

Management of Damping-Off and Root-Rot Diseases of Faba bean by Bioproducts and Inducer Resistance Chemicals

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This study was carried out to evaluate the efficiency of Plant Guard (*Trichoderma harzianum*) and Rhizo-N (*Bacillus subtilis*) as bioproducts in combination with ascorbic and shikimic acid as inducer resistance chemicals in management of faba bean damping-off and root-rot diseases caused by *Fusarium moniliforme*, *F. solani* and *Macrophomina phaseolina* under greenhouse and field conditions. *In vitro* using the tested two bioagents as well as the two inducer resistance chemicals (IRCs) and the fungicide Rizolex-T, significantly reduced the linear growth of the tested three pathogenic fungi. Rizolex-T followed by *T. harzianum* then ascorbic acid were the most effective treatments in reducing the linear growth of the tested fungi. Meanwhile, shikimic acid followed by *B. subtilis* were the lowest efficient ones in this regard. Under greenhouse conditions, soaking faba bean seeds in ascorbic acid then coating with Plant Guard (after adding talc powder) or Rhizo-N were the most effective treatments in managing the tested fungi. However, dressing faba bean seeds with the fungicide Rizolex-T was the superior treatment in this regard. In field experiments, soaking faba bean seeds in ascorbic or shikimic acid then coating with Plant Guard were the most effective treatments in reducing the natural infection by damping-off and root-rot diseases. Moreover, these treatments improved the estimated crop parameters as well as increased the percentages of total nitrogen and protein constitute of the dry seeds compared with the control treatment. Also, dressing faba bean seeds with the fungicide Rizolex-T was the superior treatment in this respect.

Keywords: Biological control, damping-off, faba bean, inducer resistance chemical, protein constitute and root-rot.

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%) (Chavan *et al.*, 1989). However, soil-borne plant pathogens are the main threat for its profitable productivity.

Hammerschmidt (1999) mentioned that plant defence mechanisms are both constitutive and inducible. The former include the physical barriers opposing pathogen penetration and the pre-formed antibiotic or biostatic compounds, while the latter involve *de novo* synthesized products, such as new material apposition, active anti-microbial chemicals and pathogenesis-related proteins.

Singh *et al.* (2012) mentioned that nitrogen fixed by roots of faba bean plants results in increasing residual soil nitrogen for subsequent crops. It can also be used as green manure which has potential to fix free nitrogen at 150 to 300 kg N ha⁻¹. Faba bean is seen as an agronomical viable alternative to cereal grains. It is a good source of lysine rich protein as well as levodopa (L-3,4-dihydroxyphenylalanine (L-dopa)). Levodopa, a precursor of dopamine, can be potentially used as medicine for the treatment of Parkinson's disease. It is also a natriuretic agent, which might help in controlling hypertension. Persons consuming faba bean may suffer from favism (type of anaemia) if they lack an enzyme called glucose-6-phosphatase dehydrogenase (G6PD).

Damping-off, 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. 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 seed yield (Abdel-Kader *et al.*, 2011). Moreover, seeds of faba bean can be contaminated by mycotoxins secreted by some fungi grown on these seeds. Mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Khlanguiset *et al.*, 2011).

Management of plant diseases is important for most crops, and it is particularly critical for the production of high-quality seeds. Plant pathogens can reduce the quantity and quality of the harvested seeds and in additions they can be preserved in seed lots in the case of seed-borne pathogens. In this way, seeds can inadvertently provide an efficient means of plant pathogen dissemination (Abdel Monaim, 2013 and Mancini and Romanazzi, 2014). Coating seeds of many crops with biocontrol agents such *Trichoderma* spp., *Bacillus subtilis*, *Pseudomonas fluorescens* was one of the most effective treatments for controlling seed and root-rot pathogens (Nayaka *et al.*, 2004 and Begum *et al.*, 2011). However, El-Mohamedy and Abd-Alla (2013) and El-Mohamedy *et al.* (2015) reported that practical using of bio-priming seed treatment to control root-rot soil-borne plant pathogens as a substitute of chemical fungicides is possible without any risk to human, animal and the environment. Biological control of soil-borne pathogens is often attributed to improve nutrition that boosts host defense or to direct inhibition of pathogen growth and activity. Amendment with certain abiotic factor (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 (De Boer *et al.*, 1999), increase the bio-control activity and stimulate the expression of plant defense proteins (Roby *et al.*, 1987 and Muthami *et al.*, 2007). All these effects may culminate in enhancing plant protection. Similarly, salicylic acid (SA) and H₂O₂ amendment was tested in combination with bioagents. Saikia *et al.* (2003) tested the efficiency of *P. fluorescens* with or without SA amendment in chickpea against *Fusarium* wilt infection. The application of *P. fluorescens* (Pf4-92) with SA recorded the highest protection of chickpea seedlings against wilt disease.

The objective of this research is to evaluate the inhibitory effect of two bioproducts and two inducer resistance chemicals (IRCs) on the linear growth of *Fusarium moniliforme*, *F. solani* and *Macrophomina phaseolina* responsible for causing faba bean damping-off and root-rot diseases. Also, to evaluate these materials as alternatives for managing damping-off and root-rot diseases of faba bean under greenhouse and field conditions. The work was expanded to assess the effect of these treatments on some crop parameters of faba bean plants, percentage of total nitrogen and protein constitute of dry seeds in field experiments.

Materials and Methods

1. Source of the pathogens:

Virulent isolates of *F. moniliforme*, *F. solani* and *Macrophomina phaseolina*, previously isolated from faba bean roots, were obtained from Legume Dis. Dept., Plant Pathol. Res. Inst., ARC.

2. Source of the bioagents:

Two commercial bioproducts, *i.e.* Rhizo-N (*Bacillus subtilis* 3×10^7 cfu / ml) and Plant Guard (*Trichoderma harzianum*, 3×10^7 cfu/ml), were used. Loops from each product were streaked on nutrient agar and PDA media, respectively to obtain the bioagent of each product and maintained in slants contained nutrient agar and PDA medium, respectively.

3. Source of faba bean seeds:

Faba bean seeds cv. Giza 429 were obtained from Crops Res. Inst., ARC, Giza.

4. Effect of the tested bioagents, IRCs and the fungicide Rizolex-T on the linear growth of the tested fungi:

This experiment was conducted to evaluate the effect of *T. harzianum*, *B. subtilis*, ascorbic acid (vitamin-c) and shikimic acid (phenolic compound) and the fungicide Rizolex-T 50% (Tolclfus-methyl) on the linear growth of *F. moniliforme*, *F. solani* and *M. phaseolina*. In this respect, Petri-plates containing PDA medium were inoculated with a disc (5 mm in diameter) taken from 7 days-old cultures of the pathogenic fungi. The pathogenic fungi were inoculated at one side of the Petri-dish at 5 mm a part, whereas the opposite side was inoculated with *T. harzianum*. Meanwhile in case of *B. subtilis*, the pathogenic fungi were inoculated at the sides of the Petri-dish at 5 mm a part and *B. subtilis* was streaked in the center of the Petri-dish.

The IRCs, *i.e.* ascorbic acid and shikimic acid (at 8 mM) as well as Rizolex-T were added to PDA medium after sterilization (at 250 ppm). Petri-dishes were inoculated with 5 mm disks for 7 days-old fungal culture. Petri-dishes containing PDA only were inoculated with the tested fungi only were used as control. Three replicates were used for each treatment and the control. All previous plates were incubated at $25 \pm 1^\circ\text{C}$ for 5 days then examined. Percentages of reduction in fungal growth were calculated as the growth in different treatments relative to those in check treatment.

5. *Seed soaking in the IRCs:*

Disinfected faba-been seeds were soaked for 12 h in any of the tested IRCs (ascorbic and shikimic acid) at 8 mM just before sowing or before coating with the two tested commercial bioproducts.

6. *Preparation of Plant Guard as powder:*

Plant Guard was mixed with sterilized talc powder at the rate of 1:1 (v/w), then dried at room temperature and kept in a refrigerator at 10 °C until usage .

7. *Seed coating with the bioproducts:*

Faba bean cv. Giza 429 was sprayed with 1% Triton-B as sticker after soaking in the IRCs then coated with the two commercial bioproducts each alone , *i.e.* Plant Guard (*T. harzianum* 3×10^7 cfu/ml) and Rhizo-N (*Bacillus subtilis*, 3×10^7 cfu/ml) at the rate of 4.0 g/kg seed and left to air dry then sown.

8. *Seed dressing with the fungicide:*

Faba bean seeds sprayed with 1% Triton-B as sticker were dressed with Rizolex-T 50 % at the recommended dose (3 g/kg seeds) then sown in pots filled with the infested soil with the tested fungi and also in the field under the natural soil infestation.

9. *Effect of the tested bioproducts, IRCs and the fungicide Rizolex-T on the incidence of damping-off and root-rot severity under greenhouse conditions:*

This experiment was carried out to evaluate the efficiency of different seed treatments, *i.e.* Rhizo-N, Plant Guard, ascorbic and shikimic acid, each alone and in combinations, *i.e.* ascorbic acid + *T. harzianum*, shikimic acid + *T. harzianum*, asorbic acid + *B. subtilis*, shikimic acid + *B. subtilis* on the incidence of damping-off and root-rot severity under greenhouse conditions in comparison with the fungicide Rizolex-T (positive control) and untreated seeds (negative control).

Formalin disinfested loamy soil was infested with 2% inoculum level of any of *F. moniliforme*, *F. solani* and *M. phaseolina*, grown on autoclaved barley medium in 500 glass bottles . The infested soil was distributed in plastic pots (25 cm in diameter) and irrigated one week before sowing the seeds. Faba bean seeds (cv. Giza 429) were treated with the following treatments just before sowing:

- 1- Coating with Rhizo-N at the rate of 4g/kg seed.
- 2- Coating with Plant Guard at the rate of 4 g/kg seed.
- 3- Soaking in ascorbic acid (8 mM) for twelve hrs.
- 4- Soaking in shikimic acid (8 mM) for twelve hrs.
- 5- Soaking in ascorbic acid (8 mM) for twelve hrs. then coating with Rhizo-N at the rate of 4g./kg. seeds.
- 6- Soaking in shikimic acid (8 mM) for twelve hrs, then coating with Plant Guard at the rate of 4g/kg. seeds.
- 7- Dressing with the fungicide Rizolex-T at the rate of 3g/kg seed (positive control)
- 8- Untreated seeds with any material.

Five faba bean seeds of any of the aforementioned treatments were sown in each pot and six pots were used as a particular treatment. Disease incidence was recorded as the percentages of pre- and post-emergence damping-off, as well as survived plants 15, 30 and 45 days after sowing, respectively. Also, root-rot severity was assessed 60 days after sowing using the devised scale (0-5%) according to Salt (1982) as follows:

$$\text{Root-rot severity \%} = \frac{\text{Sum of (nxv)}}{5N} \times 100$$

Where:

- n= Number of roots in each category,
- v= Numerical value of each category,
- N= Total number of roots in the samples.

10. Field experiments:

Field experiments were carried out at Sers El-Lyain Res. Stat., Menoufia governorate, Egypt during two successive growing seasons (2014/2015 and 2015/2016), in a piece of land has a back history of high infestation with soil-borne pathogens responsible for causing damping-off and root-rot diseases of faba bean. The experiment aimed to study the effect of: a) Single treatments: seeds coated with Plant Guard and Rhizo-N, seeds soaked in ascorbic and Shikimic acid, b) Combined treatments: ascorbic acid + Plant Guard, ascorbic acid + Rhizo-N, shikimic acid + Plant Guard, shikimic acid + Rhizo-N, c) Seeds dressed with the fungicide Rizolex-T 50 % and d) Control (untreated seeds).

The field was divided into plots of 10.5 m² (3.5 × 3 m) with 8 rows of 3m. long. The treated seeds with the aforementioned treatments were sown at the rate of two seeds in each hill with 25 cm. between the two hills on one side of the row. Complete Randomized Block Design with three replicates (plots) for each particular treatment was done.

The incidence of pre- and post-emergence damping-off was determined 15 and 30 days after sowing. Also, the survived plants were counted 45 days after sowing. Sixty days after sowing, ten randomized plants were gently pulled-off, 5 days after irrigation to assess the severity of root-rot as mentioned before. In addition, some crop parameters, *i.e.* plant height, number of branches/plant, number of pods/plant, weight of 100 seeds and weight of seed yield/plot were assessed.

11. Effect of the tested bioproducts, the IRCs and the fungicide Rizolex-T on chemical quality of faba bean seeds:

Random samples of faba bean pods were taken from the yield of each treatment in each growing season and the following chemical analysis were determined. The percentage of nitrogen in the seeds was determined according to the method described by Hafez and Mikkelsen (1981). In addition, protein percentage was calculated by multiplying nitrogen content by 6.25.

12. Statistical analysis:

All the obtained data were subjected to the proper statistical analysis of variance (ANOVA) of randomized complete block design (Gomez and Gomez, 1984).

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Results

Effect of the tested bioproducts, IRCs and the fungicide Rizolex-T on the linear growth of the tested fungi in vitro:

The effect of the tested bioagents, IRCs and the fungicide Rizolex-T on the linear growth of *F. moniliforme*, *F. solani* and *M. phaseolina*, the causal of damping-off and root-rot of faba bean is shown in Table 1. Data indicate that all treatments significantly reduced the linear growth of the tested fungi. The tested fungi failed to grow at 250 ppm. of the fungicide Rizolex-T. The average linear growth of the tested fungi recorded 35.8, 37.4, 41.9 and 43.4 mm. when *B. subtilis*, *T. harzianum*, shikimic acid and ascorbic acid were tested, respectively. In addition, the linear growth of the fungus *F. solani* was the most affected by the tested materials followed by *F. moniliforme* then *M. phaseolina*, being 38.0, 41.4 and 44.9 mm, respectively.

Table 1. Effect of two bioagents and IRCs as well as the fungicide Rizolex-T on the linear growth of *F. moniliforme*, *F. solani* and *M. phaseolina* grown on PDA medium, 5 days after incubation at 25±1°C

Treatments	Average linear growth (mm) of			Mean
	<i>F. moniliforme</i>	<i>F. solani</i>	<i>M. phaseolina</i>	
<i>B. subtilis</i>	35.0	32.0	40.3	35.8
<i>T. harzianum</i>	38.3	30.0	44.0	37.4
Ascorbic acid	45.0	35.3	50.0	43.4
Shikimic acid	40.0	40.7	45.0	41.9
Rizolex-T	0.0	0.0	0.0	0.0
Control	90.0	90.0	90.0	90.0
Mean	41.4	38.0	44.9	----

L.S.D. at 5 % for: Treatment (T) = 3.1 , Linear growth (L) = 2.2 and TxL = 4.3.

Effect of the tested IRCs, bioproducts and the fungicide Rizolex-T on the incidence of faba bean damping-off and root-rot severity caused by the tested fungi under greenhouse conditions:

The effect of different seed treatments on managing of damping-off and root-rot severity caused by the main root-rot pathogens (*F. moniliforme*, *F. solani* and *M. phaseolina*) of faba bean was evaluated under greenhouse conditions. Results shown in Tables 2 a, b and c show that , in general , all the tested seed treatments, i.e. IRCs, bioproducts and the fungicide Rizolex-T significantly decreased pre- and post-emergence damping-off as well as root-rot severity caused by the three tested fungi compared to the control treatment. The fungicide Rizolex-T was the superior treatment in this regard. However, the combined treatments between any of the two IRCs (ascorbic and shikimic acid) or the two bioproducts (Plant Guard and Rhizo-N) caused great reduction to both damping-off and root-rot severity. In addition, the combination between ascorbic acid and Plant Guard was the most efficient combination in reducing pre- and post-emergence damping-off and root-rot severity caused by any of the three tested fungi, being 6.7 , 10.0 and 18.9 % for *Egypt. J. Phytopathol.*, Vol. 45, No. 1 (2017)

F. moniliforme; 10.0, 10.0 and 22.2 % for *F. solani*; 13.3 , 10.0 and 27.3 % for *M. phaseolina*, respectively. Furthermore, both *F. moniliforme* and *F. solani* were greatly affected by the tested treatments than *M. phaseolina*. On the other hand, the lowest effect was occurred due to using seeds coated with Rhizo-N followed by seeds soaked in shikimic acid.

Table 2a. Effect of the tested IRCs, bioagents and the fungicide Rizolex-T on the incidence of damping-off and root-rot severity of faba bean caused by *F. moniliforme* under greenhouse conditions

Treatment	<i>F. moniliforme</i>			
	% Damping-off		% Survived plants	% Root-rot severity
	Pre-emergence	Post-emergence		
Seeds soaked in ascorbic acid	10.00	13.3	76.7	25.6
Seeds soaked in shikimic acid	13.3	16.6	70.0	32.2
Seeds coated with Plant Guard	13.3	13.3	73.3	30.0
Seeds coated with Rhizo-N	16.7	13.3	70.00	28.6
Seeds soaked in ascorbic acid and coated with Plant Guard	6.7	10.0	83.3	18.9
Seeds soaked in ascorbic acid and coated with Rhizo-N	10.0	10.0	80.0	20.0
Seeds soaked in shikimic acid and coated with Plant Guard	10.0	13.3	76.7	27.3
Seeds soaked in shikimic acid and coated with Rhizo-N	13.3	13.3	73.3	28.6
Seeds dressed with the fungicide Rizolex-T	3.3	0.0	96.7	6.7
Control (untreated)	26.7	22.0	51.3	53.3
L.S.D at 5%	2.8	2.6	4.0	3.3

Table 2b. Effect of the tested IRCs, bioagents and the fungicide Rizolex-T on the incidence of damping-off and root-rot severity of faba bean caused by *F. solani* under greenhouse conditions

Treatment	<i>F. solani</i>			
	% Damping-off		% Survived plants	% Root-rot severity
	Pre-emergence	Post-emergence		
Seeds soaked in ascorbic acid	13.3	16.7	70.00	30.0
Seeds soaked in shikimic acid	16.7	20.00	63.3	35.3
Seeds coated with Plant Guard	13.3	16.7	70.0	28.9
Seeds coated with Rhizo-N	20.0	13.3	66.7	33.3
Seeds soaked in ascorbic acid and coated with Plant Guard	10.0	10.0	80.0	22.2
Seeds soaked in ascorbic acid and coated with Rhizo-N	13.3	10.0	76.7	25.6
Seeds soaked in shikimic acid and coated with Plant Guard	13.3	13.3	73.7	27.3
Seeds soaked in shikimic acid and coated with Rhizo-N	13.3	16.7	70.0	30.6
Seeds dressed with the fungicide Rizolex-T	3.3	3.3	93.3	8.9
Control (untreated)	30.0	26.7	43.3	58.6
L.S.D at 5%	3.0	2.8	4.3	2.5

Table 2c. Effect of the tested IRCs, bioagents and the fungicide Rizolex-T on the incidence of damping-off and root-rot severity of faba bean caused by *M. phaseolina* under greenhouse conditions

Treatment	<i>M. phaseolina</i>			
	% Damping-off		% Survived plants	% Root-rot severity
	Pre-emergence	Post-emergence		
Seeds soaked in ascorbic acid	16.7	16.7	66.7	35.3
Seeds soaked in shikimic acid	20.0	20.0	60.0	39.6
Seeds coated with Plant Guard	16.7	13.3	70.0	30.0
Seeds coated with Rhizo-N	16.7	16.7	66.7	33.6
Seeds soaked in ascorbic acid and coated with Plant Guard	13.3	10.0	76.7	27.3
Seeds soaked in ascorbic acid and coated with Rhizo-N	16.7	10.0	73.3	28.6
Seeds soaked in shikimic acid and coated with Plant Guard	13.3	16.7	70.0	32.2
Seeds soaked in shikimic acid and coated with Rhizo-N	16.7	20.0	63.3	37.3
Seeds dressed with the fungicide Rizolex-T	6.6	3.3	90.0	11.6
Control (untreated)	33.3	26.7	40.0	62.2
L.S.D at 5%	2.5	2.4	3.4	2.3

Effect of treatment faba bean seeds with the IRCs, bioproducts and Rizolex-T on the incidence of damping-off and root-rot severity under field conditions:

The effect of the two IRCs, bioproducts and Rizolex-T on the incidence of damping-off and root-rot severity is presented in Tables 3a and b. The obtained results reveal that these treatments significantly reduced pre- and post-emergence damping-off and root-rot severity compared to the untreated plants (control). Rizolex-T resulted in the highest level of disease reduction with the highest values of survived plants compared to the check treatment. Moreover, Rizolex-T gave the best results in decreasing the percentages of pre- and post-emergence damping-off and root-rot severity during the two growing seasons. Shikimic acid was the lowest effective treatment in this regard.

Table 3a. Effect of the tested IRCs, bioproducts and the fungicide Rizolex-T on the incidence of damping-off and root-rot severity of fabe bean under field conditions during 2014/2015 growing season

Treatment	2014/2015			
	% Damping-off		% Survived plants	% Root-rot severity
	Pre-emergence	Post-emergence		
Seeds soaked in ascorbic acid	10.0	11.0	79.0	15.6
Seeds soaked in shikimic acid	11.3	12.7	76.0	0.0
Seeds coated with Plant Guard	9.1	10.0	80.9	16.7
Seeds coated with Rhizo-N	10.1	1.3	78.6	18.9
Seeds soaked in ascorbic acid and coated with Plant Guard	6.2	7.2	86.6	11.1
Seeds soaked in ascorbic acid and coated with Rhizo-N	8.2	9.4	82.4	12.2
Seeds soaked in shikimic acid and coated with Plant Guard	7.2	8.1	84.7	13.3
Seeds soaked in shikimic acid and coated with Rhizo-N	9.2	9.5	81.3	14.4
Seeds dressed with the fungicide Rizolex-T	00.0	3.3	96.7	6.7
Control (untreated)	26.2	20.0	53.8	40.2
L.S.D at 5%	7.3	7.5	11.71	1.8

Table 3b. Effect of the tested IRCs, bioagents and the fungicide Rizolex-T on the incidence of damping-off and root-rot severity of fabe bean under field conditions during 2015/2016 growing seasons

Treatment	2015/2016			
	% Damping-off		% Survived plants	% Root-rot severity
	Pre-emergence	Post-emergence		
Seeds soaked in ascorbic acid	10.2	11.0	78.8	20.0
Seeds soaked in shikimic acid	11.5	12.0	76.5	22.2
Seeds coated with Plant Guard	9.5	11.0	79.5	18.9
Seeds coated with Rhizo-N	10.9	12.0	77.1	20.0
Seeds soaked in ascorbic acid and coated with Plant Guard	7.0	8.0	85.0	12.2
Seeds soaked in ascorbic acid and coated with Rhizo-N	8.1	9.0	82.9	13.3
Seeds soaked in shikimic acid and coated with Plant Guard	7.9	8.0	84.1	15.6
Seeds soaked in shikimic acid and coated with Rhizo-N	8.8	9.2	82.0	17.8
Seeds dressed with the fungicide Rizolex-T	3.3	3.3	93.7	8.9
Control (untreated)	27.9	22.1	50.0	44.1
L.S.D at 5%	7.0	8.1	12.8	2.0

Effect of the tested IRCs, bioproducts and Rizolex-T on growth and crop components of faba bean under field conditions:

The effect of treating faba bean seeds with the tested IRCs, bioproducts and Rizolex-T on some crop parameters under field conditions was assessed during 2014-2015 and 2015-2016 growing seasons. Data in Tables 4a and b indicate that the combination between the bioproducts and the IRCs resulted in considerable improve to faba bean growth and branching more than using each of them individually. The combination between ascorbic acid and Plant Guard was the most effective treatment, where recorded the highest plant height and number of branches in both seasons as well as recorded the highest yield components, *i.e.* number of pods/plant, weight of 100 seeds and total yield (kg / plot) in both seasons.

Table 4a. Effect of the tested bioagents, IRCs and the fungicide Rizolex-T on some growth and crop parameters of faba bean plants under field conditions during 2014/2015 growing seasons

Treatment	Plant height (cm)	No. of branches / plant	No. of Pods/ plant	100 seeds (w/g)	Yield kg/ plot
Seeds soaked in ascorbic acid	103.0	3.2	19.2	71.8	1.84
Seeds soaked in shikimic acid	105.0	3.2	15.2	70.0	1.82
Seeds coated with Plant Guard	110.0	3.2	18.0	72.0	1.87
Seeds coated with Rhizo-N	105.0	3.0	17.0	70.1	1.75
Seeds soaked in ascorbic acid and coated with Plant Guard	115.0	3.8	19.2	75.3	1.93
Seeds soaked in ascorbic acid and coated with Rhizo-N	110.0	3.5	19.0	73.0	1.89
Seeds soaked in shikimic acid and coated with Plant Guard	109.0	3.4	18.0	74.0	1.90
Seeds soaked in shikimic acid and coated with Rhizo-N	106.0	3.4	17.2	72.0	1.85
Seeds dressed with the fungicide Rizolex-T	110.0	4.0	21.0	76.1	1.97
Control (untreated)	102.0	1.7	13.7	65.0	1.42
L.S.D at 5%	3.1	1.0	2.1	1.9	18.0

Effect of the tested bioproducts, IRCs and Rizolex-T on total nitrogen and protein constitutes of faba bean dry seeds:

Data presented in Table 5 show that all the tested treatments significantly increased total N and crude protein content in faba bean seeds compared with untreated treatments. Also, the combination between ascorbic acid and Plant Guard recorded the highest total N and crude protein followed by the combination between shikimic acid and Plant Guard in both seasons. Meanwhile, both shikimic acid and Rhizo- N individually recorded the lowest increase in this respect.

Table 4b. Effect of the tested bioagents, IRCs and the fungicide Rizolex-T on some crop parameters of faba bean plants under field conditions during 2015/2016 growing season

Treatment	Plant height (cm)	No. of branches / plant	No. of Pods/ plant	Weight of 100 seed (g)	Seed yield (kg) / plot
Seeds soaked in ascorbic acid	100.0	3.1	17.0	70.0	1.80
Seeds soaked in shikimic acid	104.0	3.1	13.3	72.0	1.78
Seeds coated with <i>T. harzianum</i>	106.0	3.1	17.0	71.1	1.83
Seeds coated with <i>B. subtilis</i>	103.0	2.9	16.2	69.2	1.70
Seeds soaked in ascorbic acid and coated with <i>T. harzianum</i>	112.0	3.4	18.9	73.2	1.90
Seeds soaked in ascorbic acid and coated with <i>B. subtilis</i>	107.0	3.2	18.7	71.0	1.85
Seeds soaked in shikimic acid and coated with <i>T. harzianum</i>	106.0	3.3	16.0	72.0	1.89
Seeds soaked in shikimic acid and coated with <i>B. subtilis</i>	102.0	3.3	15.4	69.9	1.81
Seeds dressed with the fungicide Rizolex-T	109.0	4.0	20.1	75.0	1.93
Control (untreated)	99.0	1.3	12.6	63.0	1.38
L.S.D at 5%	3.0	0.9	2.0	1.7	17.3

Table 5. Percentages of total nitrogen and protein constitutes of faba bean dry seeds as affected by the tested IRCs, bioagents and the fungicide Rizolex-T during 2014/2015 and 2015/ 2016 growing seasons

Treatments	2014/2015		2015/2016	
	% Total Nitrogen	% Protein	% Total Nitrogen	% Protein
Seeds soaked in ascorbic acid	4.20	26.25	3.98	24.88
Seeds soaked in shikimic acid	4.10	25.63	3.90	24.38
Seeds coated with <i>T. harzianum</i>	4.19	26.19	4.10	25.63
Seeds coated with <i>B. subtilis</i>	4.02	25.13	3.95	24.69
Seeds soaked in ascorbic acid and coated with <i>T. harzianum</i>	5.18	32.38	4.15	25.94
Seeds soaked in ascorbic acid and coated with <i>B. subtilis</i>	4.95	30.94	4.80	30.00
Seeds soaked in shikimic acid and coated with <i>T. harzianum</i>	4.80	30.00	4.60	28.75
Seeds soaked in shikimic acid and coated with <i>B. subtilis</i>	4.60	28,75	4.30	26.88
Seeds dressed with the fungicide Rizolex-T	4.30	26.87	4.20	26.25
Control (untreated)	3.95	24.69	3.70	23.13
L.S.D at 5%	0.29	2.41	0.35	2.35

Discussion

Plant growers are interested in reducing dependence on chemical inputs. Therefore, disease management should rely more on principles of Integrated Pest Management (IPM) such as sanitation, biological control, resistant cultivars, induced disease resistance. In this regard, model describing the several steps required for a successful IPM has been developed by Mc Spadden and Fravel (2002), where modern agriculture and horticulture must combine several objectives that seem to be almost mutually exclusive: to satisfy the nutritional needs of an increasing human population and to minimize the negative impact on the environment. In addition, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Nowadays, there are strict regulations on chemical pesticide use. Additionally, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Among these alternatives are those referred to as biological controls (Pal and Mc Spadden, 2006).

It is known that chemical pesticides are highly effective and convenient to use but they are a potential threat for the environment and all kinds of life on earth. Therefore, the use of biological control agents for the management of plant pathogens is considered as a safer and sustainable strategy for safe and profitable agricultural productivity. *Bacillus*-based biocontrol agents play a fundamental role in the field of biopesticides. Many *Bacillus* species have proved to be effective against a broad range of plant pathogens. They have been reported as plant growth promoter, systemic resistance inducer, and used for production of a broad range of antimicrobial compounds (lipopeptides, antibiotics and enzymes) and competitors for growth factors (space and nutrients) with other pathogenic microorganisms through colonization (Shafi *et al.*, 2017).

Soil-borne diseases of faba bean including damping-off, root-rot and / or wilt cause considerable loss to the plant stand and the yield. On the other hand, biological control of root diseases is largely based upon competition between the bioagents and the pathogens. Competitive colonization of the rhizosphere by the bioagents and the use of available resources are thought to exclude many rhizosphere soil-borne pathogens (Mc Spadden and Fravel, 2002). The mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable broad-spectrum disease control that could be used preventatively to bolster general plant health, and resulting in long lasting protection (Kessmann *et al.*, 1994).

The obtained data *in vitro* revealed that both bioagents (*T. harzianum* and *B. subtilis*), IRCs (ascorbic and shikimic acid) and the fungicide Rizolex-T caused significant reduction to the linear growth of the three pathogenic fungi, *i.e.* *F. moniliforme*, *F. solani* and *M. phaseolina*. Also, these treatments caused significant reduction to pre- and post-emergence damping-off as well as root-rot severity and increased the survived plants either in greenhouse or in field experiments, compared to the control. The combination between any of the two IRCs and any of the two bioproducts was more effective in this regard than when each of them was used individually. The combination between ascorbic acid and Plant Guard recorded the highest efficiency in this respect. These results are in agreement with those reported by Abd-Alla (1994). Seeds soaked in any of the IRCs and coated with any of the two bioproducts (Plant Guard and Rhizo-N) resulted in significant reduction to damping-off and root-rot diseases during the growing seasons of 2014/2015 and 2015/2016 with considerable increase to the assessed crop parameters as well as considerable increase in the percentages of total nitrogen and protein constitute in the dry faba bean seeds. Similar results were obtained by Rajkumar *et al.* (2008); Morsy (2011) and Yobo *et al.* (2011).

Gram-positive *Bacillus* species, however, possess several advantages that make them good candidates for use as biological control agents (BCA). First, their antagonistic effect is caused by their ability to produce different types of antimicrobial compounds, such as antibiotics *e.g.*, bacilysin, iturin, mycosubtilin and siderophores (Shoda, 2000). Second, they are able to induce growth and defense responses in the host plant (Raupach and Kloepper, 1998). Furthermore, *Bacillus* is able to produce spores resistant to UV light and desiccation, which

allows them to resist adverse environmental conditions, and permits easy formulation for commercial purposes (Raaijmakers *et al.*, 2002).

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 modification of plant cell wall by deposition of callose (Veit *et al.*, 2001).Also, Xing *et al.*(2003) declared that *Bacillus* spp. grow very fast and occupy the court of infection 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. Moreover, *Bacillus* species have a unique ability to replicate rapidly, resistant to adverse environmental conditions as well as they have broad spectrum of biocontrol ability. Volatile compounds produced by *B. subtilis* also play an important role in plant growth promotion and activation of plant defence mechanism by triggering the induced systemic resistance (ISR) in plants (Compant *et al.*, 2005).

Trichoderma spp. are the most promising and effective bioagents against various plant pathogenic fungi. *Trichoderma* as antagonist for controlling wide range of microbes was well documented and demonstrated for more than seven decades ago but its use under field conditions came much later (Ragab *et al.*,2015) and their mechanism of mycoparasitism is much more complex, that is nutrient competition, hyperparasitism, antibiosis, space and cell wall degrading enzymes (Abd-El-Khair *et al.*, 2010). *Trichoderma* spp. are known to control pathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and enhancing plant defensive mechanisms and antibiosis, or directly by inhibition of growth and sporulation of the pathogen mechanisms such as mycoparasitism and enzyme production (Bouhassan *et al.*, 2004, Faheem *et al.*, 2010 and Ragab *et al.*, 2015). El-Mougy *et al.* (2013) reported an announced reduction in root-rot incidence of bean and cowpea, caused by the pathogens *R. solani* and *F. solani* that was achieved using of *T. harzianum*.

Biological control of damping-off diseases has been successfully applied using *B. subtilis* (Harris and Adkins 1999 and Schmidt *et al.*, 2004) and *T. harzianum* (Ragab *et al.*,2015 and Ahmed, 2016). In this respect, *Trichoderma* spp. are known to control pathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and enhancing plant defensive mechanisms and antibiosis, or directly by inhibition of growth and sporulation of the pathogen mechanisms such as mycoparasitism and enzyme production (Zimand *et al.*, 1996 and Bouhassan *et al.*, 2004).

Abd-Alla (1994) concluded that phenolic compounds at low concentration (100 µM) can be used to improve soybean crop performance by stimulating nodulation, plant growth and ammonia assimilation. In addition, the results obtained here are in agreement with the results of Aldesuquy and Ibrahim (2000) who stated that seed priming with shikimic acid increased growth and yield of cowpea plants grown
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under greenhouse conditions through increasing the total soluble sugars, protein content and photosynthetic activity. Application of antioxidants, *e.g.* ascorbic, salicylic, cumaric, benzoic acids and propylgalate as either seed soaking or soil drench proved sufficient protection against cumin diseases caused by *Fusarium oxysporum* f. sp. *cumini* and *Acremonium egyptiacum* (Mostafa, 2006).

Ascorbic acid (vitamin-C) plays an important role as an antioxidant and protects the plant during oxidative damage by scavenging free radicals and ROS that are generated by various stresses (Schulthesis *et al.*, 2002 and Elwan and El-Hamahmy, 2009). Higher content of ascorbic acid might maintains relatively lower levels of ROS in pepper and tomato fruit resulting in less damage caused by ROS after stress. In addition, Reddy *et al.* (2005) reported that the increase in soluble nitrogen may be ascribed to increased hydrolysis of proteins. In addition, Khan *et al.* (2011) reported that ascorbic acid can act efficiently in plants as immunomodulators when applied at the appropriate concentration and the current stage of plant development. They added that, ascorbate is implicated in plant responses to biotic stresses and to undergo profound changes in plants interacting with pathogens.

Conclusion

In conclusion, priming faba bean seeds with any of ascorbic and shikimic acid, Plant Guard and Rhizo-N significantly reduced both damping-off and root-rot diseases with considerable improve to crop parameters and increase the percentages of total nitrogen and protein content in the dry seeds. In addition, the combination between any of the two IRCs and the two bioproducts was better treatment than using any of them individually.

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مكافحة أمراض سقوط البادرات وعفن الجذور في الفول البلدي باستخدام المركبات الحيوية والكيماويات المستحثة للمقاومة

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وجد أن أمراض سقوط البادرات وعفن الجذور في الفول البلدي تُسبب مشكلة كبيرة في مصر، وللبحث عن حل لهذه المشكلة فقد تم استخدام المُكافحة الحيوية والكيماويات المستحثة للمقاومة ومبيد الريزولكس-تي لتقليل الضرر الناتج من الفطريات المُسببة لهذه الأمراض. تم إجراء هذه الدراسة لتقييم فعالية كل من البلاننت جارد (*Trichoderma harzianum*) والريزو-إن (*Bacillus subtilis*) كمركبات حيوية مع الحمضين أسكوربيك، شيكيمك ككيماويات مُستحثة للمقاومة في مكافحة أمراض سقوط البادرات وعفن الجذور المتسببة عن *Fusarium moniliforme*, *F. solani* and *Macrophomina phaseolina* تحت ظروف الصوبة والحقل. تحت ظروف الصوبة كان نفع بذور الفول في حمض الأسكوربيك ثم تغليفها بالمركب الحيوي بلاننت جارد (بعد إضافة بودرة التلك له) أو الريزو- إن أكثر المعاملات فعالية في خفض الإصابة بالفطريات الثلاثة المختبرة. ومع ذلك، فقد كان تغطية بذور الفول بالمبيد الفطري الريزولكس-تي المعاملة السوبر في هذا الصدد. أما في تجارب الحقل كان نفع بذور الفول في أي من الحمضين أسكوربيك وشيكيمك ثم تغطيتها بالمركب الحيوي بلاننت جارد أكثر المعاملات فعالية في خفض الإصابة الطبيعية بأمراض سقوط البادرات وعفن الجذور. علاوة على ذلك فقد أدت هاتين المعاملتين إلى تحسين الصفات المحصولية التي تم تقديرها وكذلك زيادة النسبة المئوية للنيتروجين الكلي والمحتوى البروتيني للبذور الجافة مقارنة بمعاملة المقارنة. أيضا كان لتغطية بذور الفول بالمبيد الفطري الريزولكس-تي المعاملة السوبر في هذا المضمرا.