American Journal of Life Sciences
2014; 2(6-2): 19-25
Published online November 25, 2014 (http://www.sciencepublishinggroup.com/j/ajls)
doi: 10.11648/j.ajls.s.2014020602.13
ISSN: 2328-5702 (Print); ISSN:2328-5737 (Online)

Management Fusarium wilt of sweet pepper by Bacillus strains

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To cite this article:
Abada K. A., M. A. Ahmed. Management Fusarium Wilt of Sweet Pepper by Bacillus Strains. *American Journal of Life Sciences.* Special Issue: Role of Combination between Bioagents and Solarization on Management of Crown-and Stem-Rot of Egyptian Clover. Vol. 2, No. 6-2, 2014, pp. 19-25. doi: 10.11648/j.ajls.s.2014020602.13

Abstract: Isolation trials from the roots of wilted sweet pepper plants yielded *Alternaria* spp., *Fusarium oxysporum, Pythium* spp., *Rhizontonia solani , Sclerotium rolfsii* and *Trichoderma* spp. The isolates of the fungus *F. oxysporum* were selected to test their pathogenicity and Kalubia isolate was the most virulent one. The fungus was virulent to sweet pepper and low infection was found in case of the other five tested plants. Therefore, the fungus *F. oxysporum* named *Fusarium oxysporum* f.sp. *capsici.* All the five Bacillus strains, *i.e. Bacillus chitinosporus ,B. coagulans , B. humilus ,B.subtilis* and *B. thuringiensis* caused significant reduction to the radial growth of *F.o.*f.sp.*capsici* compared with control treatment. In addition, the growth of the tested pathogen was completely inhibited by *B.subtilis* and *B.thuringiensis* at the concentration of 60%. Furthermore, *B.thuringiensis* was the most efficient bioagent in this regard followed by *B.subtilis* then *B.pumilus*. Adding the three tested bioagents *,i.e.B.pumilus .B.subtilis* and *B. Thuringiensis* to soil infested with *F.o.*f.sp.*capsici* resulted in significant reduction to sweet pepper wilt with significant increase to the plant height as well as the number of pods and their weight / plant compared with control treatment. The symptoms of the disease were obvious both on the foliage growth and the xylem vesicles, but the severity of the disease was more higher on the xylem vesicles than on the foliage growth. In addition, plants grown in soil infested with Bacillus strains were of high values of plant height and fruit yield (number and weight / plant) than that grown in the control (uninfested soil). The total phenolic compounds were greatly increased in the bacterial treated plants as compared to untreated plants with the bioagents and that infested with the pathogen only. These results give a potential of these bacterial strains for use as plant protection agents against Fusarium wilt of sweet pepper. This work was performed to investigate the potential of some bacterial bioagents *,i.e. B. humilus , B.subtilis* and *B. thuringiensis* on management of sweet pepper Fusarium wilt and the formation of phenolic compounds in the plants.

Keywords: *Bacillus* spp,Bioagents, FusariumWilt, Sweet Pepper, Total Phenolic Compounds

1. Introduction

Sweet pepper (*Capsicum annum* L.) is considered one of the most important vegetable crops in Egypt for local consumption and exportation. The economic importance of sweet pepper cultivation in the world could be explained by its high nutritional value of antioxidants, vitamins and some other compound. Therefore, improving the bioproduction of this crop is one of the objectives in agriculture in many countries (Akram *et al.*, 2013).

The total cultivated area with sweet pepper in Egypt in the open field during 2013 growing season reached about 65240 feddan with a total production of 387964 ton at the rate of 5.95 ton /feddan in addition to about 1000 feddan for protected cultivation with a production of about 7500 ton with an average of 7.5 ton / feddan (Food Legume Statics Dept., Field Crops Res. Instit., ARC.,2014).

Sweet pepper is liable to be attack by many bacterial, fungal, viral and nematode diseases as well as physiological disorder. However, Fusarium wilt is considered the major devastative and destructive disease affecting crop production of pepper (Black *et al.*,1991 and Attia and Abada, 1994).

Due to the cultivated area in Egypt is limited, therefore crop rotation is not applied and this caused great difficult, especially in case of specific soil borne pathogens such as

Fusarium wilt. On the other hand, the use of chemical control against such diseases sometimes gives good results. However, improper use of fungicides leads mostly to environmental pollution, disasters throughout the world and the phenomena of resistance to the plant pathogens (Brewer and Larkin, 2005). Hence, to overcome these difficulties, it is urgent to apply biological control as an alternative safe efficient method against this disease (Akram *et al.,*2013). Biological control is considered an important approach of agricultural biotechnology in recent years for controlling many fungal plant pathogens (Zaher *et al.*,2013and Abada and Eid,2014).

Resistance to plant disease is supposed to be a dynamic and multifactorial process. It is assumed that plant defense response can be activated by specific recognition of some microorganisms by the plant. There may be whole organisms or products secreted by microorganisms under the influence of which plants initiate defense response (Albersheim *et al.,* 1975 and 1978 and Akram *et al.,*2013].

This work was planned to management of Fusarium wilt of sweet pepper by induce systemic resistance by *Bacillus* spp. Also, phenolic compounds constituent to plant defense was also assayed.

2. Materials and Methods

2.1. Isolation, Purification and Identification of the Associated Fungi

Sweet pepper plants (Balady cv.) showing characteristic symptoms of wilt were collected from Dakahlia, Menofia, Kalubia and Giza governorates. The infected root samples were thoroughly washed in running tap water and cut into small pieces with lesion having half healthy and half diseased tissue. The pieces were surface sterilized with 2 % sodium hypochlorite for two minutes. The tissue pieces were subsequently washed in three changes of sterile water to eliminate excess sodium hypochlorite and then the pieces were transferred onto PDA medium in Petri dishes. Plates were incubated at 25 ± 2°C and observed periodically for growth of the fungi. The pure cultures of the isolated fungi were obtained by hyphal tip method and/ or single spore technique and maintained on PDA slants throughout the investigation. The emerged fungi were identified on the basis of cultural, morphological characteristics and the description of Booth (1971) and Domsch *et al*. (1980).

2.2. Pathogenicity Test

The inoculum of *F. oxysporum* was prepared by culturing the fungus on potato dextrose agar (PDA) medium for 5 days in Petri plates. Conidial suspension was prepared by pouring 20 ml of sterile distilled water in each Petri plate. The concentration of conidia was adjusted to $1x10^3$ conidia per milliliter using haemocytometer. Pathogenicty test was carried out by two ways:

2.2.1.Using Seeds

Sweet pepper seeds (Balady cv.) were surface sterilized with 1.0% solution of sodium hypochlorite for 1 min and then thoroughly washed with tap water. Five seeds were sown in each plastic pot (25 cm. in diameter) contained infested clay soil by spore suspension of any of the four isolates of *Fusarirum oxysporum* ($1x10^3$ conidia / ml water) at the rate of 50 ml / pot . Also, Five seeds were, also, sown in each plastic pot (25 cm. in diameter) contained disinfested clay soil and served as control. Five pots were used for each treatment. The plants were thinned into three seedlings / pot one month after sowing and left to grow for four months under greenhouse conditions (Fac. Agric., Cairo Univ.). The plants were examined for the severity of infection by the tested fungus monthly as mentioned under disease assessment and the averages were recorded. The pots were irrigated when it was necessary and fertilized with recommended doses as recommended by Min. of Agric. and Land Reclamation.

2.2.2.Using Transplants

Three transplants (Balady cv.) of 35 day-old, grown in disinfested soil, were transplanted in each plastic pot (25 cm. in diameter) contained infested clay soil by spore suspension of any of the four isolates of *Fusarirum oxysporum* ($1x10^3$ conodia / ml water) at the rate of 50 ml / pot . Also, Three transplants were, also, transplanted in each plastic pot (25 cm. in diameter) contained disinfested clay soil and served as control. Five pots were used for each treatment. The plants were left to grow for three months after transplanting under greenhouse conditions (Fac. Agric., Cairo Univ.). The plants were examined for the severity of infection by the tested fungus monthly as mentioned under disease assessment and the averages were recorded. The pots were irrigated when it was necessary and fertilized with recommended doses as recommended by Min. of Agric. and Land Reclamation.

2.3. Isolation, Purification and Identification of the Antagonists

Rhizosheric soil samples collected from sweet pepper plants have severe infection by wilt (grown at Kalubia governorate) were used to isolate the antagonists. Serial dilution plate technique was used to isolate and select native *Bacillus* spp. on nutrient agar medium (Oedjijono and Dragar, 1993).The isolated bacteria were purified and identified depending on the description of Parry *et al.* (1983) and Holt and Krieg (1984). The identification was confirmed by the Biolog-System technique (Biological control of faba bean chocolate spot disease project, Plant Pathol. Res. Instit., A.R.C., Giza, Egypt)

2.4. Effect of the Culture Filtrate on the Radial Growth

The effect of the culture filtrate of five isolates of *Bacillus* spp. on the radial growth of *F. o.* f.sp. *capsici* was studied. One hundred ml. of nutrient medium were put in

each 250 ml flask and sterilized by steamer for three successive days. The medium was inoculated with a loop of the bacterial bioagents taken from two day-old culture. Inoculated flasks were incubated on a rotary shaker at 200 rpm for 2 days at 30 ± 2°C . The culture filtrate was filtered through Whatman No.1 filter paper and the filtrate was collected in a flask. The culture filtrate of the bioagents was mixed with the component of PDA medium in different proportion (20, 40, 60 and 80 %). The medium was then sterilized and poured into the Petri-dishes (20 ml/plate). After solidification the Petri plates were inoculated with 5 mm. discs of the test pathogen cut from the five days old culture. PDA plates inoculated with the test pathogen, but not amended with the culture filtrate of *Bacillus* spp. were maintained as control. Plates were then incubated in an incubator at 30±2°C. Five replications were maintained for each treatment. The radial growth was measured when the plates of the control treatment covered with the fungal growth. Inhibition percentage of mycelial growth of the tested pathogen was calculated by the formula:

$$I = (C- T)/C X100$$

Where;

I = Percent of inhibition in growth of the tested pathogen,
C = Radial growth of the pathogen (mm) in control,
T= Radial growth of the pathogen (mm) in treatment.

2.5. Effect on Wilt Severity and some Crop Parameters

Ten seeds (Balady cv.) were surface sterilized with 1.0% solution of sodium hypochlorite for 1 min and then thoroughly washed with tap water. Seeds were sown in plots (70 x30 x 40 cm.) containing formalin sterilized clay soil and kept under greenhouse conditions. When the seedlings were of 3 weeks old, they were thinned into four seedlings in each plot and one week later they received the following treatments:

1 The seedlings supplied with the bacterial suspension of *B.pumilus* at the rate of 500 ml ($1x10^3$ cfu) / plot.
2 The seedlings supplied with the bacterial suspension of *B.subtilis* at the rate of 500 ml ($1x10^3$ cfu) / plot.
3 The seedlings supplied with the bacterial suspension of *B. thuringiensis* at the rate of 500 ml ($1x10^3$ cfu) / plot.
4 The seedlings supplied with the bacterial suspension of *B.pumilus* at the rate of 500 ml (1x103 cfu) / plot, two weeks before infestation with FOC .
5 The seedlings supplied with the bacterial suspension of *B.subtilis* at the rate of 500 ml ($1x10^3$ cfu) / plot, two weeks before infestation with FOC.
6 The seedlings supplied with the bacterial suspension of *B. thuringiensis*at the rate of 500 ml ($1x10^3$ cfu) / plot, two weeks before infestation with FOC.
7 The seedlings left to grow without supplying with any additional from *Bacillus* spp., but received 500 ml water only (control uninfested soil).
8 The seedlings left to grow without supplying with any additional from *Bacillus* spp., but infested with the spore suspension of the causal fungus at the rate of 500 ml ($1x10^3$ conidia) / pot.

After another three weeks the grown sweet pepper plants received the previous treatments. Six plots were used for each treatment, where three plots were used to assess disease severity and the three others for estimation of plant height (cm) the number and weight of pods / plant.

2.6. Disease Assessment

Disease severity was assessed four months after sowing or three months after transplanting using the devised scale (0 to 5) by Amini and Sidovich (2010) on the foliage growth using the following scale:

Where:

0 = No foliar symptoms,
1 = Chlorosis and/or wilt restricted to cotyledons or first leaf,
2 = Chlorosis and/or wilt extending beyond the first leaf,
3 = Moderate to severe foliar symptoms usually with some abscised leaves,
4 = Severe foliar symptoms on the entire plant, and
5 = Dead plant.

$$\text{Disease severity on foliage growth \%} = £ (nxv) / 5N X 100$$

Where:

n = Number of infected leaves in each category.
v = Numerical values of each category.
N = Total number of the infected leaves.

The plants were also rated for vascular discoloration using the devised scale (0-5) by Ulloa *et al.*(2006) using the following scale:

Where:

0 = No discoloration,
1 = Light discoloration evident as spotty areas in the cross-section of the stem,
2= More continuous discoloration covering an area between one quarter and one half of the cross-section stem but light in color,
3= Vascular discoloration (moderate in color) evident in a band encircling almost the entire stem cross-section,
4 = Vascular discoloration darker in color than in 1 or 2, and evident across most of the vascular tissue in a cross section of the stem, and
5= Plant severely damaged, vascular discoloration evident throughout cross-section of the stem.

$$\text{Disease severity on the vascular \%} = £ (nxv)/5 N X 100$$

Where:

n = Number of infected vascular in each category.
v = Numerical values of each category.
N = Total number of the infected vascular.

Also, plant height (cm) of the grown plants, the average No. of pods / plant and weight of pods (g) / plant were assessed and recorded.

2.7. Estimation of Total Phenolic Compounds

One gram of sweet pepper leaves sample was extracted with 10 ml of 80% methanol at 70 °C for 15 min. Reaction mixture was containing 1 ml of methanolic extracts, 5 ml of distilled sterilized water, and 250 µl of Folin–Ciocalteau reagent (1N). 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 the standard. The amount of phenolic composunds was expressed as mg gallic acid per g plant material (Zieslin and Ben-Zaken, 1993).

2.8. Statistical Analysis

Data were statistically analyzed using the standard procedures for complete randomize block and split designs as mentioned by Snedecor and Cochran (1967). The averages were compared at 5% level using least significant differences (L.S.D) according to Fisher (1948).

3. Results

3.1. Isolation, Purification and Identification of the Associated Fungi

Isolation trials from sweet pepper plants (Balady cv.) showing characteristic symptoms of wilt collected from Dakahlia, Menofia, Kalubia and Giza governorates yielded many fungal isolates. The isolated fungi were purified and identified as : *Alternaria*spp., *Fusarium oxysporum, Pythium* spp., *Rhizontonia solani , Sclerotium rolfsii* and *Trichoderma s*pp.

The isolates of the fungus *F. oxysporum* were selected and tested for their pathogenicity and the most virulent isolate was chosen.

1 .*Pathogenicity Test of the Four Isolates of F. oxysporum*

Pathogenicity test of the four isolates of *F. oxysporum* (Table 1) reveals that the four tested isolates were pathogenic to sweet pepper plants and showing typical wilt symptoms on the foliage growth and the xylem vesicles. Data indicate that the fungus was more virulent when the seeds were used than using of the transplants in this experiment. In addition, the symptom of the disease was obvious on both the foliage growth and the xylem vesicles. Furthermore, the isolate of Kalubia governorate of the fungus *F. oxysporum* was the highest virulent one either on the foliage growth and the xylem vesicles. Therefore, it was used in the following experiments. Also, testing of cucumber (Amira cv) ,eggplant (Balady black cv.), sweet pepper (Balady cv.) , strawberry (Camarosa cv.) , tomato (GS cv.) and water melon (Giza 1 cv.) to their susceptibility to the infection by *F. oxysporum* indicated that the highest infection by the fungus was occurred only on sweet pepper and low infection was found in case of the other plants. Therefore, the fungus *F. oxysporum* named *Fusarium oxysporum* f.sp. *capsici.*

Table 1. *Pathogenicity test of the four isolates of F. oxysporum using seeds and transplants of sweet pepper (Balady cv.) , greenhouse experiments.*

Isolates	% Disease severity of sown seeds on the		Mean	% Disease severity of transplanting on the		Mean
	Foliage Growth	Xylem vesicles		Foliage growth	Xylem vesicles	
Dakahlia	36.8	42.1	39.5	32.4	40.2	33.3
Menofia	40.8	45.7	43.3	39.0	41.4	40.2
Kalubia	45.8	52.5	49.2	42.8	50.3	46.6
Giza	42.3	50.8	46.5	40.2	47.8	44.0
Control	0.0	0.0	0.0	0.0	0.0	0.0
Mean	33.1	38.2	-----	30.9	35.9	----

3.2. Effect of Culture Filtrate of Five Bacillus Strains on the Radial Growth of F. o.f.sp. Capsic

Table 2. *Effect of culture filtrate of five Bacillus strains on the radial growth of F. o.f.sp. capsici , five days after incubation at 30±°C.*

Bacillus strains	Average radial growth (mm) at concentration of (%)				
	20	40	60	80	Mean
B.chitinosporus	76.8	50.0	32.2	12.0	32.8
B. coagulans	79.6	56.8	43.0	29.4	52.2
B.pumilus	74.4	47.2	29.0	10.8	40.4
B.subtilis	70.6	35.0	0.0	0.0	26. 3
B.Thuringiensis	66.4	30.2	0.0	0.0	24.2
Control	90.0	90.0	90.0	90.0	90.0
Mean	76.3	61.8	38.8	28.4	

L.S.D. at 5% for: Bacillus strains (B)= 2,3 , Concentration(C)= 1.8 and B x C = 3.1.

Data presented in Table (2) reveal that all the five Bacillus strains caused significant reduction to the radial growth of *F.o.*f.sp.*capsici* compared with control treatment. This reduction was gradually increased by increasing the concentration of the tested bioagents. In addition, the growth of the tested pathogen was completely inhibited by *B.subtilis* and *B.thuringiensis* at the concentration of 60%. Furthermore, *B.thuringiensis* was the most efficient bioagent in this regard followed by *B.subtilis* then *B.pumilus* , being 24.2 , 26.3 and 32.8mm, respectively. Therefore, they were chosen to test their capability to the biocontrol of the tested pathogen under greenhouse conditions. Meanwhile, *B. coagulans* was the lowest efficient one in this regard (52.2 mm).

3.3.Effect of Three Bacterial Bioagents on the Severity of Pepper wilt as Well as some Crop Parameters

Table (3) shows that no symptoms of the disease were observed on the foliage growth and the roots of the plants in case of infestation the soil with the bacterial strains , *i.e. B.pumilus,.B.subtilis* and *B. Thuringiensis* as well as control treatment (uninfested soil). In addition. the three tested bioagents resulted in significant reduction to sweet pepper wilt with significant increase to the plant height as well as the number of pods and their weight / plant when added to the soil infested with the causal pathogen compared with infestation with the causal pathogen only. The symptoms of the disease were obvious both on the foliage growth and the vascular (xylem vesicles), but the severity of the disease was more higher on the xylem vesicles than on the foliage growth, being 10.5 and 13.0 %, respectively. In addition, plants grown in soil infested with Bacillus strains were of high values of plant height and fruit yield (number and weight / plant) than that grow in the control (uninfested soil).

Table 3. *Effect of some bacterial bioagents on the severity of sweet pepper wilt as well as plant height and the produced pod yield / plant , greenhouse experiment.*

Treatments	%Disease severity on		Mean	Plant height(cm)	Average No. of pods / plant	Average weight of pods (g) / plant
	Foliage growth	Xylem vesicles				
B.pumilus (BP)	0.0	0.0	0.0	79.7	38.2	1161.6
B.subtilis (BS)	0.0	0.0	0.0	82.3	39.6	1218.0
B.thuringiensis (BT)	0.0	0.0	0.0	95.9	42.0	1371.1
F.o.capsici (FOC)	63.9	71.8	67.9	36.1	20.0	369.9
BP + FOC	8.6	12.7	10.7	64.0	33.4	918.4
BS + FOC	7.4	11.0	9.2	67.5	33.8	932.2
BT + FOC	4.2	8.5	6.4	71.0	45.0	990.5
Control (Uninfested)	0.0	0.0	0.0	68.8	32.0	900.0
Mean	10.5	13.0	-------	LSD at 5%= 2.2	LSD at 5%=1.8	LSD at 5%= 12.3

LSD at 5 % for: Treatments (T)= 3.0 , Disease severity(D)=2.1 and TxD= 3.9.

3.4. Changes in the Content of Total Phenolic Compounds

Induction of defense-related biochemicals like total phenolic compounds was studied in bacterial- and pathogen-treated sweet pepper plants under different combinations (Table 4). It was noticed that Bacillus strains induced considerable higher production of phenolic compounds compared with control treatment and *F.o.* f.sp. *capsici* . However, low change was observed in pathogenic control whereas no great change was observed in total phenolic contents of untreated control (0.46 mg gallic acid / g plant fresh leaves). A great increase was observed in the total phenolic compounds of plants treated with *B. thuringiensis*, either alone or in combination with the causal pathogen, being 0.60 and 0.66 mg gallic acid / g plant fresh leaves, respectively.

Table 4. *Effect of bacterial- and pathogen-treated sweet pepper plants under different combinations on the content of phenolic compounds, 15 and 30 days after inoculation with the bioagents and the pathogen.*

Treatments	Gallic acid in mg / g plant leaves after (days)			Mean
	0.0	15	30	
B.pumilus (BP)	0.40	0.54	0.56	0.50
B.subtilis (BS)	0.40	0.56	0.59	0.52
B.thuringiensis (BT)	0.40	0.68	0.72	0.60
F.o.capsici (FOC)	0.40	0.48	0.50	0.46
BP + FOC	0.40	0.65	0.68	0.58
BS + FOC	0.40	0.68	0.71	0.60
BT + FOC	0.40	0.78	0.83	0.66
Control (Uninfested)	0.40	0.41	0.42	0.41
Mean	0.40	0.60	0.63	------

4. Discussion

F. oxysporum is a soil-borne in nature and invades vascular system of a plant internally. It is better to protect the entrance point of this fungus in plant instead of changing the entire soil mycoflora. For this purpose, some microorganisms can be used to induce resistance in plants for combating with this devastating pathogen (Akram *et al.,*2013).

The isolates of the fungus *F. oxysporum* were selected and tested for their pathogenicity and chosen the most virulent isolate . The highest infection by the fungus was occurred only on sweet pepper and low infection was found in case of the other four tested plants. Therefore, the fungus *F. oxysporum* has the name of *Fusarium oxysporum* f.sp. *capsici.*. The isolated fungi were previously isolated by Abada(1994).

It has been found that all the five *Bacillus* strains caused significant reduction to the radial growth of *F.o*.f.sp. *capsici* compared with control treatment. This reduction was gradually increased by increasing the concentration of the tested bioagents. In addition, the growth of the tested pathogen was completely inhibited by *B.subtilis* and *B.thuringiensis* at the concentration of 60%. Furthermore, *B.thuringiensis* was the most efficient bioagent in this regard followed by *B.subtilis* then *B.pumilus*, respectively.

The three tested bioagents resulted in significant reduction to sweet pepper wilt with significant increase to plant height as well as the number of pods and their weight compared with control treatment. The symptoms of the disease were obvious both on the foliage growth and the

vasculr, but the severity of the disease was more higher on the xylem vesicles (vasculr) than on the foliage growth, being 10.5 and 13.0 %, respectively. In addition, plants grown in soil infested with Bacillus strains were of high values of plant height and fruit yield (number and weight / plant) than that grown in the control (uninfested soil).

Colonization of plant roots by selected strains of nonpathogenic bacteria, such as various species of the genus *Bacillus* (Kloepper *et al.,* 2004) can induce a distinct broad-spectrum resistance response in both below- and above-ground parts of the plant. This type of resistance to diseases is named as induced systemic resistance (ISR) (van Loon *et al.,*1998; van Loon,2007 and De Vleesschauwer*et al.,*2009). The fungus *Fusarium oxysporum* is one of soil-borne plant pathogens and is widely distributed in various soil types worldwide. Recently, there has been a growing interest in nonpathogenic bacteria due to their efficacy as bioagents in many crops (Kloepper *et al.,* 2004; Yu and Chengxiang , 2011; Akram *et al*., 2013; Zaher *et al.*, 2013 and Abada and Eid, 2014). Application of some Bacillus strains to the seedlings has been found effective for suppressing soil borne diseases and has successfully induced systemic resistance in the treated plants (Kloepper *et al.,* 2004 and Szczech and Shoda, 2007). Elicitation of ISR by Bacillus strains has been demonstrated in greenhouse or field trials on tomato, bell pepper, muskmelon, watermelon, sugar beet, tobacco, *Arabidopsis* sp., cucumber, loblolly pine, and two tropical crops (Kloepper *et al.,* 2004).

Xing*et al.*(2003) mentioned that *Bacillus* sp. grow very fast and occupies the court of infection and preventing pathogen spores to reach susceptible tissues in competition for spaces. This might be due to that treatments with biopreparation induce systemic resistance as the main mechanism of activity on the plant. Also , Jacobsen *et al.*(2004) and Yu *et al.* (2011) reported *that B. subtilis* CAS15 has great potential for plant growth promotion and biological control, where reduced the incidence of Fusarium wilt in pepper significantly, by 12.5–56.9 % due to induced systemic resistance .They added that there were significant increases in plant height also enhanced the yield of pepper by shortening the time to 50 percent flowering to 17.26 days, increasing the average fruit weight 36.92%, and increasing the average yield per plant 49.68%. This research showed that *B. subtilis*CAS15 has great potential for plant growth promotion and biological control.

It is supposed that *Bacillus* spp. could be have diverse plant response involved in synthesis and accumulation of antimicrobial phytoalexins (Hammond-Kosack and Jones, 1996), induction of hypersensitive response (He *et al.*,1993), production of defense-related proteins (Yu,1995) production of activated oxygen species (Baker*et al.,*1993), and modify-cation of plant cell wall by deposition of callose (Veit *et al.*,2001).

Protection of plants from disease by induction of systemic resistance is a new approach. This is much less harmful to the environment as compared to deadly agrochemicals applied to control plant diseases.

Phenolic content is the compounds whose quantity is raised when a plant comes under attack by a pathogen (Van Peer*et al.*,1991 and Waterman and Mole, 1995). Systemic induction of phenolic compounds under influence of bacterial strains was first reported by Van Peer *et al.* (1991). However, this alone is not reliable for indication of disease resistance in plant tissues (Waterman and Mole, 1995). Akram *et al*. (2013) reported that a significant increase in total phenolic contents was observed in bacterial-treated plants. They added that pathogen alone was able to induce phenolic formation in plants but with slightly increased levels. It was noticed that Bacillus strains induced considerable higher production of phenolic compounds compared with control treatment and *F.o.*f.sp. *capsici*. However, low change was observed in pathogenic control, whereas no great change was observed in total phenolic contents of untreated control. On the other hand, a great increase was observed in the total phenolic compounds of plants treated with *B. thuringiensis*, either alone or in combination with the causal pathogen.

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