

# Improvement of Biocontrol of Damping-off and Root Rot/Wilt of Faba Bean by Salicylic Acid and Hydrogen Peroxide

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**Abstract** *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, and *Macrophomina phaseolina* were found to be associated with root rot and wilt symptoms of faba bean plants collected from different fieldes in New Valley governorate, Egypt. All the obtained isolates were able to attack faba bean plants (cv. Giza 40) causing damping-off and root rot/wilt diseases. *R. solani* isolates 2 and 5, *F. solani* isolate 8, *F. oxysporum* isolate 12 and *M. phaseolina* isolate 14 were the more virulent ones in the pathogenicity tests. Biocontrol agents (*Trichoderma viride* and *Bacillus megaterium*) and chemical inducers (salicylic acid [SA] and hydrogen peroxide) individually or in combination were examined for biological control of damping-off and root rot/wilt and growth promoting of faba bean plants *in vitro* and *in vivo*. Both antagonistic biocontrol agents and chemical inducers either individually or in combination inhibited growth of the tested pathogenic fungi. Biocontrol agents combined with chemical inducers recorded the highest inhibited growth especially in case SA + *T. viride* and SA + *B. megaterium*. Under green house and field conditions, all treatments significantly reduced damping-off and root rot/wilt severity and increased of survival plants. Also, these treatments increased fresh and weights of the survival plants in pots compared with control. The combination between biocontrol agents and chemical inducers were more effective than used of them individually and SA + *T. viride* was the best treatment in this respect. Also, under field conditions, all these treatments significantly increased growth parameters (plant height and number of branches per plant) and yield components (number of pods per plant and number of seeds per plant, weight of 100 seeds and total yield per feddan) and protein content in both seasons (2010~2011 and 2011~2012). Faba bean seeds soaked in SA + *T. viride* and SA + *B. megaterium* were recorded the highest growth parameters and yield components. Generally, the combination between biocontrol agents and chemical inducers recorded the best results for controlling damping-off and root rot/wilt diseases in greenhouse and field with addition improved plant growth and increased yield components in field.

**Keywords** Biological control, Faba bean, Growth and yield parameters, Hydrogen peroxide, Root rot and wilt, Salicylic acid

Faba bean (*Vicia faba* L.) is used as an important human food in developing countries and as an animal feed, mainly for pigs, horses, poultry and pigeons in industrialized countries. Feeding value of faba bean is high and this legume has been considered as a meat extender or substitute due to its high protein content (20~41%) [1]. Root rot and

wilt diseases caused by several soil borne fungal pathogens are wide spread and serious in many crops cultivated in different soil types. Faba bean is subjected to attack by many pathogenic organisms wherever the crop is grown. Several root rot and wilt pathogens such as *Rhizoctonia solani*, *Fusarium solani*, and *Macrophomina phaseolina* are reported to attack faba bean roots and stem base causing serious losses in seed germination and plant stand as well [2].

An investigation for controlling such diseases is considered important, especially in view of their wide prevalence in Egypt. Several attempts to control root rot and wilt diseases could be accepted. However, fungicides are considered one of several factors involving in environmental pollution, in spite of their satisfactory results in the control of plant diseases. In addition, control of disease with fungicides has proven very difficult, and almost all fungicides are effective only at phytotoxic levels [3]. Recently, the growing concern over the use of pesticides to human health and environment has brought increasing interest in the use of alternatives characterized with negative impact on the environment. Therefore, present activity focuses on finding compounds

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that are safe to human and environment. An alternative to fungicidal application, it may be possible to utilize a scheme of inducible plant defenses which provides protection against a broad spectrum of disease-causing organisms. Among synthetic inducers, salicylic acid (SA) and hydrogen peroxide ( $H_2O_2$ ) have been found to be active as antimicrobial agents in various trials as disease resistance inducers. These have been reported for inducing resistance against several plant pathogens, i.e., *F. oxysporum* in tomato [4], *R. solani* in faba bean and potato [5], *F. solani* in lentil [6], and *M. phaseolina* in water melon [7]. On the other hand, the application of biological control using antagonistic microorganisms proved to be successful for controlling various plant diseases in many countries. Biological control is proposed to be an effective and non-hazardous strategy to reduce crop damage caused by plant pathogens. In recent years the *Bacillus megaterium* and *Trichoderma viride* have been extensively used for plant growth promotion and disease control [8]. Biological control of soil borne pathogens is often attributed to improved nutrition that boosts host defenses or to direct inhibition of pathogen growth and activity. Amendment with certain abiotic factors (inducers) appears to stimulate the disease resistance by indirectly stimulating indigenous populations of microorganism that are beneficial to plant growth and antagonistic to pathogens. For example chitin amendment of soil has been found to stimulate the growth of chitinolytic microorganisms [9], increase the biocontrol activity and stimulate the expression of plant defense proteins [10]. All these effects may culminate in enhancing plant protection. Similarly, SA and  $H_2O_2$  amendment was tested in combination with biocontrol agents. Saikia *et al.* [11] tested the efficiency of *Pseudomonas fluorescence* with or without SA amendment in chickpea against *Fusarium* wilt infection. The application of *P. fluorescence* (pf4-92) with SA recorded highest protection of chickpea seedlings against wilting.

Thus, the aim of this study is to 1) screen biocontrol agents and chemical inducers individually or in combination a capable of inhibiting the growth of *R. solani*, *F. solani*, *F. oxysporum*, and *M. phaseolina*; 2) determine the biocontrol efficiency of *B. megaterium* and *T. viride*, alone and in combination with chemical inducers ( $H_2O_2$  and SA) amendment against damping-off and root rot/wilt diseases of faba bean; and 3) determine the effect of *B. megaterium* and *T. viride*, alone and in combination with chemical inducers ( $H_2O_2$  and SA) amendment on promoting growth and yield components of faba bean in field.

## MATERIALS AND METHODS

**Source of faba bean seeds and growth of plants.** Faba bean (*V. fabae* L.) cultivar Giza 40 used in this study, was obtained from Legume Crop Research Department, Field Crop Res. Inst., Agric. Res. Center, Ministry of Agriculture, Egypt. Seeds were planted in plastic pots 30 cm diameter (2.4 kg soil), filled with a pasteurized mixture of soil and sand (4:1 w/w). Six seeds were sown in each pot and these

pots were irrigated every three days.

**Isolation, purification and identification of the causal organism(s):** Samples of faba bean plants showing root rot and wilt symptoms were collected from different farms located in New Valley governorate. The infected roots were thoroughly washed with running tap water, cut into small fragments, superficially sterilized with sodium hypochlorite (5%) for 2 min, washed several times with sterile distilled water and dried between sterilized filter paper. The sterilized pieces were transferred into potato dextrose agar (PDA) medium supplemented with penicillin (20 Iu/mL) and incubated at  $25 \pm 1^\circ C$ , then examined daily for fungal growth. The fungal colonies were purified using single spore or hyphal tip techniques suggested by Booth [12] and Dhingra and Sinclair [13] and then identified according to their morphological and microscopical characters as described by Booth [12] and Barnett and Hunter [14]. Identification was confirmed by Assiut University Mycological Centre (AUMC), Assiut University, Assiut, Egypt. The obtained isolates were maintained on PDA slants and kept in refrigerator at  $5^\circ C$  for further study.

**Pathogenicity tests:** The pathogenicity tests of the obtained fungal isolates was carried out at New Valley Agric. Res. Station, on Giza 40 faba bean cultivar in pots containing soil infested with the obtained isolates individually using homogenized culture technique according to Muthomi *et al.* [15].

**Preparation of the fungal inocula:** The inocula of the obtained isolates were prepared from one week old culture grown on 50 mL potato dextrose broth (PDB) medium in conical flask (250 mL) and incubated at  $25 \pm 1^\circ C$ . The content of flask were homogenized in a blender for one min. Plastic pots were filled with sterilized soil and mixing with fungal inocula at rate 100 mL homogenized culture per pot, seven days before planting. Five pots were used as replicates per isolate and another 5 pots with equal amount of sterile PDB medium without fungal inoculation were severed as control. Five sterilized surface seeds were sown in each pot. Percentage of damping-off was recorded 30 days after planting, respectively. While severity of root rot/wilt was determined after 90 days according to Abdou *et al.* [16] using a rating scale of 0 to 5 on the basis of root discoloration or leaf yellowing as follows: 0, neither root discoloration nor 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 more than one leaf wilted; and 5, completely dead plants. For each replicate a disease severity index (DSI) similar to that one described by Liu *et al.* [17] was calculated as follows:

$$DSI = \frac{\sum d}{d \max \times n} \times 100$$

, where d is the disease rating possible, d max is the maximum disease rating and n is the total number of

plants examined in each replicate.

**In vitro studies.** The used antagonistic organisms were *T. viride* isolate TVM2 and *B. megaterium* isolate BSM5. These isolates provided by Dr. Montaser, F. Abdel-Monaim Plant Pathol. Res. Inst., Agric. Res. Center [8]. While, chemical inducers used were SA and hydrogen peroxide ( $H_2O_2$ ) at concentration 4 mM and 1 mM, respectively. The effect of biocontrol agents and chemical inducers individually and/or in combination on faba bean pathogenic fungi was studied as follows.

**Efficacy of antagonistic biocontrol agents:** *Trichoderma viride* and the tested pathogenic fungi (*R. solani*, *F. solani*, and *M. phaseolina*) were cultured on PDA medium for 7 days at  $25 \pm 1^\circ C$ . then a disc (0.7 cm diameter) of the antagonistic fungal colony was cut and placed opposite to the colony of the pathogenic fungal isolates on PDA medium. On the other hand, *B. megaterium* isolate was streaked at opposite ends of PDA plates near edge and incubated at  $25 \pm 1^\circ C$  for 24 hr, then a mycelial disc (0.7 cm) of the tested fungi was placed in the center of each plate.

**Efficacy of chemical inducers:** The effect of chemical inducers on the growth of pathogenic isolates was evaluated in PDA medium. Twenty mL of PDA medium containing 4 mM SA, 1 mM  $H_2O_2$  was poured in the plates and inoculated with the pathogenic fungi.

**Efficacy of combination between biocontrol agents and chemical inducers:** Flasks (250 mL) containing 200 mL PDA medium were amended with 4 mM SA and 1 mM  $H_2O_2$  individually, then each flask was poured in 10 plates. These plates were inoculated with antagonistic isolates and pathogenic isolates as before.

For control treatment, the agar plug of only pathogen isolates was placed on PDA plates. The inoculated plates incubated at  $25 \pm 1^\circ C$  until colony of control grew to full plate. At this point, colony diameter was measured using ruler. Percentage of growth inhibition of pathogen was calculated using the formula below:

$$\text{Inhibition (\%)} = (A - B)/A \times 100$$

, where,

A = Colony diameter of pathogen in control,

B = Colony diameter in treated plates.

### In vivo studies.

**Greenhouse experiments:** The effects of biocontrol agents (*T. viride* and *B. megaterium*) and chemical inducers (SA and  $H_2O_2$ ) individually or in combination against faba bean damping-off and root rot diseases incited by *R. solani*, *F. solani* and *M. phaseolina* were evaluated under greenhouse conditions. *Trichoderma viride* grown on PDA at  $25 \pm 1^\circ C$  for 7 days was prepared in sterilized water to  $10^6$  cfu/mL. Cell suspension of *B. megaterium* grown on nutrient broth medium for 3 days at  $25 \pm 1^\circ C$  were adjusted to  $2.5 \times 10^8$  cfu/mL. SA and  $H_2O_2$  were prepared as solutions (water or biotic suspension) at 4 mM and 1 mM,

respectively. The in combination between biocontrol agents and chemical inducers prepared with dissolving chemical inducers in suspension of biocontrol agents.

Faba bean seeds soaked for 6 hr in the following treatments: 1, SA (4 mM); 2,  $H_2O_2$  (1 mM); 3, *T. viride* ( $10^6$  cfu/mL); 4, *B. megaterium* ( $2.5 \times 10^8$  cfu/mL); 5, SA + *T. viride*; 6, SA + *B. megaterium*; 7,  $H_2O_2$  + *T. viride*; 8,  $H_2O_2$  + *B. megaterium*; and 9, control. Plastic pots were filled with sterilized soil and mixed with fungal inocula at rate 100 mL homogenized culture per pot, seven days before planting, then sown by 6 seeds of each treatment. Five replicates were used for each treatment. In control treatment, faba bean seeds soaked in water for 6 hr. and sown the same rate. Also, treated and untreated seeds were sown in pots containing uninfested soil to study the effect of these treatments on plant growth. Pots were irrigated as needed.

All pots were examined after 30 and 90 days to record the percentage of damping-off and root rot/wilt severity, respectively. Also, the end of experiment, the survival plants were weighted to record fresh weight per plant then dried at  $80^\circ C$  for 24 hr. to record the dry weight per plant.

**Field experiments:** Field experiment was carried out at New Valley Res. Station Farm during 2009~2010 and 2010~2011 growing seasons, to evaluate the efficiency of the tested biocontrol agents (*T. viride* and *B. megaterium*) and chemical inducers (SA and  $H_2O_2$ ) individually or in combination for controlling damping-off and root rot diseases and its effect on growth and yield parameters under field conditions. The experimental design was a complete randomized block with three replicates. The experimental unit area was  $10.5 \text{ m}^2$  ( $3.5 \times 3 \text{ m}$ ). Each unit included 5 rows; each row was 3.5 m in length and 60 cm width. Faba bean seeds (cv. Giza 40) were soaked in treatments described above for 6 hr. The seeds treated were sown in hills 25 cm apart on both sides of 6 cm ridges in both seasons, 2 seed per hill. In control treatment, faba bean seeds were soaked in water for 6 hr and sown the same rate. The normal cultural practices of growing faba bean were followed.

Percentages of damping-off and root rot/wilt severity were recorded 30 and 90 days after sowing. At harvest, plant height (cm), number of branches/plant, number of pods/plant, and number of seeds/plant, 100-seed weight and total yield (kg/feddan) were measured. Protein percentage content in seeds was recorded using the method of Jackson [18].

**Statistical analysis.** All experiments were performed twice. Analyses of variance were carried out using MSTAT-C, 1991 program ver. 2.10 [19]. Least significant difference was employed to test for significant difference between treatments at  $p \leq 0.05$  [20].

## RESULTS

### Isolation and identification of the causal organisms.

Twenty isolates of different soil-borne fungi listed in Table 1, were isolated from wilted and rotten roots of faba bean

**Table 1.** Pathogenicity of fungi isolated from root of faba bean diseased plants

Isolates No.	Damping-off (%)	Root rot/wilt (%)	Survival plants (%)
<i>Rhizoctonia solani</i>			
1	18 j	25.33 f	56.67 e
2	60 a	35.67 d	4.33 n
3	52 c	14.33 h	33.67 i
4	44 d	12.69 hi	43.31 g
5	44 d	39.67 c	16.33 l
<i>Fusarium solani</i>			
6	24 i	12.97 hi	63.03 d
7	28 h	20.33 g	51.67 f
8	56 b	33.6 d	10.4 m
9	52 c	20.14 g	27.86 j
<i>F. oxysporum</i>			
10	32 g	10.00 i	58 e
11	40 e	35.00 d	25 j
12	36 f	50.67 a	13.3 lm
13	28 h	44.33 b	27.67 j
<i>Macrophomina phaseolina</i>			
14	50 c	28.67 e	21.33 k
15	28 h	12.67 hi	59.33 e
16	40 e	22.54 fg	37.46 h
17	20 j	12.56 hi	67.44 c
Other fungi			
18	8 l	2.59 j	89.41 a
19	4 m	5.64 j	90.36 a
20	12 k	5.28 j	82.72 b

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $p \leq 0.05$ ).

plants, showed root rot and wilt symptoms, cultivated in different fields in New Valley governorate. These isolates were identified as *R. solani* (5 isolates), *F. solani* (4 isolates), *F. oxysporum* (4 isolates), *M. phaseolina* (4 isolates) as well as 3 isolates not identified.

**Pathogenicity tests.** The obtained isolates were tested on faba bean cv. 40 under green house conditions in pots.

Results presented in Table 1 reveal that all tested isolates could infect the roots of faba bean causing damping-off, root rot and wilt symptoms thus reduce the survived plants. The highest damping-off infection (60%) was recorded by *R. solani* (isolate 2) followed by *F. solani* isolate 8 (56%) then *R. solani* isolate 3 and *F. solani* isolate 9, where recorded 52% damping-off. On the other hand, all tested isolates caused root rot/wilt ranging between 2.59~50.67%. *Fusarium oxysporum* isolates 12 and 13 followed by *R. solani* isolate 5 caused the highest root rot/wilt severity, whereas recorded 50.67%, 44.33%, and 39.67%, respectively.

It is concluded from results that the isolates *R. solani* isolates 2 and 5, *F. solani* isolate 8, *F. oxysporum* isolate 12 and *M. phaseolina* isolate 14 were the more virulent ones whereas recorded the lowest survival plants (4.33%, 16.33%, 10.4% 13.33%, and 21.33%, respectively).

As mentioned before in the pathogenicity test experiments, the highly pathogenic fungal isolates obtained from faba bean roots, i.e., *R. solani* (2), *F. solani* (8), *F. oxysporum* (12), and *M. phaseolina* (14) were chosen to complete the further studies based on their pathogenic abilities.

#### Effect of antagonists and chemical inducers on fungal linear growth of tested pathogens.

Data in Table 2 reveal that the tested biocontrol agents and chemical inducers either individually or combination have significantly reduced linear growth of all tested fungi. In general, the combination between biocontrol agents and chemical inducers were more effective than used any of them individually. SA + *T. viride* and SA + *B. megaterium* were the most effective where they recorded the highest percentages of reduction in all the tested pathogenic fungi. However, SA followed by  $H_2O_2$  recorded the lowest reduction of growth in all tested fungi. Generally, biocontrol agents were able to reduce linear growth of the tested pathogenic fungi more than chemical inducers.

#### Effect of biocontrol agents and chemical inducers on damping-off and root rot/wilt, fresh and dry weights under greenhouse conditions.

**Diseases incidence:** Data presented in Table 3 show that

**Table 2.** Effect of biocontrol agents and chemical inducers individually or combination on growth of pathogenic tested fungi *in vitro*

Treatments	Inhibition of linear growth (%)			
	<i>Rhizoctonia solani</i>	<i>Fusarium solani</i>	<i>F. oxysporum</i>	<i>Macrophomina phaseolina</i>
<i>Trichoderma viride</i>	38.47 d	55.58 c	44.69 d	60.14 b
<i>Bacillus megaterium</i>	32.33 e	49.22 d	40.23 e	46.20 d
Salicylic acid (SA)	8.87 f	13.56 e	10.55 g	11.58 f
Hydrogen peroxide ( $H_2O_2$ )	11.36 f	16.25 e	14.00 f	15.58 e
SA + <i>T. viride</i>	57.00 a	70.54 a	62.35 a	64.30 a
SA + <i>B. megaterium</i>	46.41 b	63.25 b	58.65 b	56.36 c
$H_2O_2$ + <i>T. viride</i>	42.24 c	59.25 bc	52.31 c	56.14 c
$H_2O_2$ + <i>B. megaterium</i>	40.14 cd	57.47 c	44.96 d	53.34 c

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $p \leq 0.05$ ).

**Table 3.** Effect of seed soaking in biocontrol agents and chemical inducers individually or combination for controlling damping-off, root rot and survival plants under greenhouse conditions

Treatments	<i>R. solani</i>				<i>F. solani</i>				<i>F. oxysporum</i>				<i>M. phaseolina</i>			
	% Damping-off	% Root rot	% Survival plants	% Damping-off	% Root rot	% Survival plants	% Damping-off	% Root rot	% Survival plants	% Damping-off	% Root rot	% Survival plants	% Damping-off	% Root rot	% Survival plants	
<i>Trichoderma viride</i>	24 c	6.59 de	69.41 c	24 b	8.67 bc	67.33 e	2 b0	18.23 b	61.77 e	12 c	8.67 bc	79.33 cd				
<i>Bacillus megaterium</i>	28 b	11.7 b	60.3 d	20 c	10.23 b	69.77 e	2 b0	20.23 b	59.77 e	1 b6	10.33 b	73.67 d				
Salicylic acid (SA)	16 g	7.67 g	76.33 f	12 h	6.47 f	81.53 g	12 f	12.36 f	75.64 g	8 f	6.67 e	85.33 f				
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	20 d	8.9 c	71.1 c	16 d	7.25 cd	76.75 d	16 c	15.56 c	68.44 d	8 d	10.33 b	81.67 bc				
SA + <i>T. viride</i>	8 f	4.33 f	87.67 a	4 g	3.6 e	92.4 a	4 f	6.36 e	89.64 a	e4	5.6 d	90.4 a				
SA + <i>B. megaterium</i>	12 e	5 ef	83 ab	8 f	5.67 d	86.33 b	8 e	8.23 de	83.77 b	6 de	6.33 d	87.67 ab				
H <sub>2</sub> O <sub>2</sub> + <i>T. viride</i>	12 e	6.58 de	81.42 b	12 e	6.00 d	82.00 c	12 d	9.36 d	78.64 c	6 de	6.33 d	87.67 ab				
H <sub>2</sub> O <sub>2</sub> + <i>B. megaterium</i>	12 e	6.89 d	81.11 b	8 f	6.00 d	86.00 b	8 e	10.25 d	81.75 bc	6 de	8.33 c	85.67 abc				
Control	60 a	26.54 a	13.46 e	52 a	32.33 a	15.67 f	32 a	50.25 a	17.75 f	48 a	33.52 a	18.48 e				

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $p \leq 0.05$ ).

**Table 4.** Effect of seed soaking in biocontrol agents and chemical inducers individually and/or combination on fresh and dry weight (gm plant<sup>-1</sup>) of root and shoot healthy and infected faba bean plants

Treatments	Uninfected				Infected with			
	<i>R. solani</i>		<i>F. solani</i>		<i>F. oxysporum</i>		<i>M. phaseolina</i>	
	FW	DW	FW	DW	FW	DW	FW	DW
<i>Trichoderma viride</i>	20.66 cd	4.38 c	17.59 bc	3.58 bc	16.85 cde	3.96 bcd	14.36 cd	3.37 bc
<i>Bacillus megaterium</i>	20.01 cd	4.22 c	15.99 cd	3.24 cd	15.21 de	3.47 de	13.89 de	3.21 c
Salicylic acid (SA)	18.66 f	3.94 f	14.84 f	3.01 f	12.28 g	2.69 g	10.55 g	2.36 e
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	17.30 de	3.55 d	13.63 d	2.71 d	14.21 e	3.16 e	11.66 e	3.11 c
SA + <i>T. viride</i>	26.90 a	5.43 a	21.2 a	4.14 a	21.12 a	4.72 a	18.36 a	3.96 a
SA + <i>B. megaterium</i>	24.62 ab	5.24 a	20.17 ab	3.84 ab	20.02 ab	4.45 ab	17.45 ab	3.85 ab
H <sub>2</sub> O <sub>2</sub> + <i>T. viride</i>	22.54 bc	4.73 b	18.43 abc	3.53 bc	19.03 abc	4.15 bc	16.56 abc	3.58 abc
H <sub>2</sub> O <sub>2</sub> + <i>B. megaterium</i>	21.91 bc	4.53 bc	16.85 c	3.21 cd	17.53 bcd	3.82 cd	15.42 bcd	3.37 bc
Control	15.27 e	3.05 e	9.32 e	1.77 e	8.08 f	1.72 f	6.45 f	1.38 d

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $p \leq 0.05$ ).

FW, Fresh weight; FD, Dry weight.

all treatments could highly significantly reduced damping-off and root rot/wilt severity and increased survival plants of faba bean which have been artificially infested with tested pathogens (*R. solani*, *F. solani*, *F. oxysporum*, and *M. phaseolina*), compared to untreated control treatment. Soaking seeds in biocontrol agents combined with chemical inducers reduced damping-off and root rot/wilt severity more than used any of them individually. Chemical inducers were more effective to reduce damping-off and root rot/wilt than biocontrol agents.

On the other hand, the combination of SA and *T. viride* recorded the highest protection against to any of the tested fungi followed by SA + *B. megaterium* while seed soaked in *B. megaterium* or *T. viride* recorded the lowest ones.

**Fresh and dry weight:** In the present study, biocontrol agents (*T. viride* and *B. megaterium*) and chemical inducers (SA and H<sub>2</sub>O<sub>2</sub>) individually or combination recorded significant highly increased fresh and dry weights either in presence or absence of pathogenic fungi (Table 4). Biocontrol agents combined with chemical inducers were increased fresh and dry weights more than used any of them individually. Also, biocontrol agents gave fresh and dry weights higher than usage chemical inducers. Generally, combined between SA and *T. viride* recorded the highest fresh and dry weights of survival plants either in uninfested or infested soil with pathogenic fungi followed by SA + *B. megaterium*. On other hand, H<sub>2</sub>O<sub>2</sub> was recorded the lowest fresh and dry weights in this respect.

**Effect of biocontrol agents and chemical inducers on disease incidence under field conditions.** The efficacy of chemical inducers and biocontrol agents individually and/or combination as seed soaking against the incidence of damping-off and root rot/wilt diseases of faba bean, were evaluated under field conditions. Data in Table 5 clearly demonstrate that all treatments significantly reduced damping-off and root rot/wilt severity compared with the control. Faba bean seed soaked in biocontrol agents

together chemical inducers were more effective than using either alone. Also, the obtained data show that seed soaked in any chemical inducers (SA or H<sub>2</sub>O<sub>2</sub>) was more effective to reduce damping-off and root rot/wilt than any biocontrol agents. SA + *T. viride* recorded the highest reduction of damping-off and root rot/wilt in both seasons, whereas recorded 92% and 93.33% survival plants compared with 52.27% and 59% in control plants in both seasons, respectively. In the contrary, faba bean seeds soaked in *B. megaterium* or *T. viride* were recorded the lowest ones in both seasons (72%, 75% survival plants in first seasons and 76.34%, 78.50% in second season, respectively).

**Effect of biocontrol agents and chemical inducers on growth parameters, yield components and protein content under field conditions.**

The effect of faba bean seed soaked in biocontrol agents (*T. viride* and *B. megaterium*), chemical inducers (SA and H<sub>2</sub>O<sub>2</sub>) on growth parameters and yield components under field conditions during seasons 2010~2011 and 2011~2012 were studied. Data in Table 6 indicate that seed the combination between and biocontrol agents and chemical inducers significantly improvement faba bean growth and branching more than used individually. Also, seed soaked in any of biological agents were more effective in this respect than used any chemical inducers. The combination between SA and *T. viride* were the most effective treatments; they recorded the highest plant height (122.6 and 132.4 cm) and number of branches (4.1 and 4.2 branch/plant) in both seasons, respectively. Also, this treatment recorded the highest yield components, i.e., number of pods (19.3 and 20.9 pods/plant), number of seeds (47.3 and 53.2 seeds/plant), weight of 100 seeds (83.9 and 85.8 g) and total seed yield (1,851 and 1,986 kg/feddan) in both seasons, respectively. On the contrary, faba bean seed soaked in H<sub>2</sub>O<sub>2</sub> was less effective in both seasons compared the other treatments.

On the other hand, the obtained data show that all treatments significantly increased protein content in seeds

**Table 5.** Effect of seed soaking in biocontrol agents and chemical inducers individually and/or combination on controlling damping-off, root rot/wilt, and survival plants under field conditions during growing seasons 2010~2011 and 2011~2012

Treatments	Season 2010~2011			Season 2011~2012		
	Damping-off	Root rot	Survival plants	Damping-off	Root rot	Survival plants
<i>Trichoderma viride</i>	13.33 c	10.33 b	76.34 ef	12.00 c	9.50 b	78.50 ab
<i>Bacillus megaterium</i>	18.00 b	10.00 bc	72.00 f	15.67 b	9.33 b	75.00 bc
Salicylic acid (SA)	9.67 d	6.67 de	83.66 cd	8.00 de	7.90 bc	84.10 ab
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	10.67 cd	7.96 cd	81.37 de	10.00 cd	6.33 cd	83.67 ab
SA + <i>T. viride</i>	4.00 e	4.00 f	92.00 a	3.67 g	3.00 e	93.33 a
SA + <i>B. megaterium</i>	5.67 e	5.26 ef	89.07 ab	5.00 fg	4.84 d	90.16 ab
H <sub>2</sub> O <sub>2</sub> + <i>T. viride</i>	7.33 de	4.33 f	88.34 abc	6.33 ef	5.00 d	88.67 ab
H <sub>2</sub> O <sub>2</sub> + <i>B. megaterium</i>	9.67 d	5.50 ef	84.83 bcd	8.00 de	5.30 d	86.70 ab
Control	32.33 a	15.40 a	52.27 g	28.00 a	13.00 a	59.00 c

Values are presented as percentage.

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $p \leq 0.05$ ).

**Table 6.** Effect of seed soaking in salicylic acid (SA), hydrogen peroxide, *Trichoderma viride*, *Bacillus subtilis* individually and/or combination on growth and yield parameters under field condition during growing seasons 2010~2011 and 2011~2012

	Plant height (cm)	No. of branches/ plant	No. of pods/plant	No. of seeds/plant	Weight of 100 seeds	Total yield/feddan	Protein (%)
Season 2010~2011							
<i>Trichoderma viride</i>	110.3 cd	3.2 cd	16.4 bc	41.5 cd	80.9 a	1551.4 bcd	28.3 abc
<i>Bacillus megaterium</i>	104.0 de	3.0 d	14.5 d	37.5 ef	81.2 a	1453.0 de	27.9 bc
Salicylic acid (SA)	98.5 e	3.3 cd	15.0 cd	36.4 f	81.6 a	1531.5 cd	27.7 bc
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	89.2 f	2.9 d	13.6 de	34.8 f	79.9 a	1393.3 e	26.8 c
SA + <i>T. viride</i>	126.0 a	4.1 a	19.3 a	47.3 a	83.9 a	1851.0 a	30.8 a
SA + <i>B. megaterium</i>	119.6 b	3.9 ab	17.4 b	43.2 bc	82.5 a	1633.0 bc	29.7 ab
H <sub>2</sub> O <sub>2</sub> + <i>T. viride</i>	115.3 bc	3.9 ab	17.3 b	44.9 ab	82.9 a	1662.5 b	28.1 abc
H <sub>2</sub> O <sub>2</sub> + <i>B. megaterium</i>	109.5 cd	3.6 bc	16.5 b	39.2 de	82.1 a	1547.0 bcd	28.0 bc
Control	70.3 g	2.3 e	12.5 e	26.9 g	72.4 b	1060.0 f	26.1 c
Season 2011~2012							
<i>Trichoderma viride</i>	117.2 cd	3.2 c	17.0 bc	43.2 cd	82.1 a	1644.5 cd	28.6 bc
<i>Bacillus megaterium</i>	111.5 de	3.2 c	15.3 cd	38.7 ef	83.6 a	1537.2 de	27.4 cd
Salicylic acid (SA)	103.5 f	3.4 c	17.3 bc	41.3 de	84.4 a	1604.0 d	28.3 bc
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	89.3 g	2.8 d	15.8 c	36.5 f	82.6 a	1475.8 e	27.4 cd
SA + <i>T. viride</i>	132.4 a	4.2 a	20.9 a	53.2 a	85.8 a	1986.0 a	31.0 a
SA + <i>B. megaterium</i>	126.3 b	3.9 ab	18.8 ab	47.3 b	84.8 a	1758.7 b	30.1 ab
H <sub>2</sub> O <sub>2</sub> + <i>T. viride</i>	118.5 c	4.1 ab	18.6 b	46.2 bc	84.2 a	1729.0 bc	28.7 abc
H <sub>2</sub> O <sub>2</sub> + <i>B. megaterium</i>	110.4 e	3.8 b	17.3 bc	39.9 def	84.8 a	1552.5 de	27.8 bc
Control	73.5 h	2.4 e	13.5 d	32.1 g	75.6 b	1202.8 f	25.2 d

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $p \leq 0.05$ ).

compared with untreated control. Also, SA + *T. viride* was recorded the highest protein content (30.8% and 31%) followed by SA + *B. megaterium* (29.7% and 30.1%) in both seasons, respectively. While, H<sub>2</sub>O<sub>2</sub> individually was recorded the lowest increased in this respect.

## DISCUSSION

Faba bean (*Vicia faba* L.) is one of the most important legume crops. It is infected with many fungal pathogens causing considerable yield losses where damping-off, root rot, wilt diseases are the most important fungal diseases affecting faba bean production in Egypt [2].

Sum of 20 isolates of different soil-borne fungi were isolated from roots of faba bean plants showed root rot and wilt symptoms collected from different fields growing in New Valley governorate. These isolates were identified as *R. solani*, *F. solani*, *F. oxysporum* and *M. phaseolina*. Pathogenicity test of the obtained isolates revealed that all tested isolates could infect the roots of faba bean (cv. Giza 40) causing damping-off, root rot/wilt and reduced survived plants. *R. solani* isolates 2 and 5, *F. solani* isolate 8, *F. oxysporum* isolate 12 and *M. phaseolina* isolate 14 were the more virulent ones. These results are similar to those obtained by Metwally [5] and Abdel-Kader *et al.* [2].

Sustainable farming systems strive to minimize the use of synthetic pesticides and to optimize the use of alternative management strategies to control soil-borne pathogens [21]. Antagonistic fungi and bacteria in plant root zone are

a key agent of change in soil agroecosystems. Interactions between plant root systems and rhizobacteria have a profound effect on crop health, yield and soil quality. Root zone antagonistic fungi and bacteria are able to generate a wide array of secondary metabolites which can have a positive influence on plant growth; enhancing the availability of minerals nutrients, improving nitrogen fixation ability, decreasing susceptibility to frost damage, improving plant health through the biocontrol of phytopathogens, inducing systemic plant disease resistance and facilitating plant establishment, growth and development [22]. Also, acquired resistance by using abiotic-agents as inducers seems to be one of alternatives to substitute for, or at least to decrease the use of fungicides in plant disease control. Excessive and improper use of pesticides including fungicides presents a menace to the health of human, animal and environment [23]. Plants respond to chemical elicitor treatments by activating a wide variety of protective mechanisms designed to prevent pathogen replication and spreading [24]. The defense mechanisms include the fast production of reactive oxygen species [25]; alterations in the cell wall constitution; accumulation of antimicrobial secondary metabolites known as phytoalexins [26]; activation and/or synthesis of defense peptides and proteins [27]. In various plant species, resistance can be induced with elicitors such as SA and H<sub>2</sub>O<sub>2</sub> against a wide range of pathogens [4, 8].

In the present study, it was planning to investigate the possibility of minimizing the infection with damping-off, root rot and wilt diseases of faba bean using biocontrol

agents (*T. viride* and *B. megaterium*) and chemical inducers (SA and H<sub>2</sub>O<sub>2</sub>) individually and/or in combinations as resistance inducer.

The obtained data *in vitro* revealed that both biocontrol agents and chemical inducers individually and combination caused significant reduction growth of all tested pathogenic fungi. The combination between biocontrol agents and chemical inducers were more inhibited growth than used any of them individually especially in case of SA + *T. viride* and SA + *B. megaterium* on the other hand, all treatments caused significant reduction to both damping-off and root rot/wilt diseases and increased the survival plants ether in pots or field experiments, compared with the control treatments. The combination between biocontrol agents and chemical inducers were more effective to reduce damping-off, root rot/wilt severity and increased fresh and weights of survival plants than used of them individually. SA + *T. viride* and SA + *B. megaterium* were recorded the best results in this respect. These results are in agreement with those reported by several rehearses [5, 8]. Saikia *et al.* [11] tested the efficiency of *P. fluorescence* with or without SA amendment in chickpea against *Fusarium* wilt infection. The application of *P. fluorescence* (pf4-92) with SA recorded highest protection of chickpea seedlings against wilting. Rajkumar *at al.* [28] found that pepper seeds treated with inducers (SA and chitin) alone showed a moderate degree of plant protection against *R. solani*. However, the reduction in disease was more pronounced when inducers were applied with fluorescent pseudomonades (SE21 and RD41) Amendment with chitin alone enhanced biocontrol efficiency of both SE21 and RD41. However, amendment with SA alone or in conjunction with chitin showed a moderate effect on biocontrol efficiency of the antagonists.

Seed soaking application of biocontrol agents and chemical inducers individually or combination in both seasons showed a significant increase in faba bean growth parameters, yield components and protein content in seeds. The combination between biocontrol agents and chemical inducers were recorded highly increased in all growth and yield parameters more than in used of them individually, especially SA + *T. viride* and SA + *B. megaterium*. These increases may be attributed to biotic and abiotic elicitors effect on physiological processes in plant such as ion uptake, cell elongation, cell division, enzymatic activation and protein synthesis. In this concern, low SA and H<sub>2</sub>O<sub>2</sub> doses enhanced growth and yield components of tomato [4] and alfalfa [29]. Also, *B. megaterium* and *T. viride* increased plant growth and yield components in many plant species such as chickpea [8], dry bean [30], and alfalfa [29]. Also, these results show that the biocontrol efficiency of antagonists *T. viride* and *B. megaterium* may be stimulated by SA and H<sub>2</sub>O<sub>2</sub> resulting in a significant increase in their population density and antagonistic effect against tested pathogens.

In conclusion, soaking faba bean seeds in biocontrol agent and chemical inducers individually or in combination

significantly reduced damping-off, root rot and increased survival plants either under green house or field conditions. Also, these treatments increased plant growth, yield components and protein content in seeds in field during both growing seasons (2010~2011 and 2011~2012). The combination between biocontrol agents and chemical inducers were better than used of them individually especially SA + *T. viride* and SA + *B. megaterium*.

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