INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13S–005/2013/15–6–1289–1294
http://www.fspublishers.org

Full Length Article

Screening of Native Bacillus Strains to Induce Systemic Resistance in Tomato Plants against Fusarium Wilt in Split Root System and its Field Applications

Waheed Akram[1]*, Tehmina Anjum[1], Basharat Ali[2] and Aqeel Ahmad[1]
[1]*Institute of Agriculture Sciences, University of the Punjab, Lahore, Pakistan*
[2]*Department of Microbiology and Molecular Genetics, University of the Punjab Lahore, Pakistan*
*For correspondence: meher_waheed@yahoo.com

Abstract

A study was carried out to screen some bacillus strains for their ability to induce systemic resistance against fusarium wilt of tomato under both split root system and field conditions. Fourteen bacillus strains were used for initial screening of resistance induction under split root design in green house evaluations. Increase in quantities of defense related biochemicals as total phenolics, PO, PPO and PAL enzymes were examined to document induced systemic resistance (ISR) phenomenon in tomato plants under influence of these bacterial inducers. Two *Bacillus* strains viz., *B. fortis* IAGS162 and *B. subtilis* IAGS174 provided maximum control over fusarium wilt under split root system. Calorimetric assays proved highly significant for defense related biochemicals in tomato plants under the influence of these two bacterial strains. Talc based formulations of these two strains were prepaired to check their efficacy under field conditions. These not only provided protection against fusarium wilt, but also markedly enhanced growth and fruit yield of plants under field conditions. Our study clearly indicated the importance of these microbial organisms for suppression of Fusarium wilt and growth promotion in our agriculture system.

Keywords: Bacillus strains; Fusarium wilt; Induced systemic resistance; Disease index; Control effects; Growth promotion

Introduction

Like all living organisms, plants must face infections and diseases following the attacks of a mass of plant pathogens and pests from animal, microbial or viral origin. Plant diseases are responsible for the loss of at least 10% of global food production, representing a threat to food security (Strange and Scott, 2005). Fusarium is common in both tropical and subtropical environments and some of its members are most destructive pathogens of several plant species (Nelson *et al*., 1983; Zhang *et al*., 1996; Bokshi *et al.*, 2003). Fusarium wilt of tomato is a serious problem in all tomato growing areas of the world.

Defense mechanisms of plant can be activated by external stimuli before infection of a pathogen (Pieterse and Van Loon, 1999; Stadnik, 2000). This phenomenon is called Induced systemic resistance (ISR). Both biotic and abiotic agents have been successfully used in ISR in plants against pathogens (Akram and Anjum, 2011). ISR has been successfully used for plant protection under both green house and field conditions for longer times. Inducible systemic resistance responses include cell wall strengthening by deposition of lignin and callose, production of antimicrobial compounds like phytoanticipins and overexpression of pathogenesis related PR proteins inside plant body (Cachinero *et al*., 2002; Shoresh *et al*., 2010). All these events confer resistance against penetrating pathogen and make plant safe from subsequent pathogen attack.

Biocontrol of soil borne diseases is considered as effective disease management strategy (Wenhua and Hetong, 1997; Thakore, 2006; Kavino *et al.,* 2007). Significant reduction in disease and increase in growth of crop plants in response to inoculation with certain bacterial strains have been repeatedly reported (Asghar *et al.*, 2002; Vessey, 2003; Gray and Smith, 2005; Silva *et al.*, 2006; Figueiredo *et al.,* 2008; EPA, 2011). Bacteria in the genera *Bacillus, Streptomyces, Pseudomonas, Burkholderia,* and *Agrobacterium* are the biological control agents predominantly studied (Bashan, 1998; Lucy *et al*., 2004). These bacterial strains can either produce antibiotics or siderophores that leads to induction of systemic resistance (Tenuta, 2003). According to Hallman *et al,* (1997), endophytic bacteria involved in biological control show advantages of having the same ecological niche of the pathogen and could be protected from diverse abiotic influences.

In the current investigation, we tested our hypothesis that our native bacillus strains can induce systemic resistance in tomato against Fusarium wilt disease.

To cite this paper: Akram, W., T. Anjum, B. Ali and A. Ahmad, 2013. Screening of native bacillus strains to induce systemic resistance in tomato plants against fusarium wilt in split root system and its field applications. *Int. J. Agric. Biol*., 15: 1289–1294

So the present study was aimed on screening different native bacillus strains for their ability to indue resistance in tomato against fusarium wilt disease and elucidation of mechanism behind induced resistance. This was the first study carried out by using our native Bacillus strains under both split root system and field conditions to induce resistance against Fusarium wilt of tomato.

Materials and Methods

Efficacy of Bacillus Strains against Fusarium Wilt under Split Root System

Fourteen Bacillus strains belonging to four species were obtained from bacterial conservatories of Institute of Agricultural Sciences and Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan. These strains were mostly rhizospheric in nature whise, details are provided in Table 1. Bacterial inoculum was prepared by growing in Luria Broth (LB) medium. Media containing bacterial growth was centrifuged and pellet was resuspended in sterile distilled water to obtain the final bacterial concentration of 10^4 cfu/mL by taking OD of 0.1 at 600nm. Virulent strain of *F. oxysporum* f.sp. lycopersici (Fol) was obtained from Fungal Biotechnology Lab at the University of the Punjab, Pakistan. Pathogen inoculum was prepared by harvesting both micro- and macro-conidia from seven days old cultures grown on sterile PDA media at concentration of 1 x 10^3 conidia/mL, by haemocytometer.

Tomato seedlings of vaerity 'Rio Grand' were raised in sterilized sandy loamy soil. Green house evaluations were carried under split root design. For that purpose, roots of 30 days old tomato seedlings were splited into two halves and single seedling was tranfered in two combined pots (Fig. 1). In each treatment, inducer side was provided with 50 mL of bacterial inoculum and responder side got 50 mL of pathogen inoculum. For pathogen control, inducer side got distilled sterelized water and responder side got pathogen inoculum. Untreated control got distilled sterilized water on both sides. Pots were kept in green house for incubation. Disease index and control effects were analyzed after 30 days of inoculation. To sort out disease index, first severity of wilt was determined using a rating scale of 0~4 on the basis of root discoloration or leaf yellowing: 0: no root discoloration or leaf yellowing; 1: 1~25% root discoloration or one leaf yellowed; 2: 26~50% root discoloration or more than one leaf yellowed; 3: 51~75% root discoloration plus one leaf wilted; 4: up to 76% root discoloration or completely dead plants (Epp. 1987). Disease index and biocontrol effect were calculated according to the method of Li *et al.* (2008).

$$\text{Disease index (\%)} = \frac{\sum(\text{Grade of disease severity} \pm \text{diseased plants of this grade})}{\text{Total plants assed X Highest grade of disease severity}} \text{ X100}$$

$$\text{Biocontrol effect (\%)} = \frac{(\text{Disease index of pathogen control} - \text{diseased index of bacterial control})}{\text{Disease index of pathogen control}} \text{ X 100}$$

Table 1: Potential of Bacillus strains to control Fusarium wilt in three different varieties of tomato under split root experiment

Treatments		Disease	Control
Inducer Side	Responder Side	Index (%)	Effect (%)
B. fortis IAGS 324	Fol	20.01±2.31c-e	26.73±3.83e
B. fortis IAGS 223	Fol	23.34±1.58bc	14.53±1.53f
B. fortis IAGS 162	Fol	14.83±3.05g	52.37±4.64a
*B. thuringiensis*IAGS 199	Fol	19.16±2.86d-f	30.21±2.10de
*B. thuringiensis*IAGS 002	Fol	18.00±2.60ef	32.42±2.91cd
B. subtilis MCR7	Fol	24.17±3.46bc	11.46±2.93fg
B. subtilis IAGS 170	Fol	18.16±0.96ef	33.37±2.73cd
B. subtilis IAGS174	Fol	16.82±2.26f	48.14±3.37bc
B. subtilis FBL10	Fol	26.30±3.43b	03.68±0.57h
B. megaterium ZMR-4	Fol	23.67±1.75b-d	13.32±1.53f
B. megaterium ZMR-6	Fol	19.34±3.67d-f	30.15±2.44de
B. megaterium ZMR-3	Fol	23.41±2.26b-d	14.23±2.72f
B. megaterium MCR-8	Fol	17.41±3.92ef	36.51±3.62bc
B. megaterium OSR-3	Fol	26.34±1.08b	06.84±1.05gh
Water	Fol	57.31±4.23a	-
water	water	-	-

Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05) as governed by ANOVA and DNMRT. UC=Untreated Control. PC=Pathogen Control. Fol=*F. oxysporum* f. sp. Lycopersici

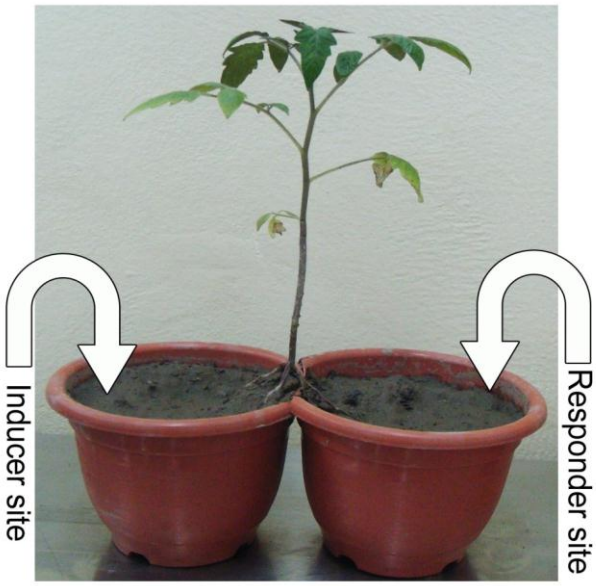

Fig. 1: Split Root Design

Elucidation of Biochemicals Basis of ISR

Quantifications of defense related biochemicals were performed at regular intervals of five days from the day of inoculation to final harvest and their mean values were used for comparison. For that purpose, root samples were taken from responde sides of the tomato plants for each treatment and total phenolics, peroxidase (PO), polyphenoloxidase (PPO) and phenyl ammonia lyase (PAL) were quantified by following methods.

Quantifications of total phenolics: One gram plant material was extracted with 10 mL of 80% methanol at 70°C for 15 min. Reaction mixture was containing 1 mL of methanolic extracts and 5 mL of distilled sterilized water 250 µL of Folin-Ciocalteau reagent (1 N). This solution was kept at 25°C. The absorbance of the developed blue color was measured using a spectrophotometer at 725 nm. Gallic acid was used as standard. The amount of total phenolics was expressed on gallic acid equivalent basis (Zieslin and Ben-Zaken, 1993).

Quantifications of PO, PPO and PAL activity: One gram of plant material was homogenized with 2 mL of 0.1 M sodium phosphate buffer (pH 7.0) in ice bath for enzyme assays. The homogenates were then centrifuged at 10,000 g for 10 min. Supernatants were used to analyze the PO, PPO and PAL activities.

Method of Fu and Huang (2001) was used to estimate the PO activity. For this purpose 50 µL of enzyme extract was added to 2.85 mL of 0.1 M phosphate buffer (pH 7.0) and mixed with 0.05 mL of 20 mM guaiacol reagent. The reaction was started by the addition of 0.02 mL of 40 mM hydrogen peroxide to the mixture. Rate of increase in absorbance at 470 nm was measured over 1 min. PPO activity was determined according to method proposed by Mayer *et al.* (1965). The reaction mixture was containing 200 µL enzym*e* extract and 1.5 mL of 0.01 M catechol. Activity was expressed as changes in absorbance at 495 nm.

PAL activity was determined according to method of Burrell and Rees (1974). The reaction mixture contained 0.03 M L-phenylalanine and 0.2 mL enzyme extract in a total 2.5 mL of sodium borate buffer (pH 8.8). This reaction mixture was kept in a water bath at 37°C for 1 h and 0.5 mL of 1 M trichloroacetic acid was added. The amount of trans-cinnamic acid formed from L-phenylalanine was measured spectrophotometerically at 290 nm.

Development of Talc Based Inoculum

Two best performing strains were selected for field evaluations. Their inoculum was prepared on sterilized Talc for application under field conditions. Three types talc formulations were prepaired viz. Both bacterial strains were grown in LB broth media separately. After overnight growth at 35°C, bacterial cells were collected by centrifugation at 4000 rpm for 15 min. Bacterial cell pellets were resuspended in sterilized distilled water at concentrations of 10^4 cfu/mL. Fifty mL of this bacterial inoculum was mixed in 100 gram of sterilized talc. Formulation in which both bacterial strains were added, 25 mL inoculum of each strain was taken and mixed with 100 gram of sterilized talc.

Field Experiment

Field experiment was performed twice in years of 2011 and 2012 in tomato growing season in Agriculture Research Station of Institute of Agricultural Sciences, University of the Punjab, Lahore Pakistan. Randomized split plot design was used for field experiments with three replicates per treatment. Main plots were further divided into subplots of 2×3 m^2. Tomato seedlings were raised in sterilized potting media as described in previous section. Fol inoculum was developed on sweet sorghum grains and applied in allotted subplots at rate of 100 g/plot and left for two weeks for establishment of pathogen. Tomat oseedlings were primed with talc based bacterial formulations and transferred in field. Details of treatments are provided in Table 4. Data regarding disease index, and control effect was noted after 60 days of transplantations as described prevously. Plant height and fruit yield was also noted at final harvest to observe grown promoting capabilitis of our selected strains.

Statistical Analysis

All the results were analyzed by performing ANOVA and DNMRT (Steel and Torrie 1980) with the help of computer aided program "DSASTAT".

Results

Efficacy of Bacillus Strains against Fusarium Wilt under Split Root System

The purpose of this experiment was to screen bacterial strain capable of inducing systemic resistance in tomato plants against fusarium wilt using split root experiment, which tried to avoid direct antagonism between pathogen and bacterial strains. During incubation period in greenhouse, symptoms first appeared as mild temporary wilting then effecting whole plant. In case of bacterial treated plants along with pathogen, delay in symptoms appearance was observed as compared to pathogen control plants. Some of our bacterial strains significantly controlled fusarium wilt disease as compared to pathogen control. Conspicuously, disease severity and control effect index (Table 1) represented that *B. fortis* IAGS162 (T3) and *B. subtilis* IAGS174 (T8) performed batter in this regard with minimum disease index of (Fig. 2). These two strains provided maximum protection against fusarium wilt and were used for field evaluations.

Elucidation of Biochemical Basis of Defense Induction

Presence of bacillus strains induced tomato plants for significantly (P>0.05) higher production levels of phenolics, PO, PPO and PAL as compared to pathogen alone and untreated controls (Table 2). *B. fortis* IAGS162 (T3) and *B. subtilis* IAGS174 (T8) increased phenolics quantities up to 67.15 and 55.47% as compared to untreated control (Table 2). In the same way, an increase of 56.70, 41.56 and 57.57% was recorder in PO, PPO and PAL activities under influence of *B. subtilis* IAGS174 (T8) compared to untreated control. Such differences (P>0.05) in quantities of total phenolics,

Table 2: Effect of bacterial inducers on elicitation of defense related biochemicals in tomato plants under split root system

Treatments		Phenolics	% IOUC	PO Activity	% I OUC	PPO Activity	% I OUC	PAL Activity	% I OUC
Inducer Side	Responder side	(µg/h/gfw)		(µg/h/gfw)		(µg/h/gfw)		(µg/h/gfw)	
B. fortis IAGS 324	Fol	1.86±0.07d-f	35.76±4.25fg	1.33±0.08cd	37.11±2.53d	6.07±0.59bc	42.48±3.07c-e	2.23±0.64bc	16.16±2.46e
B. fortis IAGS 223	Fol	1.61±0.06f-h	17.51±2.67j	1.17±0.15e-g	20.61±1.19g	5.89±0.61e-g	27.67±1.52f	2.07±0.39bc	04.54±0.82fg
B. fortis IAGS 162	Fol	2.29±0.09b	67.15±4.28b	1.45±0.09b	49.48±3.82b	7.31±0.88a	71.59±9.21a	3.27±0.51a	65.15±7.39a
*B. thuringiensis*IAGS 199	Fol	1.92±0.10c-e	40.14±3.92e	1.29±0.13d	32.98±2.51e	5.69±0.37f-i	25.13±3.43f	2.33±0.08b	12.62±2.03e
*B. thuringiensis*IAGS 002	Fol	2.03±0.08cd	48.17±2.36ef	1.34±0.67cd	41.23±2.43c	6.17±0.70d-f	44.83±2.30cd	2.85±0.13ab	43.93±6.53d
B. subtilis MCR7	Fol	1.83±0.09d-f	33.57±3.91gh	1.29±0.18d	32.98±2.18e	5.86(±0.62e-g	37.55±4.48e	2.13±0.42bc	07.57±1.04f
B. subtilis IAGS 170	Fol	1.77±0.11e-g	29.19±2.42h	1.16±0.07e-g	19.98±1.08gh	5.99±0.82fg	40.16±5.69de	2.01±0.71bc	01.51±0.13g
B. subtilis IAGS174	Fol	2.13±0.09c	55.47±4.52c	1.52±0.12a	56.70±3.44a	7.29±0.72ab	41.56±3.55c-e	3.12±0.36a	57.57±4.63b
B. subtilis FBL10	Fol	1.89±0.08d-f	37.95±2.63ef	1.12±0.19g	15.46±1.47h	5.26±0.61hi	19.01±2.27g	2.26±0.41b	14.14±2.21e
B. megaterium ZMR-4	Fol	1.90±0.12c-e	38.68±3.82ef	1.23±0.07d-f	26.80±1.92f	6.10±0.32d-g	30.16±3.27f	2.98±0.76ab	50.42±3.58c
B. megaterium ZMR-6	Fol	2.36±0.09b	72.26±6.53a	1.14±0.13fg	17.52±1.50gh	6.62±0.53cd	46.94±2.82c	3.01±0.11a	50.20±2.68c
B. megaterium ZMR-3	Fol	1.93±0.08c-e	40.87±2.24e	1.23±0.06d-f	26.80±2.83f	6.73±0.72bc	57.98±4.67b	2.88±0.28a	45.42±3.05d
B. megaterium MCR-8	Fol	2.08±0.15cd	51.82±3.50cd	1.16±0.08e-g	19.58±3.49gh	5.23±0.60i	18.54±2.07g	2.12±0.94bc	07.05±1.14f
B. megaterium OSR-3	Fol	1.70±1.05e-g	24.08±1.05i	1.38±1.05bc	42.26±1.05c	6.30±0.95c-e	47.88±5.36c	2.10±0.26bc	06.31±1.26f
Water	Fol	1.54±1.05gh	12.40±1.05k	1.13±1.05fg	16.49±1.05gh	5.51±0.24g-i	29.34±2.75f	2.06±0.17bc	04.75±0.85fg
water	water	1.37±1.05h	-	0.97±0.08h	-	4.26±0.34j	-	1.98±0.06cd	-

Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05) as governed by ANOVA and DNMRT. UC=Untreated Control. PC=Pathogen Control. IOUC=Increase over untreated control

Table 3: Potential of selected bacillus strains on fusarium wilt management under field conditions

Treatments	Experiment 1		Experiment 2	
	Disease index (%)	Control effect (%)	Disease index (%)	Control effect (%)
BS	37.86±4.23c	58.07±5.42b	28.81±7.43bc	62.34±5.80b
BF	46.29±5.77b	47.29±4.63c	31.11±3.29b	64.08±7.43b
BS±BF	23.87±3.92d	68.15±8.61a	19.57±2.41d	76.87±11.81a
PC	83.67±8.61a	ND	75.93±09.51a	ND
UC	ND	ND	ND	ND

Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05) as governed by ANOVA and DNMRT. BS=*B. subtilis* IAGS174. BF=*B. fortis* IAGS 162. UC=Untreated Control. PC=Pathogen Control

PO, PPO and PAL were observed for *B. fortis* IAGS162 (T3) when comparisons were made between control treatments (Table 2).

Field Experiment

Like split root experiment, our bacterial inducers provided promising protection against fusarium wilt under field conditions. Treatments in which we used combination of strains provided excellent protection against fusarium wilt. This treatment provided biocontrol effect of more than 60% in both field experiments (Table 3).

Along with protection agiainst Fusarium wilt, our bacterial inducers also promoted growth and yield of tomato plants under field conditions (Table 4). Tomato seedlings that were primed with bacterial inducers, provided significantly (P>0.05) higher plant height and biomasses as compared to untreated control plots. Treatments in which both straisn were applied, promoted height of plant upto 37 and 28% in experiment I and II, respectively. Samely, yield of tomato plants was positively influenced by bacterial inducers at significant levels (Table 4). These data provide strong evidence in favor of our bacterial inducers under field conditions.

Discussion

This study showed that our native bacillus strains have potential benefits in practical agriculture. Numerous reports show that beneficial microbes can protect plants against a wide range of disease (Raaijmakers *et al*., 1995; Latha *et al*., 2009). Bacterial inducers remain restricted up to root system of the plant. But the phenomenon of ISR is because of lipopeptides of the surfactin and fengyci, that play the role of elicitor for activation of pant defense system (Ongena *et al*., 2007; Jourdan *et al*., 2009). Bacterial inducers comprised variable degree of disease controlling phenomenon in our current investigation. This can be attributed the differences that might result from the different origins of each isolate (Raaijmakers *et al*., 1995; Mercado-Blanco and Bakker, 2007). In our split root investigations, *B. fortis* IAGS162 and *B. subtilis* IAGS174 showed better suppression of disease as compared to other bacilli strains. Efficacy of bacillus genera to control other plant diseases has also been documented against several other plant diseases. *B. subtilis* proved effective to control *B. cinerea* on grapes (Magnin-Robert *et al*., 2007; Trotel-Aziz *et al*., 2008).

Under field conditions, combination of these two microbes provided better level of protection against fusarium wilt disease. Some researchers showed that combination of bacterial strains provided better protection against diseases. In a research, mixture of bacterial inducers provided ISR against a cucumber leaf spot disease (Raupach and Kloepper, 2000). It is suggested that use of bacterial inducers in combination display increased defense related biochemicals inside plant body (Raupach and Kloepper, 2000; Jetiyanon and Kloepper, 2002). It is also proposed that use of combination of bacterial inducers can provide protection against a wide range of pathogens to plants.

Considering the spatial separation of applied bacteria at the root level, the disease protection by bacteria results

Table 4: Effect of bacillus strains on plant growth parameters under field conditions

Treatment	Experiment 1				Experiment 2			
	Plant Height (cm)	Total Biomass (g) Fresh	Dry	Number of Fruits	Plant Height (cm)	Total Biomass (g) Fresh	Dry	Number of Fruits
BS	33.27±2.16b	113.29±16.73bc	13.90±04.13ab	15.18±3.96bc	38.07±5.14b	127.29±15.50b	21.38±4.71b	14.11±3.17c
BF	30.38±4.29bc	102.55±11.25c-e	10.74±02.82b-d	17.73±2.72b	35.36±4.18bc	112.55±19.72c	16.97±2.98c	11.53±2.25cd
BS±BF	38.92±5.14a	123.94±13.52ab	15.03±02.36a	23.43±4.80a	41.26±3.26ab	159.49±17.53a	26.72±5.31a	21.37±4.30a
PC	18.27±3.36e	51.29±08.61g	06.75±01.17de	07.06±1.18d	21.07±3.52e	66.92±07.58d	09.73±3.76e	04.42±1.06e
UC	27.64±3.04cd	94.26±07.43ef	08.96±02.25cd	16.65±3.95b	32.93±5.24cd	123.26±22.48b	14.19±1.58cd	17.61±2.16bc

Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05) as governed by ANOVA and DNMRT. BS=*B. subtilis* IAGS174. BF=*B. fortis* IAGS 162. UC=Untreated Control. PC=Pathogen Control

from an ISR in plants (Magnin-Robert *et al*., 2007). Bacillus strains can induce resistance against a number of diseases in field crops (Akram and Anjum, 2011). Previously, mostly researchers have performed ISR experiments in single pot system but we applied split root system in our initial screening that confirms ISR phenomenon and negated chances of direct antagonism. Bacterial organisms have the potential to elicit ISR in plants. This activated defense system of plant then responds very quickly against fungal pathogens by producing fungitoxic environment in plants (Morsy *et al*., 2009). This concept is entirely in accordance with our present investigation.

Phenolics in plants have numerous functions as stability of structures, protection form herbivory and biocidal effect against fungal and bacterial plant pathogens (Heldt, 1997). As we observed increased activities of total phenolics in plants with lesser disease severity, that were under influence of our bacterial inducers. In the same way, resistance in plants is accompanied by increased activities of enzymes involved in phenylpropenoid pathway viz: PO, PPO and PAL (Trotel-Aziz *et al.,* 2008; Jourdan *et al.,* 2009; Radjacommare *et al*., 2010; Akram and Anjum, 2011). PO, PPO and PAL play role in production of quinones and some other phytoalexins in plants that destroy pectolytic enzymes produced by pathogens (Li and Stiffens, 2002; Kavino *et al* 2008). These enzymes are also associated with induced resistance in plants by production of defense barriers in plants as lignin and reactive oxygen species (Van Loon, 1999). We also recorded high levels of these enzymes in tomato plants that surpass Fol attack in response to induced resistance by some bacterial strains.

In parallel with disease suppression, plant growth promotion is also observed under influence of bacterial inducers in many plants (Adhikari *et al.,* 2001; Bacon and Hinton, 2002; Nihorimbere *et al*., 2010). These inducers play dual role of induced resistance along with growth promotion. This beneficial effect of bacillus strains on plant development is because of diverse mechanism (Gupta *et al*., 2000; Ping and Boland, 2004; Berg, 2009). As we observed in current investigation, growth was significantly increased in bacterial treated plants under field conditions. Growth is stimulated under the influence of bacillus strains because of production of hormones like compounds as auxins and cytokinins. These bacterial organisms improve nutrients acquisition by plants either by nitrogen fixation or by solubilization of phosphorus, iron and other oligoelements (Ryu *et al.,* 2003). Seemingly increasing fruit was observed set when experiment was performed under field conditions.

In conclusion this work illustrates the effectiveness of bacillus strains to induce systemic resistance and growth promotion in tomato plants under both greenhouse and field conditions. Based on the results of our studies, inoculum of these strains can be provided commercially to local farmers for dual benefits. This study provides a cheap and environmental friendly solution for management of this nasty pathogen in our fields.

References

Adhikari, T.B., C.M Joseph, G. Yang, D.A. Phillips and L.M. Nelson, 2001. Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling disease of rice. *Can. J. Microbiol*., 47: 916–924

Akram, W. and T. Anjum, 2011. Use of bioagents and synthetic chemicals for induction of systemic resistance in tomato against diseases. *Int. R. J. Agric. Sci. Soil. Sci*., 1: 286–292

Asghar, H.N., Z.A. Zahir, M. Arshad and A. Khalig, 2002. Plant growth regulating substances in the rhizosphere: microbial production and functions. *Adv. Agron.*, 62: 146–151

Bacon, C.W. and D.M. Hinton, 2002. Endophytic and biological control potential of *Bacillus mojavensis* and related species. *Biol. Cont*., 23: 274–284

Bashan, Y., 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. *Biotechnol. Adv*., 16: 729–770

Berg, G., 2009. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *App. Microbiol. Biotechnol.*, 84: 11–18

Bokshi, A.I., S.C. Morris and B.J. Deverall, 2003. Effects of benzothiadiazole and acetylsalicylic acid on beta-1,3-glucanase activity and disease resistance in potato. *Plant. Pathol*., 52: 22–27

Burrell, M.M. and T.A. Rees, 1974. Metabolism of phenylalanine and tyrosine in rice leaves infected by *Pyricularia oryzae. Physiol. Plant Pathol*., 4: 497–508

Cachinero, J.M., A. Hervas, R.M. Jimenez-Diaz, and M. Tena, 2002. Plant defense reactions against Fusarium wilt in chickpea induced by incompatible race 0 of *Fusarium oxysporum* f.sp. ciceris and nonhost isolates of *F. oxysporum'. Plant Pathol*., 51: 765–776

EPA., 2011. *Regulating Biopesticides*. http://www.epa.gov/oppbppd1/biopesticides/index.htm. February 27

Epp, D., 1987. Somaclonal variation in banana: a case study with Fusarium wilt'. in *Banana and Plantain Breeding Strategies*, pp: 140–150. Persley, G.J., E.A. and D. Langhe, (eds.). ACIAR Publications

Figueiredo, M.V.B., H.A. Burity, C.R. Martinez and C.P. Chanway, 2008. Alleviation of water stress effects in common bean (*Phaseolus vulgaris* L.) by co-inoculation *Paenibacillus* x *Rhizobium tropici*. *Appl. Soil Ecol*., 40: 182–188

Fu, J.H.B., 2001. Involvement of antioxidants and lipid peroxidation in the 284 adaptation of two cool-season grasses to localized drought stress. *Environ. Exp. Bot.*, 45: 105–114

Gray, E.J. and D.L. Smith, 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil. Biol. Biochem.*, 37: 395–412

Gupta, A., M. Gopal and K.V. Tilak, 2000. Mechanism of plant growth promotion by rhizobacteria. *Ind. J. Exp. Biol.*, 38: 856–862

Hallman, J., A. Quadt-Hallman, W.F. Mahafee and J.W. Kloepper, 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol.*, 43: 895–914

Heldt, H.W., 1997. *Plant Biochemistry and Molecular Biology*, 1st edition. Oxford University Press, UK

Jetiyanon, K. and J.W. Kloepper, 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biol. Cont.*, 24: 285–291

Kavino, M., S. Harish, N. Kumar, D. Saravanakumar, T. Damodaran, K. Soorianathasundaram and R. Samiyappan, 2007. Rhizosphere and endophytic bacteria for induction of systemic resistance of banana plantlets against bunchy top virus. *Soil. Biol. Biochem.*, 39: 1087–109

Kavino, M., S. Harish, N. Kumar, D. Saravanakumar, and R. Samiyappan, 2008. Induction of systemic resistance in banana (Musa spp.) against Banana bunchy top virus (BBTV) by combining chitin with root-colonizing Pseudomonas fluorescens strain CHA0. *Eur. J. Plant Pathol.*, 120: 353–362

Jourdan, E., G. Henry, F. Duby, J. Dommes, J.P. Barthelemy, P. Thonart and M. Ongena, 2009. Insights into the defense-related events occurring in plant cells following perception of surfactin-type lipopeptide from *Bacillus subtilis. Mol. Plant-Microbe. Interact*., 22: 456–468

Latha, P., T. Anand, N. Ragupathi, V. Prakasam and R. Samiyappan, 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and zimmu leaf extract against *Alternaria solani*. *Biol. Cont.*, 50: 85–93

Li, W., J.C. Hu and S.J. Wang, 2008. Growth-promotion and biocontrol of cucumber fusarium wilt by marine Bacillus subtilis 3512A. *J. Shenyang Agric. Univ*., 39: 182–185

Li, L. and J.C. Stiffens, 2002. Over expression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta*, 215: 239–247

Lucy, M., E. Reed and B.R. Glick, 2004. Applications of free living plant growth-promoting rhizobacteria'. *Rev. Ant. Van. Lee*, 86: 1–25

Magnin-Robert, M., P. Trotel-Aziz, D. Quantinet, S. Biagianti and A. Aziz, 2007. Biological control of *Botrytis cinerea* by selected grapevine-associated bacteria and stimulation of chitinase and b-1,3 glucanase activities under field conditions. *Eur. J. Plant. Pathol.*, 118: 43–57

Mayer, A.M. and E. Harel, 1997. Polyphenol oxidases in plants. *Phytochemistry*, 18: 193–215

Mercado-Blanco, J. and P.A.H.M. Bakker, 2007. Interactions between plants and beneficial Pseudomonas spp. exploiting bacterial traits for crop protection. *Ant. van Lee.*, 92: 367–389

Morsy, E.M., K.A. Abdel-Kawi and M.N.A. Khalil, 2009. Efficiency of *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents against *Fusarium solani* on tomato plants. *Egy. J. Phytopathol*., 37: 47–57

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1983. *Fusarium Species*. An Illustrated Manual for Identification. Pennsylvania State University Press, University Park, Pennsylvania, USA

Nihorimbere, V., M. Ongena1, H. Cawoy, Y. Brostaux and P. Kakana, 2010. Beneficial effects of *Bacillus subtilis* on field-grown tomato in Burundi: Reduction of local *Fusarium* disease and growth promotion. *Afr. J. Microbiol. Res.*, 4: 11–19

Ongena, M. and P. Jacques, 2008. *Bacillus lipopeptides*: versatile weapons for plant disease biocontrol. *Trend Microbiol.*, 16: 115–125

Pieterse, C.M.J. and L.C. Van Loon, 1999. Salicylic acid-independent plant defense pathways. *Trend. Plant Sci.*, 4: 52–58

Ping, L.Y. and W. Boland, 2004. Signals from the underground: bacterial volatiles promote growth in Arabidopsis. *Trend. Plant Sci.*, 9: 263–266

Raaijmakers, J.M., M. Leeman and M.M.P. Van-Oorschot, I. van der Sluis, B. Schippers and P.A.H.M. Bakker, 1995. Dose–response relationships in biological control of Fusarium wilt of radish by Pseudomonas spp. *Phytopathology*, 85: 1075–1081

Radjacommare, R., S. Venkatesan and R. Samiyappan, 2010. Biological control of phytopathogenic fungi of vanilla through lytic action of Trichoderma species and Pseudomonas fluorescens. *Arch. Phytopathol. Plant. Prot*., 43: 1–17

Raupach, G.S. and J.W. Kloepper, 1998. Mixtures of plant growthpromoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology*, 88: 1158–1164

Raupach, G.S. and J.W. Kloepper, 2000. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. *Plant Dis.*, 84: 1073–1075

Ryu, C.M., M.A. Farag, C.H. Hu, M.S. Reddy, H.X. Wei, P.W. Pare and J.W. Kloepper, 2003.Bacterial volatiles promote growth in Arabidopsis. *Proc. Natl. Acad. Sci. USA*, 100: 4927–4932

Shoresh, M., G. Harman and F. Mastouri, 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu. Rev. Phytopathol*., 48: 21–43

Silva, V.N., L.E.S.F Silva and M.V.B. Figueiredo, 2006. Atuac¸a˜o de rizo´bios com rizobacte´rias promotoras de crescimento em plantas na cultura do caupi (Vigna unguiculata L. Walp). *Act. Sci. Agron*., 28: 407–412

Stadnik, M.J., 2000. Inducao de resistencia a Oidios. *Summa. Phytopathol*., 26: 175–177

Strange, R.N. and Scott, P.R., 2005. Plant disease: A threat to global food security. *Annu. Rev. Phytopathol.*, 43: 83–116

Steel, R.G.D. and J.H. Torrie, 1980. *Principles and Procedures of Statistics: A Biometrical Approach*', 2nd edition. McGraw Hill Inter, Book Co, Tokyo, Japan

Tenuta, M., 2003. http://www.umanitoba.ca/afs/ agronomists_conf/2003/pdf/tenuta_rhizobac-teria.pdf.

Thakore, Y., 2006. The biopesticide market for global agricultural use. *Ind. Biotechnol.*, 2: 194–208

Trotel-Aziz, P., M. Couderchet, S. Biagianti and A. Aziz, 2008.Characterization of new bacterial biocontrol agents Acinetobacter, Bacillus, Pantoea and Pseudomonas spp. mediating grapevine resistance against *Botrytis cinerea. Environ. Exp. Bot.*, 64: 21–32

Van Loon, L.C., 1999.Occurrence and properties of plant pathogenesis-related proteins. *In*: '*Pathogenesis-related Proteins in Plants*', pp: 1–19. Datta, S.K., S. Muthukrishnan, (eds.). CRC Press LLC, Boca Raton

Vessey, J.K., 2003. Plant growth-promoting rhizobacteria as biofertilizers. *Plant Soil*, 255: 571–586

Wenhua, T. and Y. Hetong, 1997. Research and application of biocontrol of plant diseases and PGPR in China'. *In*: '*Plant Growth-promoting Rhizobacteria – present Status and Future Prospects'*, pp: 2–9. Ogoshi, A., K. Kobayashi, Y. Homma, F. Kodama, N. Kondo and S. Akino, (eds.). OECD OCDE, Sapporo, Japan

Zhang, J.X., C.R. Howell, J.L. Starr and M.H. Wheeler, 1996. Frequency of isolation and the pathogenicity of Fusarium species associated with roots of healthy cotton seedlings. *Mycol. Res.*, 100: 747–752

Zieslin, N. and R. Ben-Zaken, 1993.Peroxidase activity and presence of phenolic substances in peduncles of rose flower. *Plant. Physiol. Biochem.*, 31: 333–339

(Received 10 March 2013; Accepted 15 August 2013)