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INDUCTION OF RESISTANCE AGAINST RHIZOCTONIA ROOT ROT OF COMMON BEAN

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ABSTRACT

The effect of seed treatments of common bean plants (cv. Nebraska) with Bion, Salicylic Acid (SA) and *Paenibacillus polymyxa* on infection with *Rhizoctonia solani* under greenhouse and field conditions was investigated. In greenhouse experiment, all treatments decreased percentages of pre and post-emergence damping- off compared with control grown in infested soil by *R. solani*. The highest percentage of survival plants was achieved from treatment with Bion 5mM (80%) followed by each of Bion 3mM and SA 5mM (76.7%) compared with control (40.1%). Meantime, significant increases in the values of shoot length, shoot dry weight and root dry weight over the control treatment were achieved.

Under field experiments during summer 2009 and 2010 growing seasons, all the tested treatments significantly increased the percentage of survived plants compared with the control. There were no significant differences between the treatments with Bion 3mM and fungicide (Rhizolex-T 3g/Kg seeds) 89.4% and 89.3 %, respectively compared with untreated control 67.9% calculated as means of the two seasons. Also, the treatments with salicylic acid and *P. polymyxa* were less effective regarding survival plants in 2009 and 2010 growing seasons. Moreover, all treatments significantly increased all the studied vegetative characteristics, i.e. stem length, number of leaves/plant as well as fresh and dry weight of leaves/plant compared with untreated control. Meanwhile, higher increase pe in seed yield (kg/feddan) was estimated with bion and fungicide treatments (86% and 87.7%, respectively) followed by salicylic acid and *P. polymyxa* (66.8% and 55.4 %, respectively) increasing over the untreated control calculated as means of the two seasons.

Laboratory studies indicated that, all treatments were effective in eliciting the activities of peroxidase and polyphenol oxidase. Peroxidase activity was higher with Bion followed by SA treatments; they showed 77.7% and 41.2 % increase over the untreated control, respectively. Meantime, elevation of the polyphenol oxidase activity was showed with Bion followed by SA treatments as 97.7% and 58.0% increasing over the untreated control, respectively. However, Bion treatment resulted in the highest increase in total phenols contents over the untreated control (94.3%) followed by SA and *P. polymyxa* treatments (57.9% and 52.6%) over the untreated control, respectively.

Keywords: Common bean, *Rhizoctonia solani, Paenibacillus polymyxa,* Bion, Salicylic acid, Rhizolex-T.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an ancient and versatile crop. It is characterized as a near perfect food because of its high protein content and generous amounts of iron, folic acid, complex carbohydrates and other diet essentials (Sing *et al.,* 2013). Consuming beans also have medicinal benefits as it is recognized that they contribute to treating human aliments like cancer, diabetes, and heart diseases (Singh, 2001 and Hutchins *et al.,* 2012). Meantime, common bean is one of the most important leguminous crops in Egypt. Its production reached 100,622 tonnes in 2011 and record export volumes of 47436 tonnes were achieved with gross value of 69,444 million US$ (FAOSTAT, 2013).

Rhizoctonia root rot disease caused by *Rhizoctonia solani* Kühn is a serious and economically important disease for bean production in most of the tropical, subtropical and temperate areas of the world (Tu *et al.,* 1996). Root diseases caused by *R. solani* can limit common bean production in commercial fields (Papavizas *et al.,* 1975). The fungus has a broad host range and consists of important subgroups, including some which attack roots and some which cause foliar diseases in bean plants. Losses of up to 10% have been attributed to Rhizoctonia root rot (Hall, 1994). *R. solani* anastomosis groups AG-4 and AG-2-2 were shown to be important in the etiology of the disease. Different bean cultivars planted in artificial and naturally infested soils with *R. solani* AG-4 suffered severe yield losses with significantly reduced (46 - 92%) plant stands 32 days after planting (Win & Sumner, 1988). The pathogen infects other several important rotational crops and its inoculum can survive in their residues. *R. solani* AG-4 infects alfalfa, bean, canola, peas, soybeans, sugar beet, red clover, tomato *and* potato (Yang & Li, 2012). The wide host range exhibited by these pathogens further complicates management strategies.

Traditional methods used to control the disease including seed treatment with fungicides. The hazardous effect of fungicides or their degradation products on the environment and human health strongly necessitates the search for new, harmless means of disease control. Induction of resistance by application of elicitors is one of the alternatives, either alone or as a part of an integrated control strategy. Elicitors are compounds, which stimulate the natural defence mechanisms in plants. Commonly tested chemical elicitors are salicylic acid, methyl salicylate, benzothiadiazole, benzoic acid, chitosan, and so forth which affect production of phenolic compounds and activation of various defence-related enzymes in plants. (Thakur & Sohal, 2013). In plants, a complex array of defence response is induced after detection of microorganism via recognition of elicitor molecules released during plant-pathogen interaction. Following elicitor perception, the activation of signal transduction pathways generally lead to the production of active oxygen species (AOS), reinforcement of plant cell wall associated with phenylpropanoid compounds, deposition of callose, synthesis of defence enzymes (Thakur & Sohal, 2013), phytoalexin biosynthesis (Durango *et al.,* 2013) and the accumulation of pathogenesis-related (PR) proteins, some of which possess antimicrobial properties (Van Loon & Van Strien, 1999).

The natural plant defense hormone-like substance, salicylic acid (2-hydroxybenzoic acid) plays a crucial role in plant growth and development, and serves as an endogenous signal to activate certain immune responses and to establish disease resistance (White, 1979, Ryals *et.al*., 1996 and Heil & Bostock, 2002). Various defense-related stimuli have been shown to trigger enhanced SA levels in local and systemic plant tissues. Exogenous application of SA can stimulate particular enzymes catalyzing biosynthetic reactions to produce defense compounds (Chen *et al.,* 2009 and Mandal, 2010), and induce reactive oxygen intermediates (ROI) production, pathogenesis-related (PR) gene expression and immunity against various pathogens with biotrophic or hemibiotrophic lifestyles (Glazebrook, 2005; Vlot *et al.,* 2009 and Kumar, 2014).

Benzothiadiazole or BTH (benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester) was described as a structurally related functional analogue of salicylic acid (SA) and presented as a novel class of systemic acquired resistance (SAR) inducer. Benzothiadiazole (BTH) has been shown to be effective against a wide range of pathogens on a range of crops and was released commercially under the trade names Bion (in Europe) and Actigard (in the USA) by Novartis (now marketed by Syngenta)(Friedrich *et al*., 1996; Kessmann *et al*., 1996; Lawton *et al*., 1996; Benhamou & Belanger, 1998 and Tally *et al*., 1999). Many papers have been published in the last twelve years, both on different crop/pathogen combinations and at different experimental scales (Reignault & Walters, 2007 and Walters *et al.,* 2013).

Plant growth promoting rhizobacteria (PGPR) are free-living or root-associated bacteria in the rhizosphere of many plant species that increase plant growth and suppress plant disease (Ryu *et al*., 2006 and Ahemad & Kibret, 2014). In addition to suppressing plant pathogens by secretion of antibiotics and production of allelopathic compounds in the rhizosphere some PGPR can also elicit mechanism of induced systemic resistance (ISR) against a broad range of pathogens, (Jetiyanon & Kloepper, 2002; Ryu *et al*., 2007 and Jain *et al.,* 2013). One of the reported plant growth promoting rhizobacteria (PGPR) is *Bacillus polymyxa*, now named *Paenibacillus polymyxa* (Ash *et al*., 1993). It has a range of reported properties, including nitrogen fixation (Heulin *et al.,* 1994 and Bal & Chanway, 2012); soil phosphorus solubilization (Wang *et al.,* 2012); production of antibiotics (Raza *et al.,* 2008), chitinase (Mavingui & Heulin, 1994), and other hydrolytic enzymes (Raza *et al.,* 2008).

The objective of this study was to evaluate the disease management potential of biotic and chemical inducers against Rhizoctonia root rot disease of common bean under greenhouse and field conditions.

MATERIALS AND METHODS

Plant material

Bean seeds (*Phaseolus vulgaris* L.) cv. Nebraska was obtained from the Central Administration for Seed Certification, Ministry of Agriculture and Land Reclamation, Egypt.

Pathogen

The fungus *Rhizoctonia solani* Kühn (Isolate CB 84) was isolated among many isolates from naturally infected bean plants, showing damping off and root rot symptoms, cultivated in El-Ayyat, Giza Governorate. Its pathogenicity was confirmed and identified on the basis of cultural properties and microscopic morphological characters according to Sneh *et al.,* (1991). The cultures were maintained on malt extract agar (Malt extract 20 g; Peptone 3 g and 18 g of agar) slants under a phosphate buffer (pH 6.5) at 4± 0.5 °C (Boeswinkel, 1976).

Preparation of pathogen inoculum

Inoculum of *R. solani* (isolate CB 84) was prepared by growing the fungus in glass bottles 500 cc containing sterilized sorghum medium (100 g of sorghum grains and 90 ml of water). The bottles were inoculated with equal disks (0.5 cm) of four days old *R. solani* cultures and incubated at 24 ± 1°C for 21 days, during this period the incubated bottles were shaken for 3 min. every three days to ensure uniform distribution of the fungal growth. After incubation period, the inoculum then air dried for 3days and ground in a mill to pass through a 3-mm sieve. The ground inoculum was added to soil within one week (Gaskill, 1968).

Preparation of biotic inducer

The culture of the bacterium *Paenibacillus polymyxa* (isolate 9D14), previously isolated by the authors (Shehata *et al.,* 2006) was activated on fresh slants and, after 24 hrs was transferred to many 250 ml Erlenmeyer flasks with 50 ml of nutrient yeast dextrose broth (NYDB) medium (per litre: nutrient broth 8 g, yeast extract 5 g and dextrose 10 g). The flasks were placed on a rotary shaker at 120 rpm for 66 hrs at 24±1°C.

Seed and soil treatments

Apparently healthy seeds of common bean were surface disinfected by immersing in sodium hypochlorite (1%) for 2 min, and washed several times with sterilized water, then left to dry on screen cloth with paper towel underneath to absorb the excess water at room temperature for approximately two hour.

A) Chemical inducers treatments: Bion®, Benzothiadiazole [benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] wettable granule (WG) 50%, and salicylic acid (Sigma Aldrich, USA) were used in this study. The disinfected common bean seeds were soaked in aqueous solutions of the each of two inducers (Bion, and salicylic acid) for one hour just before sowing at the rate of 3 mM and 5 mM, respectively.

B) Biotic inducer treatment: after growth of *P. polymyxa,* the liquid culture medium was then centrifuged under cooling (4°C) at 10000 rpm for 10 min. Then, the disinfected common bean seeds were soaked in supernatant for one hour. Cells of *P. polymyxa* were collected in 20cm Petri dish and bacterial slurry was obtained by adding 1-1.5 ml of 1% methyl cellulose (Sigma-Aldrich, Milwaukee, WI, USA) in sterile distilled water to bacterial cells harvested from each Erlenmeyer flask. Healthy seeds of common bean previously were soaked in supernatant, were coated with bacterial slurry, then spread on screen cloth with paper towel underneath to absorb the excess slurry, and air-dried for 19 hrs until sowing time. Enumeration of bacteria coated on seeds was performed by plate dilution method on the basis of colony forming unit (cfu/ seed) on nutrient yeast dextrose agar (NYDA) medium.

C) Fungicide treatment: seed dressing was carried out to the disinfected common bean seeds by applying the Rhizolex-T 50% WP (Tolclofos-methyl-thiram), Sumitomo Chemical Company Ltd. at the recommended dose (3 g/kg) to the 1% methyl cellulose (as sticker) moistened seeds in polyethylene bags and shaking well to ensure even distribution of the fungicide.

D) Root-nodule bacteria treatment: formulation of Rhizobium (*Phaseolus*) spp., was kindly obtained from Biofertilizers Production Unit, Soils Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt, was used to treat potted soils (infested or not-infested with pathogenic fungus) or field soil. Three grams of Rhizobium formulation were mixed in each pot during sowing and 800 g of Rhizobium formulation was mixed with approximately 50 kg of moistened fine sandy soil and added to field soil into the seed furrow during sowing, at rate of 800 g Rhizobium formulation /feddan.

E) Control, the disinfected common bean seeds were soaked in sterilized water for one hour just before sowing.

Greenhouse experiment

The trials were carried out in the greenhouse of Plant Pathology Department, Faculty of Agriculture, Ain Shams University. Plastic pots (25 cm in diameter) with a bottom drainage hole were sterilized by dipping in 5% formalin solution for 15 minutes, and left for one week until complete formalin evaporation. Pots were filled with steam disinfested Sandy clay soil 1:2 (V/V). Soil infestation was achieved by mixing the inoculum of of *R. solani* with the soil at the rate of 2% of soil weight (Papavizas & Davey, 1962). Sterilized un-inoculated grounded sorghum grains were added to the disinfested soil at the same rate for used as healthy control. The infested soil was mixed thoroughly and watered every 2 days for a week before planting to stimulate the fungal growth and ensure its distribution in the soil. Five seeds of pre-treated common bean seeds, as mentioned before, were sown in each pot and pots were irrigated directly. Six replicated pots were used for each particular treatment. All pots were irrigated when necessary, and watered once a week to near field capacity with a 0.1% 15:15:15 (N:P:K) fertilizer solution and kept in a green house under natural conditions. Other agricultural procedures were performed according to normal practice. The treatments were as follows: 1) Bion 3 mM; 2) Bion 5 mM; 3) salicylic acid 3 mM; 4) salicylic acid 5 mM; 5) *P. polymyxa;* 6) Water in infested soil (Control A, infected) and 7) Water in non-infested soil (Control B, Healthy). Twelve plants (three replicates each of four plants) were harvested 45 days after sowing, shoots length were measured and cut at the soil line. Roots were washed under running water to remove soil particles. Shoots and roots placed in a paper bags and oven dried at 70°C for 48h, then weighed.

Disease assessment

The disease incidence (DI) % was determined by recording pre-emergence damping off, post-emergence damping off and the percentage of healthy survival plants 15, 30 and 45 days after sowing, respectively according to the following formulas:

$$\text{Pre-emergence \%} = \frac{\text{Total No. of un-germinated seeds x 100}}{\text{Total No. of planted seeds}}$$

$$\text{Post-emergence \%} = \frac{\text{Total No. of rotted seedlings x 100}}{\text{Total No. of planted seeds}}$$

$$\text{Survived seedlings \%} = \frac{\text{Total No. of survived seedlings x100}}{\text{Total No. of planted seeds}}$$

Reduction or increasing % over the infected control was also calculated according to the following formula:

$$\text{Reduction or Increasing \%} = \frac{\text{DI of Control A - DI of treatment x100}}{\text{DI of Control A}}$$

Field experiments

The field experiments was carried out during the two successive summer growing seasons 2009 and 2010 near Kafr Ashma, El-Shohadaa, Menoufia Governorate, Egypt, in field known to have Rhizoctonia root rot history, in order to investigate the effect of chemical and biotic inducers for controlling damping-off and root rot diseases. The disinfected common bean seeds were treated by the same manner in a greenhouse experiment. In the control treatment, seeds were soaked in distilled water as mentioned before. The disinfected common bean seeds were sown in the field on February 25, 2009 and 2010 seasons. The field trial (15 plots) was designed in complete randomized block with three replicates. The area of each plot was 10.5 m^2 consisted of five rows; each row was 3.5 m length and 0.6 m width. All treatments were sown in hills 15 cm apart on both sides of the row ridge, with one seed per hill (90,000 plants/feddan). Number of plants/row was 48 and the total number of plants/ plot was 240. Calcium super-phosphate (15 % P_2O_5) at 200 kg /feddan was added on rows during the soil preparation. Ammonium nitrate (33% N), at 200kg/feddan and potassium sulphate (48% K_2O) at 100 Kg/feddan were applied as soil application in two times, the first was done after 18 days from sowing at the first irrigation and the second one was carried out after one month from the first addition. All recommended agricultural practices were followed according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. The treatments were as follows: 1) Bion 3 mM; 2) salicylic acid 5 mM; 3) *P. polymyxa*; 4) Rhizolex-T; 5) Water (Control). The disease incidence (DI) % was determined as mentioned before. Random samples of seven common bean plants were collected (from the inner rows) at the end of the flowering and harvest stages from each plot. Parameters of plant vegetative growth and Seed yield such as plant height (cm), number of leaves/plant, leaves fresh weight/plant (g), leaves dry weight/plant (g), number of pods/ plant, seed yield/ plant (g) and hundred-seed weight were recorded as well as seed yield Kg/ feddan was calculated by multiplying the average seed yield per plant by number of plants per feddan.

Effect of common bean seed treatment with inducer on activity of oxidative enzymes and phenol content.

An experiment was carried out to determine activity of oxidative enzymes and phenol content. Common bean plants were grown as mentioned before in greenhouse experiment. Fifteen days after sowing activity of peroxidase (PO), polyphenol oxidase (PPO) and phenol contents was determined in tissue extracts of common plants surviving from the following treatments: 1) Bion 3 mM; 2) salicylic acid 5 mM; 3) *P. polymyxa;* 4) Water in infested soil (Control, infected) and 5) Water in non-infested soil (Control, healthy).

Assay of enzymes activities

Extraction and assay of peroxidase (PO) activity were carried out according to Chakraborty & Chatterjee (2007). Enzyme activity was recorded (using pyrogallol reagent) as the change in absorbance at 430 nm using a Milton Roy 601 UV-Vis spectrophotometer, immediately after the addition of substrate (H_2O_2).

Extraction and assay of polyphenoloxidase enzyme (PPO) were carried out according to Sadasivam & Manickam (1996). The enzyme activity was measured as the change in absorbance per minute at 495 nm using a Milton Roy 601 UV-Vis spectrophotometer immediately after the addition of catechol solution, which initiated the reaction.

Determination of phenolic compounds

Extraction of phenolic compounds was carried out according to Sutha *et al.,* (1998). Total and free phenols determinations were carried out using Folin Ciocalteau reagent following the method described by Snell & Snell (1953) and results were expressed as mg equivalents of Catechol per gram fresh weight.

Statistical analysis

Completely randomized design (CRD) and randomized blocks design (RBD) were conducted in greenhouse experiment and Field experiment, respectively. The obtained data were subjected to computer statistical software (ASSISTAT) originated by Silva & Azevedo (2009). Data analyzed using analysis of variance (ANOVA), and mean values were compared using Duncan's multiple range test at a significance level of $P \leq 0.05$.

RESULTS

1- Enumeration of bacteria coated on seeds

Enumeration of bacteria coated on seeds was performed by plate dilution method on the basis of colony forming unit (CFU/seed) on nutrient yeast dextrose agar (NYDA) medium, after 21 hrs from treatment. Population densities of *P. polymyxa* were 3.2×10^5; 2.8×10^5 2.5×10^5 and 3.0×10^5 CFU per seed of common bean in greenhouse experiment, field experiments in 2009, 2010 and activity of oxidative enzymes experiment, respectively.

2- Greenhouse experiment

A-Effect of some inducers on the incidence of common bean *Rhizoctonia* damping- off disease.

In this experiment, common bean seeds were soaked in aqueous solutions (3mM and 5mM) of Bion and salicylic acid for one hour just before sowing as chemical inducers, or soaked in culture filtrate of *P. polymyxa* for one hour and then seeds were coated with bacterial cells slurry of *P. polymyxa* as biotic inducer to study their effect on the incidence of Rizoctonia damping- off disease in pots. Data in Table (1) indicate that all treatments decreased percentages of pre and post-emergence damping- off compared with control grown in infested soil. There was no significant difference among the treatments by Bion, salicylic acid and *P. polymyxa* in reducing pre and post emergence damping- off. Data also, indicated that the highest percentage of survival plants was achieved from treatment with Bion 5mM followed by each of Bion 3mM and salicylic acid 5mM.

Table 1. Effect of Bion, Salicylic Acid (SA)[1] and *Paenibacillus polymyxa*[2] as seed treatments on the percentage of damping- off disease of common bean plants caused by *Rhizoctonia solani* under greenhouse condition (artificially infection)[3].

Treatments	Damping- off				Survival plants %	Increasing %
	Pre-emergence		Post- emergence			
	Incidence %	Reduction %	Incidence %	Reduction %		
Bion 3mM	13.3 b	66.67	10.0 b	40.12	76.7 bc	91.27
Bion 5mM	10.0 b	74.94	6.7 b	59.88	80.0 b	99.50
Salicylic Acid 3mM	16.7 b	58.15	10.0 b	40.12	70.0 c	74.56
Salicylic Acid 5mM	13.3 b	66.67	6.7 b	59.88	76.7 bc	91.27
P. polymyxa	16.7 b	58.15	10.0 b	40.12	73.3 bc	82.79
Control[4] (*R. solani*)	39.9 c	0.00	16.7 c	0.00	40.1 d	0.00
Control[4] Healthy non infested soil)	3.3 a		0.0 a		96.7 a	

1- Common bean seeds (cv. Nebraska) were soaked in aqueous solutions of Bion and salicylic acid for one hour just before sowing.

2- Common bean seeds were soaked in culture filtrate for one hour, and then seeds were coated with bacterial cells slurry of *P. polymyxa.*

3- Soil infestation was achieved by mixing the inoculum of *R. solani* with the soil at the rate of 2% of soil weight

4- Control common bean seeds were soaked in sterilized water for one hour just before sowing.

5- Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

B- Effect of some inducers on the some growth parameters of common bean plants infected with *Rhizoctonia solani* under greenhouse condition.

Data presented in Table (2) reveal that Bion, salicylic acid and *P. polymyxa* treatments caused a significant increase in the values of shoot length, shoot dry weight and root dry weight over the control treatment grown in infested soil by *R. solani.* The highest values of shoot length, shoot dry weight and root dry weight of common bean plants grown in *R. solani* infested soil were recorded from treatment with Bion 3mM followed by control grown in disinfested soil. Meanwhile, there was no significant difference between the treatments with Bion 3mM and Bion 5mM in common bean plants grown in infested soil by *R. solani* and control (healthy plants) grown in disinfested soil regarding to shoot length and root dry weight.

Table 2. Effect of Bion, Salicylic Acid (SA)[1] and *Paenibacillus polymyxa*[2] as seed treatments on Shoot length , Shoot dry weight and Root dry weight of common bean plants infected with *Rhizoctonia solani* under greenhouse condition (artificially infection)[3].

Treatments	Shoot length (cm)	Increasing over control %	Shoot dry weight (g/plant)	Increasing over control %	Root dry weight (g/plant)	Increasing over control %
Bion 3mM	35.4 a	49.4	1.74 a	114.8	0.77 a	113.9
Bion 5mM	33.9 ab	43.0	1.56 c	92.6	0.73 ab	102.8
Salicylic Acid 3mM	32.9 bc	38.8	1.29 e	59.2	0.67 b	86.1
Salicylic Acid 5mM	34.4 ab	45.1	1.40 d	72.8	0.68 b	88.9
P. polymyxa	31.9 c	34.6	1.29 e	59.2	0.70 b	94.4
Control (*R. solani*)	23.7 d	0.0	0.81 f	0.0	0.36 c	0.0
Control Healthy non infested soil)	34.6 ab		1.64 b		0.79 a	

1- Common bean seeds (cv. Nebraska) were soaked in aqueous solutions of Bion and salicylic acid for one hour just before sowing.
2- Common bean seeds were soaked in culture filtrate for one hour, and then seeds were coated with bacterial cells slurry of *P. polymyxa.*
3- Soil infestation was achieved by mixing the inoculum of *R. solani* with the soil at the rate of 2% of soil weight
4- Control common bean seeds were soaked in sterilized water for one hour just before sowing.
5- Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

3- Field experiments

A- Effect of some inducers on the incidence of common bean damping-off disease.

In these experiments, the effect of treatments by Bion 3mM, salicylic acid 5mM , *P. polymyxa* and the fungicide Rizolex T on damping- off incidence and survival of common bean plants under Field conditions during summer 2009 and 2010 growing seasons was studied. Results in Table (3) exhibited that in 2009 growing season, all the treatments significantly decreased the percentage of pre and post-emergence damping-off compared with untreated control. The highest percentage of pre-emergence damping-off reduction over the control was obtained from treatment with Rhizolex-T and Bion 3mM. Meantime, the percentage of post-emergence damping-off reduction over the control was equal from treatments with Rhizolex-T and Bion 3mM but significantly different from treatments with salicylic acid and *P. polymyxa.* Also, results showed that all the tested treatment significantly increased the percentage of survived plants compared with the control. There were no significant differences between the treatments with Bion and fungicide, whilst the treatments with salicylic acid and *P. polymyxa* were less effective regarding Survival plants. To some extent, the same results were obtained in 2010 growing season except with post- emergence damping- off which showed no significant difference among treatments.

Table 3. Effect of Bion, Salicylic Acid (SA)[1], *Paenibacillus polymyxa*[2] and Rhizolex-T[3] as seed treatments on the percentage of damping-off disease of common bean plants under field condition during summer seasons 2009 (I) and 2010 (II) (natural infection).

(I): season 2009

Treatments	Damping- off				Survival plants %	Increasing %
	Pre-emergence		Post- emergence			
	Incidence %	Reduction %	Incidence %	Reduction %		
Bion 3mM	8.3 c	63.1	1.7 c	74.6	90.0 a	28.6
Salicylic Acid 5mM	11.3 b	49.8	3.3 b	50.7	84.2 b	20.3
P. polymyxa	11.7 b	48.0	3.8 b	43.3	83.3 b	19.0
Rhizolex-T[3]	7.9 c	64.9	1.7 c	74.6	90.4 a	29.1
Control[4]	22.5 a	0.0	6.7 a	0.0	70.0 c	0.0

(II): season 2010

Treatments	Damping- off				Survival plants %	Increasing %
	Pre-emergence		Post- emergence			
	Incidence %	Reduction %	Incidence %	Reduction %		
Bion 3mM	8.3 c	61.8	2.1 b	78.1	88.8 a	35.0
Salicylic Acid 5mM	12.1 b	44.2	2.9 b	69.8	84.6 b	28.6
P. polymyxa	13.8 b	36.4	2.5 b	74.0	82.9 b	26.0
Rhizolex-T[3]	8.8 c	59.4	2.1 b	78.1	88.3 a	34.1
Control[4]	21.7 a	0.0	9.6 a	0.0	65.8 c	0.0

1- Common bean seeds (cv. Nebraska) were soaked in aqueous solutions of Bion and salicylic acid for one hour just before sowing.

2- Common bean seeds were soaked in culture filtrate for one hour, and then seeds were coated with bacterial cells slurry of *P. polymyxa.*

3- Seed dressing by fungicide was carried out at the recommended dose (3 g/kg).

4- Control common bean seeds were soaked in sterilized water for one hour just before sowing.

5- Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

B) Effect of some inducers on vegetative characteristics of common bean plants

Results presented in Table (4) show the effect of Bion, salicylic acid and *P. polymyxa* treatments on vegetative characteristics, i.e. stem length, number of leaves/plant as well as fresh and dry weight of leaves/plant compared with untreated control in two growing seasons, 2009 and 2010. To some extent, the two growing seasons showed nearly similar results which indicated that all treatments significantly increased vegetative characteristics as compared to untreated control. Meantime, Bion 3mM and the fungicide (Rhizolex-T) treatments showed significantly increased in all vegetative characteristics compared with salicylic acid and *P. polymyxa* treatments.

C) Effect of some inducers on seed characteristics of common bean plants

Results presented in Table (5) show the effect of Bion, salicylic acid and *P. polymyxa* treatments on seed characteristics, i.e. number of pods/plant, one hundred seed weight (g), seed yield (g)/plant as well as seed yield (Kg)/feddan of common bean plants compared with untreated control in two growing seasons, 2009 and 2010. Results exhibited that approximately, all the treatments significantly increased seed characteristics as compared with untreated control. Bion 3mM and the fungicide (Rhizolex-T) treatments showed significantly increased number of pods/plant and seed yield (g)/plant compared with salicylic acid and *P. polymyxa* treatments during the two growing seasons 2009 and 2010. Concerning one hundred seed weight (g), there was no significant difference among the treatments by Bion, fungicide (Rhizolex-T) and salicylic acid during the two growing seasons. Meanwhile, higher increase in seed yield (kg/ feddan) was estimated with bion and fungicide treatments (86% and 87.7%, respectively) followed by salicylic acid and *P. polymyxa* (66.8 and 55.4 %, respectively) increasing over the untreated control calculated as means of the two seasons.

Table 4. Effect of Bion, Salicylic Acid (SA)[1], *Paenibacillus polymyxa*[2] and Rhizolex-T[3] as seed treatments on vegetative characteristics of common bean plants under field condition during summer seasons 2009 and 2010.

Treatments	Stem length (cm)		Number of leaves/ plant		Leaves Fresh weight/ plant (g)		Leaves Dry weight/ plant (g)	
	2009	2010	2009	2010	2009	2010	2009	2010
Bion 3mM	41.5 a	39.9 a	18.2 a	17.4 a	49.9 a	48.8 a	9.3 a	9.0 a
Salicylic Acid 5mM	40.5 b	38.4 ab	17.4 ab	16.7 b	46.4 b	44.8 b	8.6 b	8.5 b
P. polymyxa	38.7 c	36.9 b	16.8 b	16.3 b	44.9 b	43.9 b	8.3 b	8.1 b
Rhizolex-T[3]	40.6 b	40.3 a	18.3 a	17.7 a	51.3 a	50.2 a	9.5 a	9.2 a
Control[4]	32.1 d	31.3 c	13.1 c	12.7 c	35.2 c	34.1 c	6.8 c	6.4 c

1- Common bean seeds (cv. Nebraska) were soaked in aqueous solutions of Bion and salicylic acid for one hour just before sowing.

2- Common bean seeds were soaked in culture filtrate for one hour, and then seeds were coated with bacterial cells slurry of *P. polymyxa.*

3- Seed dressing by fungicide was carried out at the recommended dose (3 g/kg).

4- Control common bean seeds were soaked in sterilized water for one hour just before sowing.

5- Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

Table 5. Effect of Bion, Salicylic Acid (SA)[1], *Paenibacillus polymyxa*[2] and Rhizolex-T[3] as seed treatments on seed characteristics of common bean plants under field condition during summer seasons 2009 and 2010.

Treatments	Number of pods / plant		One hundred seed weight (g)		Seed yield / plant (g)		Seed yield (Kg) / feddan	
	2009	2010	2009	2010	2009	2010	2009	2010
Bion 3mM	16.5 a	16.2 a	49.2 a	47.8 a	22.4 a	21.8 a	1814	1738
Salicylic Acid 5mM	15.8 ab	15.1 b	46.6ab	45.3ab	21.5ab	20.5 b	1627	1558
P. polymyxa	15.4 b	14.8 b	46.1bc	44.6 b	20.3 b	19.4 b	1522	1447
Rhizolex-T[3]	16.3 a	16.2 a	48.8ab	46.9ab	22.5 a	22.1 a	1830	1756
Control[4]	11.5 c	12.0 c	43.9 c	42.1 c	15.6 c	15.7 c	982	928

1- Common bean seeds (cv. Nebraska) were soaked in aqueous solutions of Bion and salicylic acid for one hour just before sowing.

2- Common bean seeds were soaked in culture filtrate for one hour, and then seeds were coated with bacterial cells slurry of *P. polymyxa.*

3- Seed dressing by fungicide was carried out at the recommended dose (3 g/kg).

4- Control common bean seeds were soaked in sterilized water for one hour just before sowing.

5- Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05).

4- Effect of common bean seed treatment with inducer on activity of oxidative enzymes and phenol content.

A) Activity of oxidative enzymes

All inducer treatments were effective in eliciting the enzyme activities (peroxidase and polyphenol oxidase) (Table 6). The maximum activities of the two enzymes were observed with Bion treatment. Meantime, Peroxidase activity was higher with Bion treatment followed by SA treatment 77.7 and 41.2 % increasing over the untreated control, respectively. Elevation of the polyphenol oxidase activity was showed with Bion treatment followed by SA treatment as 97.7 and 58.0% increasing over the untreated control, respectively. Whereas a less increase was recognized when *P. polymyxa* was applied 34.8 and 38.9% increasing over the untreated control for Peroxidase and polyphenoloxidase, respectively. However, the least activities of the two enzymes were recorded in healthy control treatment.

Table 6.Effect of Bion, Salicylic Acid (SA)[1], and *Paenibacillus polymyxa*[2] as seed treatments on the activity of peroxidase and polyphenol oxidase in common bean plants grown in soil infested with *Rhizoctonia solani* under greenhouse condition (artificially infection)[3]

Treatments	Peroxidase activity[4] (absorbance at 430 nm)		polyphenol oxidase[4] activity (absorbance at 495 nm)	
	Activity	Increasing over control	Activity	Increasing over control
Bion 3mM	1.97	77.7	0.259	97.7
Salicylic Acid 5mM	1.57	41.2	0.207	58.0
P. polymyxa	1.49	34.8	0.182	38.9
Control[5] (*R. solani*)	1.11	0.0	0.131	0.0
Control Healthy (non infested soil)	0.83		0.091	

1- Common bean seeds (cv. Nebraska) were soaked in aqueous solutions of Bion and salicylic acid for one hour just before sowing.

2- Common bean seeds were soaked in culture filtrate for one hour, and then seeds were coated with bacterial cells slurry of *P. polymyxa.*

3- Soil infestation was achieved by mixing the inoculum of *R. solani* with the soil at the rate of 2% of soil weight

4- Enzyme activity is expressed as change in absorbance/minute /g fresh weight

5- Control common bean seeds were soaked in sterilized water for one hour just before sowing.

B) Phenol content

All inducer treatments were effective in enhancing the total phenols contents (Table 7). Bion treatment resulted in the highest increase in total phenols contents over the untreated control (94.3%) followed by salicylic acid and *P. polymyxa* (57.9 and 52.6% over the untreated control, respectively). In general, the total phenol contents, free phenols and conjugated phenols were found to be higher in plants with Bion and SA treatments over the control. Whereas a less increase was recognized in free phenols content (16%), when *P. polymyxa* was applied compared with untreated control. Moreover, the least values in total and free phenols were recorded in healthy control treatment.

Table 7.Effect of Bion, Salicylic Acid (SA)[1], and *Paenibacillus polymyxa*[2] as seed treatments on levels of phenolic compounds in common bean plants grown in soil infested with *Rhizoctonia solani* under greenhouse condition (artificially infection)[3]

Treatments	Phenolic contents (mg/g fresh weight)					
	Total phenols	Increase over control	Free phenols	Increase over control	Conjuga-ted phenols	Increase over control
Bion 3mM	3.928	94.3	2.429	55.7	1.499	167.7
Salicylic Acid 5mM	3.193	57.9	2.102	43.9	1.091	94.8
P. polymyxa	3.084	52.6	1.695	16.0	1.389	148.1
Control[4](*R. solani*)	2.021	0.0	1.560	0.0	0.560	0.0
Control Healthy (non infested soil)	1.371		1.036		0.459	

1- Common bean seeds (cv. Nebraska) were soaked in aqueous solutions of Bion and salicylic acid for one hour just before sowing.

2- Common bean seeds were soaked in culture filtrate for one hour, and then seeds were coated with bacterial cells slurry of *P. polymyxa.*

3- Soil infestation was achieved by mixing the inoculum of *R. solani* with the soil at the rate of 2% of soil weight

4- Control common bean seeds were soaked in sterilized water for one hour just before sowing.

DISCUSION

Common bean (*Phaseolus vulgaris* L.), a legume native to America, is now one of the most important crops worldwide. Rhizoctonia root rot disease caused by *Rhizoctonia solani* Kühn is a serious and economically important disease for bean production in most of the tropical, subtropical and temperate areas of the world (Tu *et al.,* 1996). Meantime, excessive and improper use of pesticides including fungicides presents a menace to the health of human, animal and environment. So, research priorities call for novel protection methods that are compatible with sustainable agriculture. Acquired resistance that increases plant resistance to subsequent pathogen attack, by using biotic (microorganisms) or abiotic (chemicals) agents as inducers seem to be one of alternatives to substitute for, or at least to decrease the use of fungicides in plant disease control. Resistance induced by these agents (resistance elicitors) has broad spectrum against numerous pathogens and long lasting, but rarely provides complete control of infection, as many resistance elicitors provide between 20 and 85% disease control. (Kuc, 1982; Kuc, 2001; da Rocha & Hammerschmidt, 2005; Walters *et al.,* 2005 and Lyon, 2007).

In the present research, seed treatment with Bion (benzothiadiazole), salicylic acid and *P. polymyxa* as seed treatments induced systemic resistance in common bean plants (cv. Nebraska) and enhanced resistance to pre emergence and post emergence damping off caused by *R. solani* under green house and field conditions, compared with the control. Furthermore, the treatments increased the vegetative and seed growth parameters of common bean plants under field conditions.

Seed treatment of common bean with Bion [benzothiadiazole (BTH)] developed significantly higher resistance to Rhizoctonia root rot infection and highest percentage of survival plants. In this respect, the benzothiadiazole (BTH) previously showed to be an efficient broad-spectrum resistance inducer against bacterial, fungal and viral diseases in different monocot and dicot crops (Walters *et al.,* 2013). Early, it was shown to induce a typical SAR response in wheat and tobacco, effective against different pathogens and resulting in induced expression of “SAR genes” (Görlach *et al.,* 1996; Friedrich *et al.,* 1996). Benzothiadiazole (BTH) is widely reported to induce resistance against a broad spectrum of pathogens in many plant species, for example in apple against *E. amylovora* (Brisset *et al.,* 2000 and 2002); in common bean against *Uromyces appendiculatus* (Iriti & Faoro, 2003); in grapevine against obligate biotrophic oomycete *Plasmopara viticola* (Perazzolli *et al.,*2008); in pea against *Uromyces pisi* (Barilli *et al.,* 2010a and 2010b); in muskmelon against *Alternaria alternata* and *Fusarium* spp. (Zhang *et al*., 2011); in tomato against *Ralstonia solanacearum* (Hong *et al*., 2011) and against *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Myresiotis *et al*., 2012); in cabbage against *Peronospora parasitica* (van der Wolf *et al*., 2012); in faba bean against *Uromyces viciae-fabae* and *Orobanche crenata* (Sillero *et al*., 2012); in rice against *R. solani* (Sood *et al.,* 2013); The mechanisms of BTH-induced plant resistance have been shown to involve the activation of SAR mechanisms based on the salicylic acid (SA) pathway (Friedrich *et al.,* 1996), with consequent up-regulation of defence genes (Bovie *et al.,* 2004) and accumulation of phenolic compounds (Iriti *et al.,* 2004); induced synthesis of chitinase and β-1,3-glucanase isozymes (Burketova *et al.,* 1999); also, activating resistance by increasing the activity of peroxidase (POD) (Sarma *et al.,* 2007). The beneficial effect of BTH in reducing the extent of fungal colonization in the root tissues is primarily associated with a massive accumulation of structural barriers i.e. wall appositions (Benhamou, 1996).

In the present work, application of salicylic acid had a significant effect on damping off reduction. In greenhouse and field experiments, the highest percentage of disease reduction over the control followed Bion treatment was obtained from treatment with salicylic acid (5mM). Salicylic acid a simple phenolic compound is an important and well-studied endogenous plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in plant defence in addition to their impact on plant growth and development (Lu, 2009; Vlot *et al.,* 2009 and Vicent & Plasencia, 2011). The role of salicylic acid in plants was recorded for the first time in 1979 (White, 1979). Treatment with SA and its derivative induced expression of pathogenesis-related (PR) proteins (Malamy *et al.,* 1990 and Gaffney *et al.,* 1993). So salicylic acid as a key plant hormone plays an important role in induction of plant defence against a variety of biotic and abiotic stresses through morphological, physiological and biochemical mechanisms (Hayat *et al*., 2009 and Kumar, 2014). It regulates the activities of various enzymes such as, peroxidase (POD), polyphenol oxidase PPO, phenylalanine ammonia lyase (PAL) etc., which are the major components of induced plant defence against biotic and abiotic stresses (Idresse, *et.al*., 2011).

On the other side, treatment with *P. polymyxa*, the genus which was created by Ash *et al.,* in 1993 to accommodate the former 'group 3' of the genus Bacillus, had also a significant effect on damping off reduction and significantly enhanced the vegetative and seed growth parameters of common bean plants under greenhouse and field conditions compared with untreated control, although, its treatment was less effective than Bion. Previous reports have shown that *P. polymyxa* controls many soil and foliar pathogens in the greenhouse and in the field (Dijksterhuis *et al.,* 1999; Helbig, 2001; Beatty & Jensen, 2002; Timmusk *et al.,* 2003; Ryu *et al.,* 2006; Khan *et al.,* 2008; Raza *et al.,* 2009; Phi *et al.,* 2010, Postma *et al.,* 2013 and Raza *et al.,* 2015). *P. polymyxa* is known for its ability to produce antimicrobial compounds (produced by some but not all strains) act against fungi, bacteria and actinomycetes including LI-F antibiotics (Deng *et al.,* 2011); fusaricidins (Beatty & Jensen, 2002) ; polymyxins and lantibiotics (He *et al.,* 2007); gavaserin and saltavidin (Pichard *et al.*, 1995); fusaricidins (Kajimura & Kaneda, 1996) and polyxin (Piuri *et al.,* 1998). More than that, *P. polymyxa* strains are capable of producing several hydrolytic enzymes, including β1,3-glucanases and chitinases which are considered key enzymes in control of fungal plant diseases (Mavingui & Heulin, 1994; Jung *et al.,* 2003 and Raza *et al.,* 2009) as well as some isolates have siderophore-producing capabilities. The production of these antibiotics could provide an advantage for establishing the population during the germination of seeds. Plant growth promotion may be an indirect effect of this antibiotic production through the suppression of plant diseases in disease-carrying soil. One of the possible explanations for growth promotion by *P. polymyxa* which have also been reported that it produces many plant growth stimulators, including auxin as indole-3-acetic acid (Lebuhn *et al.,* 1997; da Mota *et al.,* 2008 and Phi *et al.,* 2010) ; cytokinin (Timmusk *et al.,* 1999); and 2,3-butanediol (Nakashimada *et al.,* 2000).

However, the present results indicated that treatments with Bion, salicylic acid and *P. polymyxa* as seed treatments were effective in eliciting the enzyme activities (peroxidase and polyphenol oxidase), and the maximum activities of the two enzymes were observed with Bion treatment. In this respect, oxidative reduction enzymes play an important role in induced resistance, the oxidation of phenols is mediated by the enzyme polyphenoloxidase and peroxidase and the resulting quinines are effective inhibitors of SH group of enzymes which may be inhibiting to the pathogen (Goodman *et al.,* 1967). Peroxidase is reported to have an important function in secondary cell wall biosynthesis by polymerizing hydroxy and methoxycinnamic alcohols into lignin and forming rigid cross-links between cellulose, pectin, hydroxyproline-rich glycoproteins (HRGP) and lignin (Grisebach, 1981). Therefore, Peroxidase may be directly associated with the increased ability of systemically protected tissue to lignify which may restrict the penetration (Gross, 1979). Meantime, polyphenoloxidase (PPO) is a widespread enzyme found in plant cells, located in the chloroplast thylakoid membranes and plays an important role in plant resistance. However, it indicates the highest activity toward hydroxylation of monophenols to diphenols, and is capable of dehydrogenating of o-diphenols to produce o-quinones (antimicrobial compounds) as well as lignifications of plant cells during microbial invasion (Meyer, 1987). In case of oxidation of phenolic compounds in plant cells, it is responsible for initiating the browning reaction of the tissues and it is considered as indicator of invasion by pathogens (Boss *et al.,* 1995). Moreover, polyphenoloxidase induces metabolization of these phenolic compounds into more toxic forms. (Chranowski *et al*., 2003). Furthermore, the treatments led to increase the phenolic compounds content compared with the untreated control. In this respect, phenols are oxidized to quinones or semi-quinones which are more toxic and play a great role as antimicrobial substances (Farkas & Kiraly, 1962 and Gupta *et al.,* 1992).

From the obtained results, it can be concluded that treatments with Bion, salicylic acid and *P. polymyxa* as seed treatments increased plant resistance against the infection by *R. solani,* improved plant growth, yield, accumulation of some antimicrobial substances such as phenolic compounds and increasing activity of defence related enzymes. Such these treatments may be used as a part of integrated disease management for field crops in order to avoiding the use of fungicides.

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إستحثاث المقاومة فى نباتات الفاصوليا ضد مرض عفن الجذور الرايزوكتونى

شحاته طه شحاته[1]
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يهدف هذا البحث إلى دراسة تأثير معاملات بذور الفاصوليا من الصنف نبراسكا بكل من مادتى البيون وحامض السلسيلك بالإضافة إلى البكتريا *Paenibacillus polymyxa* على العدوى بالفطر *Rhizoctonia solani* تحت ظروف الصوبة والحقل. تحت ظروف الصوبة، أدت جميع المعاملات إلى إنخفاض نسبة موت البادرات قبل و بعد الظهور فوق سطح التربة مقارنة بالبذور غير المعاملة المنزرعة فى التربة المعداة بالفطر. وقد تحققت أعلى نسبة بقاء للنباتات على قيد الحياة من المعاملة بالمركب بيون بتركيز 5 مللى مول (80%)، يلي ذلك المعاملة بكل من البيون بتركيز 3مللى مول والمعاملة بحمض السلسيلك بتركيز 5 مللى مول (76.7%) مقارنة بالبذور غير المعاملة المنزرعة فى التربة المعداة بالفطر (40.1%). فى الوقت نفسه، تحققت زيادة معنوية في قيم طول المجموع الخضرى، الوزن الجاف للمجموع الخضرى و الوزن الجاف للجذور مقارنة بالنباتات الناتجة من البذور غير المعاملة المنزرعة فى التربة المعداه بالفطر *Rhizoctonia solani*.

خلال التجارب الحقلية أثناء موسمى صيف 2009 و 2010، أدت جميع المعاملات إلى زيادة معنوية فى نسب النباتات الباقية على قيد الحياة مقارنة بالنباتات الناتجة من البذور غير المعاملة. ولم توجد فروق معنوية بين المعاملة بالمركب بيون بتركيز 3 مللى مول وبين المعاملة بالمبيد الفطرى (ريزولكس- تى بمعدل 3جم/كجم تقاوى) حيث كانت نسبة النباتات الباقية على قيد الحياة 89.4 % و 89.3٪ على التوالي مقارنة بالنباتات الناتجة من البذور غير المعاملة 67.9 % كمتوسط للموسمين. على الجانب الآخر، كانت المعاملة بحمض السلسيلك بتركيز 5 مللى مول والمعاملة بالبكتريا *Paenibacillus polymyxa* أقل فعالية فيما يتعلق ببقاء النباتات على قيد الحياة خلال موسمى النمو عام 2009 و عام 2010. كما أدت المعاملات إلى زيادة معنوية فى كل الخصائص الخضرية المدروسة، وهى طول الساق وعدد الأوراق لكل نبات وكذلك وزن الأوراق الرطب والجاف لكل نبات مقارنة بالنباتات الناتجة عن البذور غير المعاملة. وكذلك، قدرت زيادة كبيرة في المحصول (كجم / فدان) مع المعاملة بمركب البيون والمعاملة بالمبيد الفطرى بنسب زيادة مئوية (86% و 87.7% على التوالي)، يلي ذلك المعاملة بحمض السلسيلك والمعاملة بالبكتريا *Paenibacillus polymyxa* بنسب زيادة مئوية (66.8 % و55.4 % على التوالي) مقارنة بالنباتات الناتجة عن البذور غير المعاملة كمتوسط للموسمين.

أشارت الدراسات المعملية، أن جميع المعاملات كانت فعالة في إستثارة نشاط إنزيمى البيروكسيديز والبوليفينول أوكسيديز. كان نشاط إنزيم البيروكسيديز أعلى مايمكن فى المعاملة بمركب البيون يليه المعاملة بحمض السلسيلك بنسب زيادة مئوية 77.7% و 41.2% ، على التوالي مقارنة بالنباتات الناتجة عن البذور غير المعاملة. فى نفس الوقت، قد ظهر ارتفاع فى نشاط إنزيم البوليفينول أوكسيديز فى المعاملة بمركب البيون يليه المعاملة بحمض السلسيلك بنسب زيادة مئوية 97.7 % و 58.0% ، على التوالي مقارنة بالنباتات الناتجة عن البذور غير المعاملة. في الوقت نفسه، أسفرت المعاملة بمركب البيون في أعلى زيادة في محتوى الفينولات الكلية (94.3 %) مقارنة بالنباتات الناتجة عن البذور غير المعاملة يلى ذلك المعاملة بحمض السلسيلك والمعاملة بالبكتريا *Paenibacillus polymyxa* بنسب زيادة مئوية (57.9 % و 52.6 % على التوالى) مقارنة بالنباتات الناتجة عن البذور غير المعاملة.