Voyage Journal of Scientific Research and Exploration (VJSRE) *Vol 2. Issue 2*
ISSN (Online): 3007-3898, ISSN (Print): 3007-388X, October 2025 to March 2026

EVALUATION OF SOME ANTAGONISTIC BACTERIA FOR THE CONTROL OF *ALTERNARIA SOLANI* (ELLIS AND MARTIN) JONES AND GROUT CAUSE OF EARLY BLIGHT DISEASE OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

[1*]Hina Zafar and [1]Khalil Ahmed Khanzada

[1]*Crop diseases research Institute, Southern-zone Agricultural Research Centre, Pakistan Agricultural Research Council, Old Blocks 9-10, University of Karachi, Karachi-75270, Pakistan.*
[*]*Corresponding Author's Email: zafarhina786@yahoo.com*

ABSTRACT

Bacteria are capable to produce a wide range of metabolites and having potential to produce antifungal compounds. The present study was conducted to examine the potential for antagonism of eight different bacterial strains, Bacillus subtilis, B. Staphylococcus aureus, B. thuringiensis, B. Lentimorbus, B. cereus, B. coagulans, Pseudomonas fluorescens and Rhizobium sp. against Alternaria solani. All isolates were subjected to preliminary tests against A. solani, i.e. screening tests, agar disc method, and slide culture method resulting antagonistic activity against A. solani were observed in mostly selected bacteria. Among the eight bacterial species, Bacillus subtilis and Pseudomonas fluorescens showed effective biological control against A. solani. On the other hand B. thuringiensis and B. coagulans reduced 50% growth in slide culture technique, while Bacillus subtilis and Pseudomonas fluorescens completely inhibited the spore germination of A. solani as compared to control. Similar tests were conducted in greenhouse and open field conditions where the treatment of Bacillus subtilis and Pseudomonas fluorescens were applied against A. solani, resulting both the bacterial strains significantly reduced the growth of A. solani and showed significant increase in number of leaves, length of stem, root length, fresh and dry weights of root and stem as compared to positive control. While maximum growth was found in negative control. However, Phenolic contents were found significantly greater under greenhouse conditions following infection with Alternaria solani and bacterial treatments in all plants as compared to -ve controls.

Keywords: Antagonistic Bacteria, *Alternaria Solani*, *Solanum Lycopersicum* L.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important and widely grown vegetable in the world. Tomato is generally grown for its edible part and it constitutes a good source of vitamins A, B, C, minerals and nutrients essential for human diet. In this respect it occupies number one position (Arunakumara, 2006). Because of its adaptability of varied climatic and high nutritive value tomato cultivation has become more popular since mid nineteenth century. Bacteria, fungi, nematodes, abiotic factors and viruses cause several diseases of tomato (Balanchard, 1992; Singh *et al*., 2017). Target spot disease or early blight incited by *Alternaria solani*, (Ellis & Martin) Jones and Grout is one of the world's most catastrophic disease among the fungal diseases. The causal organism which is responsible for fruit rot, collar rot and early blight is air borne and soil inhabiting (Datar and Mayee, 1981). Under favorable conditions the disease appears on fruits, leaves, petiole, stems and twigs resulting in drying off of twigs, defoliation and premature fruit drop and in fruit yield causing losses up to

50 to 86 percent (Mathur and Shekhawat, 1986). The majority of agrochemicals used against fungal and bacterial diseases of plants, besides contributing to current human and animal toxicity, increased production costs, persistent accumulation in natural ecosystems and when improperly applied they also lead to residue-contaminated fruits, reduction of beneficial organism, to appearance of resistant pathogens (Nordlund, 1996; Glick and Bashan, 1997). Effective microorganisms, organic fertilizers and microbial products have been included in biological control agents, which are attracting attention as alternatives to chemical agents (Fravel, 2005). Over the past two decades, for the control of disease as inducers of systemic resistance use of rhizobacteria is an alternative tool that has been widely studied. Use of rhizobacteria as biocontrol agents under both controlled and field conditions is often reported in literature in diverse patho systems (Mathre and Johnston, 1995; Wei *et al*., 1996). A number of researchers reported that several pathogens belonging to the genera *Sclerotinia, Rhizoctonia, Fusarium, Gaeummanomyces, Phytophthora* and *Pythium* are suppressed by many species of *Bacillus* including *B. subtilis*, *B. cereus*, *B. mycoides* (Cook and Baker, 1983; McKnight, 1993; Fiddaman and Rossall, 1994). Therefore, biological control is an advantageous alternative to chemical control of the fungal diseases since chemical control is not entirely satisfactory (Anjajah *et al*., 2003).

Phenolic compounds (secondary metabolites) play a vital role in ecology and their adaptation to different factors of abiotic and biotic stress, which accumulate during pathogen attack (Ewane *et al.*, 2012; Malencic *et al.*, 2012). Current findings (Horsakova *et al*., 2013; Medicpap *et al*., 2014) have also established that as a result of infection antioxidant activity is increased. Antioxidant compounds found in plants material and prevent oxidative damage by deactivating reactive oxygen, owing to their natural origin, it has greater benefit in comparison to synthetic ones (Rohman *et al*., 2010). Natural antioxidants do not persuade side effects, while synthetic have genotoxic effects (Chen *et al*., 1992).According to Pane *et al*., (2011) antioxidant compounds in post-harvest tomato inhibit *Botrytiscinerea*. Many researchers confirmed that tomato plant characterize the major source of antioxidants compound (Stewart *et al*., 2000; Slimestad and Verheul 2005; Luthria *et al*., 2010).

The present study is an effort to statement the following questions. 1. To evaluate if the eight different species of bacteria have biocontrol potential against the fungal pathogen *Alternaria solani*. 2. To test the biocontrol activity against *A. solani* under laboratory, greenhouse and field condition. 3. To determine whether accumulation of soluble Phenolic contents occurs under pathogenic stress condition, and if the bio-control fungi can also trigger the accumulation of soluble Phenolic compounds.

MATERIALS AND METHODS

Collection, isolation and identification of *A. solani*: Infected leaves and fruits samples were collected from the fields of Federal Urdu University, Karachi. To isolate the pathogen, only infected portions of leaf and fruit were surface sterilized (70% ethanol and 0.1% mercuric chloride) and cultured on Potato Dextrose Agar (PDA). Plates were incubated for seven days at 25 °C. Cultured fungus was identified based on morphological characters of spores and mycelium (Barnett and Hunter, 1998).

Collection of Bacteria: Eight different species of bacteria were used in this experiment including *Bacillus subtilis, B. aureus, B. thuringiensis, B. lentimorbus, B. cereus, B. coagulans, Rhizobium* sp. and *Pseudomonas fluorescens* that were obtained from culture collection center of Prof. Dr. A. Ghaffar, Department of Botany, University of Karachi. Bacterial cultures were maintained on Nutrient agar slants respectively at 4º C until used for further study. For the measurement of bacterial count as the number of colony-forming units (CFU) a series of dilution technique was performed. One ml bacterial suspension from final concentration 1.8×10^6 CFU (matched with McFerland Index for count bacterial colony).
Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml.

Laboratory experiment: Four mm diameter disc of an actively growing culture of *Alternaria solani* was taken by a sterile cork borer and placed in the center of a PDA plate. Bacterial species as thick bands were streaked on four opposite edges on the PDA plates. The Petri dishes were incubated at room temperature for 4-5 days. Without any bacterium *Alternaria solani* mycelia disc on PDA medium served as control (Sivantham *et al*., 2013). The above procedure was carried out on eight bacterial species separately. Treatments and control were replicated four times each.

Screening test: Screening of bacterial antagonist was carried out using dual culture assay. One 6-mm diameter disc of *Alternaria solani* was placed at the center of PDA medium in Petri dish. Bacteria were streaked on PDA medium at a distance of 2.5 cm between *Alternaria solani* disc and bacteria (Dikin *et al*., 2006). Plates were incubated for 7 days at 26 ± 2°C. The experiments were replicated four times.

Agar disc method: With the help of sterile cork borer 4 mm diameter mycelial disc of *Alternaria solani* was placed in the center of the above PDA plate separately. Using sterilized pipette 0.1 ml of the test bacterial suspension (102 CFU/ml) was transferred in the PDA plate and spread by sterile glass spreader separately. Without bacteria mycelial disc of *A. solani* on PDA medium was used as control. The cultures were incubated at room temperature in dark for 3-5 days and diameter of the fungal mycelial growth was measured (Sivantham *et al*., 2013). The experiment was replicated four times. The first three methods are referred to here as classical methods.

Slide culture method: Spore suspension of *Alternaria solani* was separately prepared from 8-day-old culture and spores counted by haemocytometer. Likewise, spores suspension of bacteria was prepared and by dilution technique concentrations were adjusted to 100 CFU/ml. Subsequently, by using micropipette 25 µl of bacterial spore suspension mixed with the 25µl of the fungal spores' suspension on cavity slides. Subsequently, to make moist chamber in the Petri plates placing a sterile filter paper and cavity slide was placed and incubated for 8 days at room temperature. For controls, spores of *Alternaria solani* were mixed in sterile distilled water (Sivantham *et al*., 2013). Treatments and controls were replicated three times.

Greenhouse Experiment: A greenhouse experiment was conducted in Federal Urdu University (Gulshan-e-iqbal campus). *Bacillus subtilis* and *Pseudomonas fluorescens* were tested against *Alternaria solani* in the form of spray. Four-week-old tomato plants were transferred in plastic pots (14cm diameter) containing sterilized sandy loam soil with four treatments and three replicate. After forty days of transplantation, tomato plants were sprayed with *A. solani* spore suspension (106 spores/ ml) while control plants remained unsprayed with spore suspension of *A. solani* serving as negative control. While positive controls were sprayed with *A. solani* spore suspension. After 25 days of inoculation of *A. solani* when symptoms of early blight were emerged in the treatment pots were sprayed with 50 ml of the test bacterial suspension (102 CFU/ml) and after fourteen days of treatment, number of leaves, stem length, root length, fresh and dry weights of stem and root were recorded.

Estimation of total soluble phenol content: Leaves were collected from greenhouse experiment after three days of inoculation of *Alternaria solani* and bacterial treatment. 10 ml of 80 % methanol and 500 mg of tomatoes leaves were homogenized and agitated for 15 min at 70 °C. Five ml of distilled water was added in one ml of the methanolic extract of leaves and 250 µl of Folin Ciocalteau reagent (1N) and the solution was kept for three minutes at 25 °C, Further 1ml of 20 % Na_2CO_3, 1 ml distilled water were also added and the mixture was incubated at 25 °C for 60 minutes. Absorbance was measured by a spectrophotometer (UV-1201.Shimadzu, Japan) at 760 nm and phenolic content was expressed as the milligrams per gram of leaf fresh weight (Zieslin and Ben-Zaken, 1993). Gallic acid was used as standard.

Field Experiment: The field experiment was performed with the randomized complete block design (RCBD) (1.5 X 1.5m, 3 rows of 3 plants each; plots were separated about 15cm high ridges approximately). Soil was sandy-loam (Sand: silt: clay, 70 : 19 : 11) of pH 8.1 and moisture holding capacity of 36.5%, with four treatments and three replications in Federal Urdu University (Gulshan-e-Iqbal campus). After five-weeks tomato plants were transferred in field and *Bacillus subtilis* and *Pseudomonas fluorescens* were tested against *Alternaria solani* (in the form of spray on plants). After forty days of transplantation, tomato plants were sprayed with *A. solani* spore suspension (100 ml/plant) at the concentration of (5×10^6 conidia/ml) while, control plants remained unsprayed with spore suspension of *A. solani* for negative control. Positive controls were sprayed with *A. solani* spore suspension. After 25 days of inoculation of *A. solani* when symptoms of early blight were visible in the field, Plants in the treatment were sprayed with 50 ml of Bacterial suspension at the concentration of (102 CFU/ml). After fifteen days of treatment, number of leaves, stem length, root length, fresh weight of stem and root, dry weights of stem and root were recorded.

Statistical analysis: Analysis of variance (ANOVA), followed by Duncan's multiple range test and Fisher's least significant difference (LSD) test at P=0.05 (Zar, 2009) were applied on results. While, results of greenhouse and field experiments were analyzed by ANOVA and multivariate methods including cluster analysis and PCA (principal component analysis) ordination. Cluster analysis was performed using Ward's clustering strategy and Euclidean distance as the resemblance function. PCA was performed on the correlation matrix between

variables and two-dimensional ordination was obtained. Both the multivariate techniques were implemented on MINITAB version 14

The results of accumulation of soluble phenolic compounds under greenhouse condition were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range test (hereafter referred to as multiple range test) and Fisher's least significant difference (LSD) test at P=0.05 (Zar, 2009).

RESULTS

Laboratory experiment: Eight bacterial strains were tested against *Alternaria solani* in which two bacterial strains including *Bacillus subtilis* and *Pseudomonas fluorescens* strongly inhibited growth of *A. solani* (Table 1).

Table 1: Mean and Standard error of Classical (Colony diameter in cm) and Slide culture method (number of spore).

S.N	Treatment	Classical Methods			Slide culture method
		Preliminary	Screening	Agar disc	
1	Control	5.65 ± 0.18	5.73±32	5.68 ± 0.09	153.75 ± 10.49
2	*Bacillus subtilis*	2 ± 0.07	2 ± 0.19	1.37 ± 0.20	10.75 ± 1.49
3	*P. fluorescens*	1.8 ± 0.32	1.55 ± 0.13	2.22 ± 0.11	19.75 ± 1.88
4	*B. thuringiensis*	3.37 ± 0.22	2.3 ± 0.09	3.27 ± 0.21	79 ± 7.24
5	*B. coagulans*	3.97 ± 0.14	3.02 ± 0.12	4.05 ± 0.06	110 ± 25.48
6	*B. aureus*	4.55 ± 0.16	2.6 ± 0.04	4.72 ± 0.14	149 ± 9.44
7	*B. lentimorbus*	4.5 ± 0.20	4.2 ± 0.16	4.87 ± 0.06	158.2 ± 11.81
8	*B. cereus*	4.9 ± 0.04	3.55 ± 0.30	17.02 ± 11.99	179.25 ± 9.55
9	*Rhizobium sp.*	4.82 ± 0.18	5.32 ± 0.31	5.2 ± 0.09	152.5 ± 7.89

$LSD_{.05}$ = 0.298$LSD_{.05}$ = 33.50

ANOVA was performed for the data set pertaining to three classical methods. It was demonstrated that there was a significant difference (P at the most 0.05) between most of the bacterial antagonists against *Alternaria solani*. However, some of them did not show any significant antagonistic activity such as *B. cereus* and *Rhizobium* sp. against *A. solani.* Whereas, in the presence of the above bacteria *A. solani* revealed approximately equal growth on PDA plate as did the controls (Table1). While ANOVA was also performed for the data set pertaining to slide culture method revealed that there was a significant difference (P at the most 0.05) between most of the bacterial antagonists against *Alternaria solani.*

Greenhouse experiment: One factor ANOVA were applied to greenhouse experiment $p<0.05$. The both antagonistic bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* showed significant effect on number of leaves, stem length, fresh and dry weight of root and stem ($p<0.001$) while fresh weight of stem and root length were found non-significance. The magnitude of growth parameters for negative control (water control) were, in general, considerably higher compared to those for both antagonistic bacteria treated plants. Number of leaves and stem length in treatments were significantly lower ($p<0.05$) compared to –ve controls. Whereas, root length and stem fresh weight were not significantly altered against

the –ve controls. However, stem and root dry weight were significantly lower ($p<0.05$) in treatments compared with –ve controls.

The results of greenhouse experiment (Table 2) were also analyzed by multivariate analysis using cluster and PCA (principal component analysis) ordination. The dendrogram derived from Ward's method of cluster analysis (Fig.1) showed cleared grouping. Two main groups were recognized in the dendrogram separating of bacteria treatments (*Bacillus subtilis* and *Pseudomonas fluorescens*) and the negative and positive controls. On the left side were the three replicates of positive control which form a separate group while, the negative controls tend to be separated on the right side because of the greater response of the growth parameters. The two bacterial treatment remains in between negative and positive controls. The two-dimensional PCA ordination also showed a cleared separation of –ve and +ve controls (Fig.2). The PCA ordination basically repeats the pattern depicted by the dendrogram. Both group 1 and group 2 obtained in cluster analysis clearly separated out (Fig 2).

Table 2: Mean effect of bacteria on the growth of tomato (*Solanum lycopersicum* L.) infected with *A. solani* 84 days of growth under greenhouse condition.

Treatment	No. of Leaves	Stem Length(cm)	Root Length(cm)	Stem Fresh Wt.(g)	Root Fresh Wt.(g)	Stem Dry Wt.(g)	Root Dry Wt.(g)
Control (+)	16.67 ± 1.45bc	29.33 ± 1.45c	16.33 ± 1.20b	5.23 ± 0.260b	0.173 ± 0.01c	1.03 ± 0.02b	0.055 ± 0.002c
Control (-)	39.33 ± 1.45a	44.33 ± 2.33a	22.33 ± 1.86a	6.2 ± 0.208a	0.41 ± 0.020a	1.14 ± 0.014a	0.088 ± 0.001a
Bacillus subtilis	30.33 ± 3.53bc	38 ± 1.73bc	21.33 ± 1.45ab	5.6 ± 0.173a	0.34 ± 0.02ab	1.08 ± 0.012b	0.076 ± 0.004b
Pseudomonas fluorescens	30 ± 2.98bc	32.6 ± 2.19bc	19 ± 1.53ab	6.43 ± 0.43ab	0.33 ± 0.073b	1.08 ± 0.01b	0.069 ± 0.003b
Lsd $_{.05}$=	8.152	6.385	4.98	0.760	0.067	0.054	0.0150

Note: Means are followed by ± Standard error (SE). Same letters in the same column are not significant, while different letters are significantly different ($p<0.05$).

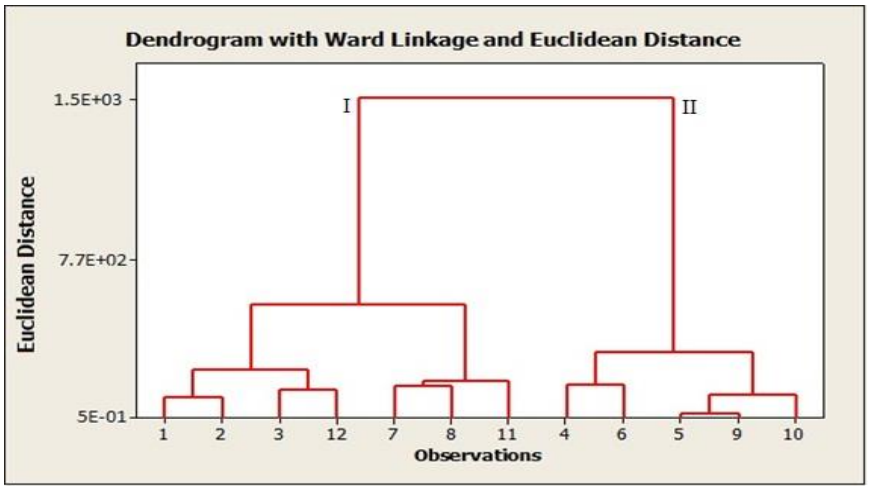


Fig. 1: Dendrogram: derived from the data of the effect of antagonistic bacteria on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under greenhouse conditions.
Note: (1,2,3) = positive control, (4,5,6) = negative control, (7,8,9) = *Bacillus subtilis* and (10,11,12) = *Pseudomonas fluorescens*

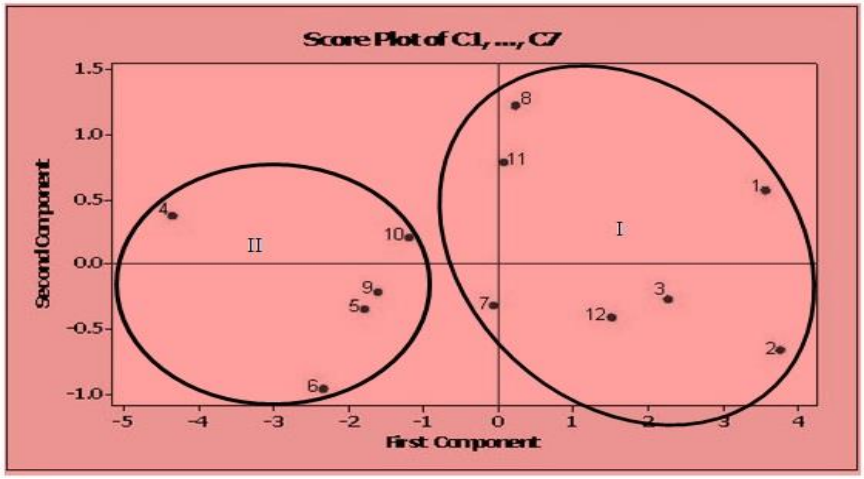
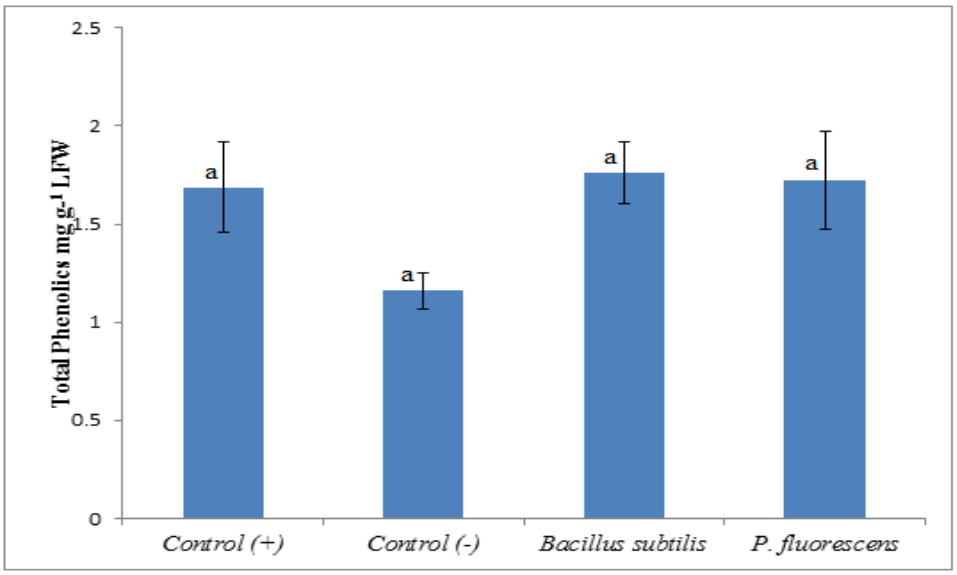


Fig. 2: Two-dimensional PCA ordination of the effect of antagonistic on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under greenhouse conditions.
Note: (1,2,3) = positive control, (4,5,6) = negative control, (7,8,9) = *Bacillus subtilis* and (10,11,12) = *Pseudomonas fluorescens.*

Phenolic compound: Total soluble phenolic content of tomato leaves were studied and the results disclosed that total phenolic content was considerably higher in all treated plants as compared to -ve control (Fig. 3). One way ANOVA was also performed on the data of phenolic content under greenhouse condition showed non-significant difference but positive control show significant difference as compare to negative control. Similarly both bacterial strain show significant difference as compare to negative control.



LSD $_{.05}$= 0.63

Fig. 3: Total phenolic compounds in tomato leaves.

Note: LFW = leaf fresh weight. same letter are not significantly different while different letter are significantly different (p<0.05).

Field experiment: Table 3 showed the growth of tomato plants at 110 days in negative and positive controls and treated plants. Both the bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* significantly increased the number of leaves, stem length, stem fresh weight, root fresh weight and root dry weights over positive controls (P at the most 0.05). However, stem dry weight and root length were reduced compared to positive control. The magnitude of growth parameters for negative control was in general, substantially higher compared to bacterial treatments (P at the most 0.05).

The results of multivariate methods including cluster analysis and PCA ordination (Fig. 4) showed considerable separation of bacterial treatments (*Bacillus subtilis* and *Pseudomonas fluorescens*) and the negative and positive controls. On the left side are the three replicates of positive control which form a separate group because of the lower magnitude of the growth parameters. The negative control and both bacterial treatments tend to be separated on the right side. The two-dimensional PCA ordination also showed a remarkable separation of treatments and –ve and +ve controls (Fig. 5) and basically represent the same results as disclosed by the dendrogram.

Table 3: Effect of bacteria on the growth of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* at 110 days of growth under field condition.

Treatment	No. of Leaves	Stem length(cm)	Root length(cm)	Stem Fresh Wt.(g)	Root Fresh Wt.(g)	Stem Dry Wt.(g)	Root Dry Wt.(g)
Control (+)	13.66 ± 0.33c	27.3 ± 1.45b	10 ± 0.57b	4.66 ± 0.17b	0.17 ± 0.01b	1.13 ± 0.044a	0.044 ± 0.003c
Control (-)	32.33 ± 1.45a	37 ± 0.57a	14.33 ± 0.82a	5.66 ± 0.12a	0.39 ± 0.06a	1.21 ± 0.029a	0.072 ± 0.0037a
Bacillus subtilis	23.33 ± 2.33b	33 ± 2.08a	12.6 ± 0.88ab	5.1 ± 0.153bc	0.25 ± 0.05b	1.16 ± 0.034a	0.045 ± 0.002bc
Pseudomonas fluorescens	24.33 ± 2.60b	32.6 ± 1.76a	12.3 ± 1.20ab	5.16 ± 0.12c	0.37 ± 0.06b	1.143 ± 0.03a	0.057 ± 0.005b
Lsd .05=	6.1917	5.1276	2.977	0.4707	0.125	0.1217	0.0129

Note: Means are followed by ± Standard error (SE). Same letters in the same column are not significant while different letters in the same column are significantly different ($p<0.05$).

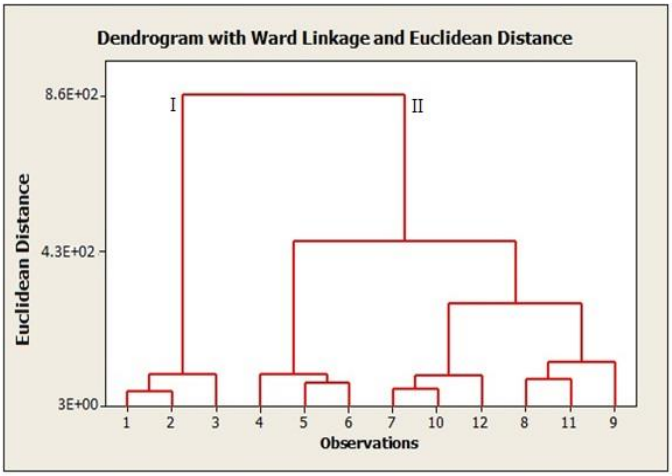


Fig. 4: Dendrogram derived from the data of the effect of antagonistic bacteria on the growth of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under field conditions.
Note: (1,2,3) = positive control, (4,5,6) = negative control, (7,8,9) = *Bacillus subtilis* and (10,11,12) = *Pseudomonas fluorescens*.

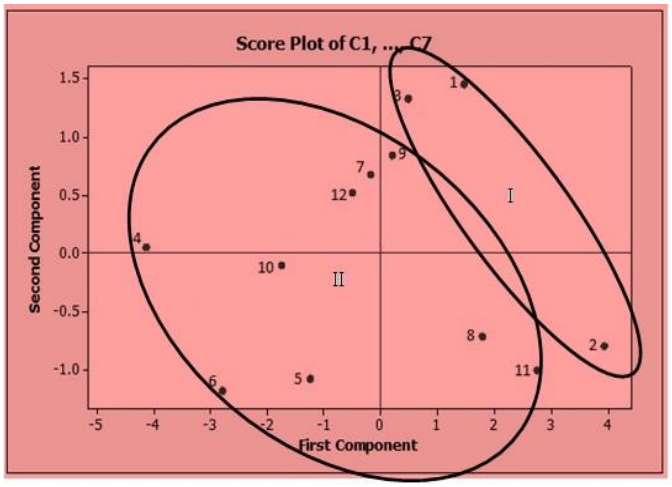


Fig. 5: Two-dimensional PCA ordination of the data of the effect of antagonistic on the growth of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under field conditions.
Note: (1,2,3) = positive control, (4,5,6) = negative control, (7,8,9) = *Bacillus subtilis*and (10,11,12) = *Pseudomonas fluorescens.*

DISCUSSION

Present research evaluated that eight bacterial strains including *Bacillus subtilis*, *B. aureus, B. thuringiensis, B. lentimorbus, B. cereus, B. coagulans, Pseudomonas fluorescens, and Rhizobium* sp. were tested against *Alternaria solani*. Out of eight, four bacterial strains showed antagonistic effects against *Alternaria solani.* In this experiment, *Bacillus subtilis* and *Pseudomonas fluorescens* showed highest antifungal activity against *Alternaria solani* cultivated on PDA medium and reduced the growth of *A. solani* presumably due to the production and secretion of

antifungal compounds. Both the bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* significantly increased the number of leaves, stem and root length, stem and root fresh and dry weight over positive controls (plants sprayed with *A. solani*). Likewise, many researchers (James and Gutterson, 1986; Smirnov and Kiprianova, 1990; Haas and Defago, 2005; Veselova *et al*., 2008) observed antagonistic activity of *Pseudomonas fluorescence* against many fungal pathogens through the secretion of compounds with antifungal and antibacterial activity. Furthermore, Srivastava and Shalni, (2008) investigated the antifungal potential of *Pseudomonas fluorescence* against pathogenic fungi including *Alternaria cajani*, *Bipolaris sp., Curvularia lunata*, *Fusarium* sp. and *Helmintho sporium.* Rakh *et al.,* (2011) observed highest antagonistic activity of *P. monteilii* by producing diffusible antibiotic, hydrogen cyanide, siderophore and volatile metabolites on *S. rolfsii.* Melentev *et al*., (2006) observed *in vitro* inhibitory activity of *Bacilli* species, *Bacillus subtilis* and *B. polymyxa* against wood decaying fungi. Nourozian *et al*., (2006) and Munimbazi *et al.*,(1998) disclosed that many species of *Pseudomonas* and *Bacillus* have potential to inhibit the growth of many species of *Penicillium*, *Aspergillus* and *Fusarium.* Kiewnick *et al*., (2000) reported that *B. subtilis*, *B. cereus* and *B. amyloliquefaciens* were very effective against *A. solani in vitro.* Alippi and Monaco, (1994) reported that *B. subtilis* secretes it urine family antifungal metabolites (subtiline, bacitracin, bacillomycin and bacillin) that confer potential to these bacterial agents and the best form of introduction in the host. Nonetheless, further studies must be conducted under field conditions to demonstrate the biocontrol potential of the selected bacterial strains.

Total soluble phenolic content of tomato leaves were studied and the results disclosed that total phenolic content was considerably higher in *Bacillus subtilis* ≥ *Pseudomonas fluorescence* ≥ +ve control ≥ -ve control. Similarly, Ruelas *et al.*, (2006) found that after pathogen attack concentration of phenolic acids increased in tomato plant. Pane *et al.,* (2011) disclosed that synthesis of phenolic compound increased in post-harvest tomato resistance to *Botrytis cinerea.* Similar results are reported here Wurms, (2005) observed that increase of phenolic compounds in the infected kiwifruit by *Botrytis cinerea*. Eguchi *et al*., (1996) confirmed that pseudomonas species have a unique strategy to produce high phenolic compounds after attack of fungal pathogen. Phenolic compounds are considered to be phytoalexins (antifungal compounds) concerned in plant defense (Daayf *et al*., 2012). Phenolic compounds are produced by the phenylpropanoid pathway (Yu *et al*., 2005; Dixon *et al*., 2002; Naoumkina *et al*., 2010). A Phenylpropanoid pathway is therefore regarded as obstructive to pathogen infection. Yachana Jha *et al*., (2011) found that phenyl propanoid is changed into L-phenylalanine, furthermore change into trans-amination of ammonia for the production of trans-cinnamic acid, which enters into diverse biosynthetic pathways to form phytoalexins and phenolics. Bordbar *et al*., (2010) observed accumulation of phenolic compound increase in apple plants after cause of blue mould of apple fruits produced by *Penicillium expansum*.

Conclusion

The study revealed that among the eight bacterial species, Bacillus subtilis and Pseudomonas fluorescens showed effective biological control against *A. solani*. While on the other hand B. thuringiensis and B. coagulans reduced 50% growth in slide culture technique. However, Bacillus subtilis and Pseudomonas fluorescens completely inhibited the spore germination of *A. solani* also in greenhouse and open field conditions where the treatment of both the bacterial strains significantly reduced the growth of *A. solani* and showed significant increase in number of leaves, length of stem, root length, fresh and dry weights of root and stem as compared to positive control.

REFERENCES

Alippi, A. and C. Monaco. 1994. Antagonismo*in vitro* de especies de *Bacillus* contra *Sclerotium rolfsii*y *Fusarium solani*. *Revista de la Facultad de Agronomía, La Plata*, 70: 91-95.

Anjaiah, V., N. Koedam, B. Nowak-Thompson, J.E. Loper, M. Hofte, J.T. Tambong and P. Cornelis. 1998. Involvement of phenazines and anthranilate in the antagonism of *Pseudomonas aeruginosa* PNA1 and Tn*5* derivatives toward *Fusarium*spp. and *Pythium*spp. *Molecular Plant-Microbe Interactions*, 11: 847-854.

Anjajah, V., P. Cornelis and N. Koedam. 2003. Effect of genotype and root colonization in biological control of *Fusarium* wilt in pigeonpea and chickpea by *Pseudomonas aeruginosa*PNA1. *Canadian Journal of Microbiology*, 49: 85-91.

Arunakumara, K.T. 2006. *Studies on Alternaria solani causing early blight disease of tomato*, Master's thesis. Dharward University of Science, India.

Balanchard, D. 1992. *A colour atlas of tomato diseases*.Wolfe Pub. Ltd., Brook House, London, P.298.

Barnett, H.L. and B.B. Hunter. 1998. *Illustrated Genera of Imperfect Fungi*, Fourth Edition 218p.

Bordbar. F.T, H.R. Etebarian , N. Sahebani and H. Rohani. 2010. Control of postharvest decay of apple fruit with *Trichoderma virens* isolates and induction of defence responses. *Journal of Plant Protection Research*, 50: 146-152.

Chen, C., M.A. Pearson and I.J. Gray. 1992. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compound. *Food Chemistry*, 43: 177-183.

Cook, R.J. and K.F. Baker. 1983. *The nature and practice of biological control of plant pathogens*, American Phytopathological Society.

Daayf, F., A. El Hadrami, A.F. El-Bebany, M.A. Henriquez, Z. Yao and H. Derksen. 2012. Phenolic compounds in plant defense and pathogen counter-defense mechanisms, in *Recent Advances in Polyphenol Research* eds Cheynier V., Sarni-Manchado P., Quideau S., editors. (Oxford, UK:Wiley-Blackwell;).

Datar, V.V. and C.D. Mayee. 1981. Assessment of loss in tomato yield due to early blight. *Indian Phytopathology*, 34: 191-195.

Dikin, A., K. Sijam., J. Kadir and I. Seman. 2006. Antagonistic bacteria against Schizophyllum commune Fr. In perinsular Malaysia. *Biotropia*, 13: 111-112.

Dixon, R.A., L. Achnine, P. Kota, C.J. Liu, M.S.S. Reddy and L. Wang. 2002. The phenylpropanoid pathway and plant defence – a genomics perspective. *Molecular Plant Pathol*ogy, 3: 371-390.

Eguchi, Y., O.F. Curtis and K. Shetty.1996. Interactionof hyperhydricity preventing Pseudomonas sp. With oregano (*Origanum vulgare*) and selection of high phenolic and rosmarinic acid producing clonal lines. *Food Biotechnology,* 10: 191-202.

Ewane, C.A., P. Lepoivre, L.L. De Bellaire and L. Lassois. 2012. Involvement of phenolic compounds in the susceptibility of bananas to crown rot. A review, *Biotechnology, Agronomy, Society and Environment*, 16: 393-404.

Fiddaman, P. and S. Rossall. 1994. Effect of substrate on the production of antifungal volatiles from *Bacillus subtilis*. *Journal of applied microbiology*, 76: 395-405.

Fravel, D.R. 2005.Commercialization and implementation of biocontrol.*Annual Reviewof Phytopathology*, 43: 337-359.

Glick, B.R. and Y. Bashan. 1997. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. *Biotechnology*, Adv, 15: 353-378.

Haas, D. and G. De´fago. 2005. Biological control of soilborne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 3: 307-319.

Horsáková, J., J. Sochor, B. Krška. 2013. Assessment of antioxidant activity and total polyphenolic compounds of peach varieties infected with the plum pox virus. *Acta Universitatis Agriculturae et SilviculturaeMendelianae Brunensis,*187: 1693-1701.

James, D.W. and N.I. Gutterson. 1986. Multiple antibiotics produced by *Pseudomonas fluorescens*HV37 and their differential regulation by glucose. *Applied and Environmental Microbiology*, 52: 1183-1189.

Kiewnick, A.B., J.B. Jacobsen and C.D. Sands. 2000. Biological control of *Pseudomonas syringae*pv. *syringae*, the causal agent of basal Kernel Blight of Barley, by antagonistic *Pantoea agglomerans*. *Phytopathology*, 90: 368-375.

Lutheria, D., A.P. Singh, T. Wilson, N. Vorsa, G.S. Banuelos and B.T. Vinyard. 2010. Influence of conventional and organic agricultural practices on the phenolic content in eggplant pulp: Plant to plant variation. *Food Chemistry*, 121: 406-411

Malencic, D., Z. Maksimovic, M. Popovic and J. Miladinovic. 2008. Polyphenol contents and antioxidant activity of soybean seed extracts. *Bioresource Technology*, 99: 6688-6691.

Mathre, D.E., R.H. Johnston. 1995. Combined biological and chemical seed treatments for control of two seedling diseases of Sh2 sweet corn. *Plant Disease*, 79: 1145-1148.

Mathur, K. and K.S. Shekhawat. 1986. Chemical control of early blight in Kharif sown tomato. *Indian Journal of Mycology Plant Pathology*, 16:235-238.

McFarland, J. 1907. Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of American medical association,* 14: 1176-8.

McKnight, S.E. 1993. *Effects of Bacillus subtilis on cotton seedling development*, Nottingham Univ. (United Kingdom).

Medić-Pap, S.S., D. Maširević and D. Prvulovic. 2014. Phenolic compounds and antioxidant activity of sunflower hybrids inoculated with broomrape. *Studia UBB Chemia*, 59: 7-16.

Melent'ev, P., L. Helisto, Yu. Kuz'mina, N.F.G.E. Aktuganov and T. Korpela. 2006. *Applied Biochemistry and Microbiology*, 42: 70-75.

Munimbazi, C. and L.B.Bullerman. 1998. Isolation and partial characterization of antifungal metabolites of *Bacillus pumilus*. *Journal of Applied Microbiology,* 184: 959-968.

Naoumkina, M.A., X. He and R.A. Dixon. 2008. Elicitor-induced transcription factors for metabolic reprogramming of secondary metabolism in *Medicago truncatula*. *BMC Plant Biology*, 8: 132.

Nordlund, D.A. 1996. Biological control, integrated pest management and conceptual models. *Biocontrol News Informat*, 17: 35-44.

Nourozian, J., H.R. Etebarian, G. Khodakaramian. 2006. Biological control of Fusariumgraminearum on wheat by antagonistic bacteria.*Songklanakarin. Journal Science and Technology*, 28: 29-38.

Pane, C., M. Parisi, M. Zaccardelli, G. Graziani and V. Fogliano. 2011. Putative role of antioxidant activity of high pigment tomato cultivars in resistance against *Botrytis cinerea* post-harvest infection. *Acta Horticulture*, Proceedings of 3rd International Symposium on Tomato Diseases, Naples, Italy, 25-30th July, 2010

Rakh, R.R., S.L. Raut, M.S. Dalvi and V.A. Manwar. 2011. Biological control of *Sclerotiumrolfsii*, causing stem rot of Groundnut by *Pseudomonas* cf. *monteilii*. *Microbiolog*, 3: 26-34.

Rohman, A., S. Riyanto, I.N. Yuniart, W.R. Saputra and I.R. Utam. 2010. Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (Pandanus conoideus Lam). *International Food Researchjournal*, 17: 97-106.

Ruelas, C., M.E. Tiznado-Hernández, A. Sánchez-Estrada, M.R. Robles-Burgueno, R. Troncoso-Rojas. 2006. Changes in phenolic acid content during *Alternaria alternata* infection in tomato fruit. *Journal of Phytopathology*, 154: 236-244.

Sivanantham, T., V. Rasaiyah, N. Satkunanathan and A.C. Thavaranjit. 2013. *In vitro* screening of antagonistic effect of soil borne bacteria on some selected phytopathogenic fungi. *Archives of Applied Science Research,* 5: 1-4.

Slimestad, R. and M.J. Verheul. 2005. Content of chalconaringenin and chlorogenic acid in cherry tomatoes is strongly reduced during post-harvest ripening. *Journal of Agriculture Food Chemistry,* 53: 7251-7256.

Smirnov, V.V. and E.A. Kiprianova. 1990. *Bacteria of Pseudomonas genus*, pp. 100-111. NaukovaDumka, Kiev, Ukraine.

Srivastava, R. and Shalni. 2008. Antifungal Activity of *Pseudomonas fluorescens* against different plant pathogenic fungi. *EJEAFChe*, 7: 2789-2796.

Stewart, A.J., S. Bozonnet, W. Mulluen, G.I. Jenkis, M.E.J. Lean and A. Crozier. 2000. Occurrence of flavonols in tomato and tomato based products. *Journal of Agriculture and Food Chemistry*, 48: 2663-2669.

Veselova A., S.H. Klein, I.A. Bass, V.A. Lipasova, A.Z. Metlitskaya, M.I. Ovadis, L.S. Chernin and I.A. Khmel. 2008. Quorum sensing systems of regulation, synthesis of phenazine antibiotics, and antifungal activity in rhizospheric bacterium *Pseudomonas chlororaphis* 449. *Russian Journal of Genetics*, 4: 1400-1408.

Wei, G., J.W. Kloepper and S. Tuzun. 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. *Phytopathology*, 86: 221-224.

Wurms, K.V., M.P. George and D.R. Lauren. 2003. Involvement of phenolic compounds in host resistance against *Botrytis cinerea* in leaves of the two commercially important kiwifruit (*Actinidia chinensis* and *A. deliciosa*) cultivars. *New Zealand Journal of Crop* and *Horticultural Science*, 31: 221-233.

Yachana. J, R.B. Subramanian and S. Patel. 2011. Endophytic bacteria induced enzymes against *Magnapor thegrisea* in *O. sativa* under biotic stress. *African Journal of Basic and Applied Sciences*, 3: 136-146.

Yu, O., W. Jung, J. Shi, R.A. Croes, G.M. Fader, B. McGonigle. 2000. Production of the isoflavones genistein and daidzein in non-legume dicot and monocot tissues. *Plant Physiology*, 124: 781-794.

Zar, J.H. 2009. *Biostatistical Analysis*. 5th ed. Prentice Hall, Englewood Cliffs, New Jersey.

Zieslin, N. and R. Ben-Zaken. 1993. Peroxidase activity and presence of phenolic substances in peduncles of rose flower. *Plant Physiology Biochemistry*, 31: 333-334.