds mainly consisted of linear chain aldehydes (5-hexenal, and octanal), allylsulfides, and disulfides (allyl disulfide allyl methyl disulfide) (Shalaby and Atia, 1996 and Ahmed *et al*., 2009).

Table (11): Effect of plant extracts concentration on tomato early blight disease incidence using intact leaves under greenhouse conditions.

Plant extracts	Concentration (%)	Disease severity	Disease severity (Reduction)	Percentage infection	Percentage infection (reduction)
Garlic	**0**	54.59	0	95.06	0
	10	26.57	51.33	67.03	29.40
	20	18.19	66.59	59.18	37.66
	50	13.18	75.76	49.33	47.98
	100	8.44	84.47	33.58	64.7
	Mean	24.19	55.63	60.83	35.94
Ginger	**0**	54.59	0	95.06	0
	10	40.02	26.55	82.21	13.36
	20	34.08	37.46	74.65	21.37
	50	30.04	44.84	69.14	27.22
	100	25.68	52.81	64.04	32.59
	Mean	36.88	32.33	77.02	18.90
L.S.D at 5%		2.84	3.15	3.79	2.34

Othman *et al.,* (1991) and Satya *et al.*, (2005), proved that the crude antiphytoviral of garlic bubbliest was thermos-table. Also this study revealed that fungi-toxic compounds were presented in ginger and garlic since they were able to inhibit the growth of the fungus tested. This agrees with earlier reports of (Udo *et al*., 2001) on the inhibition of growth. It was shown that antifungal substances found in the *Eucalyptus* species examined consist of from 1 to 4 different components (Balandrin *et al*., 1985). Cinnamon, halfa barr, laurel and ginger plant extracts with either cold distilled water (CDW) or boiling (BDW) had a strong antifungal activity with significant inhibition on the growth and hydrolytic enzymes (glucosidase, pectinlyase and protease) of *A. alternata* and *F. oxysporum* (Fawzi *et al*., 2009).

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مكافحة مرض الندوة المبكرة باستخدام بعض العوامل الحيوية وغير الحيوية

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يعتبر مرض الندوة المبكرة على الطماطم المتسبب عن الفطر الترناريا سولاني من الأمراض الاقتصادية الهامة، حيث يسبب خسارة كبيرة في كمية وجودة محصول الطماطم. وقد كانت عزلات الفطر تريكودرما فيردي، والبكتيريا باسيلس ستلس والعزلة رقم واحد (ا) من الاكتينوميسيت أكثر كائنات المقاومة الحيوية المختبرة تأثيراً على خفض نمو الفطر الترناريا سولاني. وقد خفضت كل عزلات المقاومة الحيوية مرض الندوة المبكرة على أوراق الطماطم المنزوعة وعلى النبات الكامل وذلك بعد يومين وسبع أيام من المعاملة قبل الحقن. وقد كانت عزلة الفطر تريكودرما هارزيانم أكثر العزلات كفاءة في مكافحة المرض حيث سجلت أعلى نسبة مئوية للوقاية عند الحقن بالمسبب بعد يومين وسبع أيام من المعاملة بكائنات المقاومة الحيوية، تلاها عزلة تريكودرما فيردي ثم البكتيريا بسلس ستلس على الأوراق المنزوعة على التوالي، وعلى النبات الكامل كانت عزلة الفطر تريكودرما هارزيانم أكثر العزلات كفاءة في مقاومة المرض، حيث سجلت أعلى نسبة مئوية للوقاية عند الحقن بالمسبب بعد يومين وسبع أيام من المعاملة. وقد تلاها عزلة البكتيريا بسلس ستلس، ثم عزلة الفطر تريكودرما فيردي على التوالي. وقد كانت كائنات المقاومة الحيوية أعلى كفاءة في المقاومة على الأوراق المنزوعة عن النبات الكامل.

وقد أدت التركيزات المختلفة من الشيتوزان وحمض السلسليك علي خفض نمو وإنبات جراثيم الفطر الترناريا سولاني، وقد ازداد التأثير بزيادة التركيز، حيث كان تركيز ٤ جرام /لتر أكثرها تأثيراً. وقد أدت المعاملة بتركيزات الشيتوزان وحمض السلسليك تحت الدراسة إلى استحثاث المقاومة في نباتات الطماطم ضد مسبب مرض الندوة المبكرة وذلك بعد ٧ أيام من المعاملة قبل حقن المسبب المرضي على الأوراق المنزوعة وعلى النبات الكامل.

وقد كان للمستخلصات المائية والكحولية تأثيرا فعالا علي خفض نمو وإنبات جراثيم الفطر الترناريا سولاني، وقد ازداد التأثير بزيادة التركيز، وقد كان مستخلص الثوم المائي والكحولي أكثرها تأثيراً. كما أظهر مستخلص التوم تأثيرا فعالا في تقليل عدد البقع المصابة وقطرها والمساحة المصابة وإظهار أعلى نسبة للوقاية من المرض تلاه مستخلص الزنجبيل.

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