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ORIGINAL PAPER

Induction of Systemic Resistance in Cluster Bean Against Damping-off and Root Rot Diseases

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ABSTRACT

Efficacies of three abiotic inducers (systemic resistance agents) *i.e.*, bion, humic acid (HA), salicylic acid (SA), compared to Rizolex-T50 as seed treatments were tested against damping-off and root rot diseases of cluster bean (*Cyamopsis tetragonoloba* L.) in pot and field experiments. In green house, pathogenicity test indicated that the three tested fungi were pathogenic and caused emergence damping-off. *R. solani* caused the highest percentage of pre-emergence damping-off. Moreover, the lowest percentage of survived plants was occurred under *F. oxysporum* and *R. solani* (26.7% for each). Rizolex-T50 presented the highest reduction in disease parameters in infested soil with the three fungi, Bion came next to the fungicide followed by humic acid then salicylic acid. All the investigated treatments significantly increased the activity of chitinase, β-1, 3-gluctronase, peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) enzymes. As well as total content of phenolic compounds and total content of lignin were increased in cluster bean plants grown in artificially infested soil with the three tested fungi (*Fusarium oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina*) each alone compared with untreated control. In field, pre sowing seed treatments with the desired inducer of resistance inducer caused considerable increase in the photosynthesis pigments (chlorophyll-a, chlorophyll-b and total chlorophyll) and seed quality (total nitrogen and total protein). In general, the highest figured data of the increase in the cluster bean tissue were associated with the inducer resistance agents *i.e.*, Bion, salicylic acid and humic acid, respectively, followed by seed treatment with the fungicide Rizolex-T50. Whereas the lowest increase was shown in control treatment. It could be concluded that any of bion, HA or SA can act as inducer of systemic resistance in cluster bean plant against infection by each of *Fusarium oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* infections. Consequently, these agents could be recommended for management damping-off and root rot diseases in cluster bean plants and improving photosynthetic pigments and seed quality.

Keywords: Cluster bean, *Cyamopsis tetragonoloba*, damping-off, root rot, inducer resistance chemicals, biochemical analysis.

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INTRODUCTION

Cluster bean or guar [(*Cyamopsis tetragonoloba*) (L.) Taub. (Syn. *C. psoraliodes*)], commonly known as guar is important crop belonging to family Fabaceae (Singh, 2014).

Cluster bean is primarily grown for seed, animal feed, fodder, vegetable and green manuring purposes. Cluster bean is important source of high-quality galactomannan gum and protein (40-50%), with availability as meal animal feed. Seed gum is used in various industries such as textiles, paper, cosmetics, explosives and food processing. Besides the gum preparation, cluster bean is emerging as a potential source of vegetable protein for human beings (Kumar and Singh, 2002).

Cluster bean tender pods are nutritionally rich in energy, protein, fat, carbohydrate, Vitamin C and iron (Kumar and Singh, 2002).

The importance of this crop has increased in Egypt, especially in the new reclaimed area, where have many beneficial effects on soil physical structure and could be considered as a friendly crop to the environment related to its efficient nitrogen fixation system, in addition to its improvement to the traditional cereal rotation and protein supply in low input farming systems (Akande *et al*., 2007; Abdel-Monaim, 2018 and Abd-El-Rahman *et al*., 2018).

Pathogenic microorganisms cause various plant diseases that usually weaken or destroy plant tissues and reduce crop yields varying from 25-100% (Frisvad and Samson, 1991). Root diseases are estimated to cause 10-15% yield losses annually in the world (Bajoria *et al*., 2008). *Rhizoctonia solani* Kuhn (teleomorph:

Thanatephorus cucumeris (Frank). Donk is an ecologically diverse soil-borne fungus that causes root rot disease on cluster bean plants.

Damping-off and root rot diseases are the most damaging soil and seed borne diseases that attack cluster bean and affect germination and plant growth as well as yield. It is known to suffer from many fungi, *i.e*., *M. phaseolina*, *Rhizoctonia salami, and F. oxysporum*, which are the most common fungi causing considerable yield losses (Matloob and Juber, 2013; Abdel-Monaim, 2018 and Abd-El-Rahman *et al*., 2018). These pathogens are difficult to control because of their persistence in the soil and their wide host range. Some Chemicals are effective in controlling these diseases, but these chemicals are expensive and not environmentally friendly. Thus, there is a growing need to develop alternative approaches for the management of these pathogens. An acceptable approach that is being actively investigated involves the use of abiotic agents such as bion, humic acid and salicylic acid, which using as a resistance inducer for induction of systemic resistance in plant.

MATERIALS AND METHODS

Isolation, purification and identification of fungal pathogens:

Cluster bean plant samples infected with root rot were gathered from diverse localities of Dakahliya and Damietta governorates, Egypt. The infected roots were cut into minor pieces and surface sterilized with 2% sodium hypochlorite for two min. The sterilized pieces washed with sterilized water and dried between tow sterilized filter papers. The sterilized samples placed onto potato dextrose agar (PDA) medium supplemented with streptomycin-sulfate (100 µg ml^{-1}) and incubated at 25°C. The growing fungi were isolated and purified using the hyphal tip and single spore techniques. The isolated fungi were identified based on their cultural, morphological, and microscopic characters according to Barnett and Hunter (1972); Booth (1977); Dhingra and Sinclair, (1978); and Sneh *et al*. (1992). The isolated fungi were sub-cultured in PDA slants and kept at 6°C in a refrigerator.

Pot experiments:

Pathogenicity tests:

The pathogenicity tests of the three isolated fungal, *F. oxysporum*, *M. phaseolina* and *R. solani*, were carried out at Hosinia Agric. Res. Stat, Sharkia Governorate, Egypt.

Preparation of fungal inocula and soil infestation

Sterilized sorghum medium (200 g sorghum / bottle of one liter capacity and enough water to cover the sorghum) was used for preparation of each fungal inoculum. The media were mixed and autoclaved for 20 minutes then inoculated with the inoculum of the desired fungus, each alone, and incubated at 28±2°C for 15days.

Clay silty soil was disinfested with 5% formalin solution under plastic sheet and left for two weeks until formalin evaporates.

The sterilized soil was infested with the inoculum of each fungus alone, at the rate of 2 % (W/W) and distributed in plastic pots (25cm in diameter). Infested soils were mixed thoroughly and watered for one week to insure even distribution of the inoculum. Cluster bean seeds were sown at the rate of ten seeds / pot. A set of three replicates was used for each fungus. Also, three pots containing non-infested soil (sterilized) were used as control. Percentages of pre-and post-emergence damping-off were recorded at 15 and 30 days after sowing, respectively, according to the following formulas:

$$\textbf{Pre-emergence damping-off \%} = \frac{\textbf{No. of non-germinated seeds after 15 d.}}{\textbf{Total no. of planted seeds}} \times 100$$

$$\textbf{Post-emergence damping-off \%} = \frac{\textbf{No. of dead seedlings after 30 d.}}{\textbf{Total no. of planted seeds}} \times 100$$

The dead plants due to the infection by root rot and / or wilt were assessed 90 days after sowing and recorded according to Muthomi *et al*. (2007**).**

$$\textbf{Dead plants \%} = \frac{\textbf{No. of dead plants after 90 d.}}{\textbf{Total no. of planted seeds}} \times 100$$

Seed treatments with chemical inducers:

Cluster bean seeds were soaked for 3 hours in 50 mM of the inducer resistance chemicals *i.e.*, bion, HA and SA and 1.5g/L of the fungicide Rizolex-T50 each alone before sowing.

The treated seeds were sown in pots (25 cm. in diameter) contained infested soil with any of the three tested pathogenic fungi, *viz., F. oxysporum*, *M. phaseolina* and *R. solani* at the rate of 2%. Ten seeds were sown in each pot, for comparison un-treated seeds were sown in soil infested with the tested pathogenic fungi, each alone. A set of three replicates was used for each treatment. Percentages of pre-and post-emergence damping-off as well as root rot were recorded at 15, 30 and 90 days after sowing, respectively.

Biochemical changes under fungal infection:

Estimation of pathogenesis related protein, oxidative-reductive enzymes, phenolic compounds and lignin:

The activity of pathogenesis related protein (Chitinase and β-1, 3-glucanase) and oxidative-reductive enzymes *viz.* peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) as well as well as content of each of phenolic compounds and lignin in cluster bean leaves under treatment with inducer resistance chemicals and the fungicide Rizolex-T50 were studied at artificial infection by each of *F. oxysporum*, *R. solani* and *M. phaseolina* alone in pots.

One month after sowing, one g. fresh leaf samples were taken from the treated cluster bean plants with the previous treatments grown in soil infested and un-infested with the tested pathogenic fungi, individually, then extracted according to Maxwell and Bateman (1967). One g. of plant tissue was homogenized in 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6.8) containing 1M NaCl, 1% polyvinylpyrrolidone, (PVP),1 mM EDTA and 10 mM β-mercaptoethanol (Biles and Martyn, 1993). Sample was filtrated through cheesecloth; the homogenates were centrifuged at 8000 rpm at 4°C for 25 min. The supernatants (crude enzyme extract) were stored at −20°C then immediately used for determination of chitinase and β-1, 5–glucanase, peroxidase (PO), Polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL) enzymes activities.

Enzymes related plant defense:

Chitinase activity:

The chitinase activity was determined using the method of Monreal and Reese (1969). High polymeric chitin labeled covalently Remazol Brilliant Violet 5R (CM-Chitin*-RBV. Comp. Loewe Biochemica) was used as substrate. The reaction mixture consisted of 0.50 mL 0.01 M Na-Acetate buffer pH 5.2 with 5% (v/v) glycerin, 0.25 mL plant extract and 0.25 mL dye labeled substrate CM-*RBV solution (2 mg/mL). Tested samples were incubated in a water bath at 37°C for 120 min. The enzyme reaction was terminated by adding 0.25 mL 2 NHCl. After centrifugation (8000 rpm; 25 min), supernatants containing soluble, dye labeled degradation products were transferred to cuvet. Absorbency was measured spectrophotometrically at 550 nm; sodium acetate buffer was added to blanks instead of plant extract. Enzyme activity was expressed as enzyme unit/min/mg carbomethyl-substituted.

B-1, 3-glucanase activity:

B-1, 3-glucanase enzyme activity was assayed by the laminarin dinitrosalicylic acid method (Pan *et al*., 1991). Plant samples (1 g) were homogonized with 2 mL of 0.05 M sodium acetate buffer (pH 5.0) and centrifuged at 16 000g for 15 min at 4°C. The supernatant was used in the enzyme assay. The reaction mixture consisted of 62.5 µl of 4% laminarin and 62.5 µl of enzyme extract. The reaction was carried out at 40°C for 10 min. The reaction was then stopped by adding 375 µl of dinitrosalicylic acid and heating for 5 min on boiling water, vortexes and its absorbance was measured at 500 nm. The enzyme activity was expressed as µg glucose released min^{-1} mg^{-1} dinitro salicylic acid.

Peroxidase activity:

The activity of PO enzyme was determined by using the direct spectrophotometric method (Hammerschmidt *et al*., 1982) using guaiacol as common substrate for peroxidase. The reaction mixture consisted of 0.2 mL crude enzyme extract and 1.40 mL of a solution containing guaiacol, hydrogen peroxide (H_2O_2) and sodium phosphate buffer (0.2 mL 1% guaiacol+0.2 mL 1% H_2O_2+1 mL 10 mM potassium phosphate buffer), was incubated at 25°C for 5 min and the initial rate of increase in absorbance was measured over 1 min at 470 nm using spectrophotometer. Peroxidase activity was expressed as units of PO $min^{-1}mg^{-1}$ guaiacol (Urbanek *et al*., 1991).

Polyphenol oxidase activity:

The activity of PPO enzyme was determined by adding 50 µl of the crude extract to 3 mL of a solution containing 100 mM potassium phosphate buffer, pH 6.5 and 25-mM catechol. The increase of absorbance at 410 nm, for 10 min at 30°C, was measured (Gauillard *et al*., 1993). One PPO unit was expressed as the variation of absorbance at 410 nm per milligram of soluble catechol / minute.

Phenylalanine ammonia-lyase activity:

Phenylalanine ammonia layse (PAL) activity was determined using the direct spectrophotometric method adapted by Cavalcanti *et al.* (2007). Two hundred microliters of the crude enzyme extract previously dialyzed overnight with 100 mM Tris- HCl buffer, pH 8.8, were mixed to obtain a solution containing 200 µl 40 mM phenylalanine, 20 µl 50 mM β-mercaptoethanol and 480 µl 100 mM Tris-HCl buffer, pH 8.8. After incubation at 30°C for 1 hr., the reaction stopped by adding 100 µl 6 N HCl. Absorbance at 290 nm was measured and the amount of trans-cinnamic acid formed was evaluated by

comparison with a standard curve (0.1-2 mg trans-cinnamic acid/mL) and expressed as units of PAL /min/ mg cinnamic.

Determination of phenolic compounds:

To assess phenolic content, 1g plant fresh shoot was homogenized in 10 mL 80% methanol and agitated for 15 min. at 70°C. One mL of the extract was added to 5 mL of distilled water and 250 µl of 1 N Folin-Ciocalteau reagent and the solution was kept at 25°C. The absorbance was measured with a spectrophotometer at 725 nm. Catechol was used as a standard solution. The amount of phenolic content was expressed as phenol equivalents in mg catechol/ gm fresh tissue **(**Malik and Singh, 1980).

Determination of lignin:

Plant samples were refluxed with acid detergent solution to remove the water soluble and materials other than the fibrous component. The left-out materials are weighed after filtration, dried, treated with 72% H_2So_4 and filtered, dried and ashes. The loss of weight on lignin gives the acid detergent lignin (A.O.A.C, 2000).

Field experiments:

The field experiments were carried out in naturally infected soil of Sharkia and Dakahliya governorates to study the efficacy of seed soaking in the previous abiotic inducers on photosynthetic pigments content in cluster bean leaves and seed quality.

Estimation of Photosynthetic Pigments:

The blade of the 3rd leaf from plant tip (terminal leaflet) cluster bean plants were taken to determine photosynthetic pigments (chl a, b and carotenoids) which extracted with methanol 90% after adding traces of calcium carbonate (Robinson and Britz, 2000) and determined according to Mackinney,1941. Total phenols (mg catecole 100 g^{-1} fresh weight) in fresh shoot were determined using the Folin-Cocalteau reagent (Singleton and Rossi, 1965).

Seed quality:

The effect of the tested inducer resistances and the fungicide Rizolex-T50 on nitrogen and protein content of cluster bean was estimated in random samples of cluster bean seeds of plants grown in both experiments of Sharkia and Dakahliya governorates. 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 (Bradford, 1976).

Statistical analysis:

The data were arranged in one- way randomized complete black design using Duncan's multiple array test (1955) at probability value of ≤0.05. All data statistical analyses were performed by the statics software package Costate 2005 version 6.4, Cohort Software, USA.

RESULTS

Pathogenicity test of the isolated fungi:

Fungi belonging to three genera were isolated and identified as *Fusarium oxysporum*, *Rhizoctonia solani* (Kuhn), and *Macrophomina phaseolina* (Tassi) Goid. All the isolates were tested for their pathogenic capabilities on cluster bean plants. Data presented in Table (1) showed that the three tested fungal genera were pathogenic to cluster bean plants. However, they were varied in their pathogenicity. In this respect, *F. oxysporum* failed to cause pre-emergence damping-off. While *R. solani* caused the highest plateau of pre-emergence damping-off (36.7%) followed by *M. phaseolina* (23.3%). On the other side, the lowest percentage of survived plants was occurred by *F. oxysporum* and *R. solani* (26.7% for both fungi). No infection by damping-off and no dead plants were found in the control treatment.

Table (1): Pathogenicity tests of the tested fungi under greenhouse conditions.

Fungal isolates	% Damping-off		% Dead plants*	% Survived plants
	Pre-emergence	Post-emergence		
F. oxysporum	0.0d	26.7 a	46.7 a	26.7 d
M. phaseolina	23.3 b	26.7 a	13.3 c	36.7 c
R. solani	36.7 a	26.7 a	10.0 d	26.7 d
Control	0.0 d	0.0 c	0.0 e	100 a

*Dead plants: Resulted from the infection by root-rot or wilt and assessed 90 days after sowing.
Figures in the same column followed by the same letter(s) is not significantly different (p≤0.05) based on Duncan's multiple range test.

Effect of chemical inducers on damping-off and root rot under artificially infested soil:

The efficacy of chemical inducers and Rizolex-T50 fungicide for controlling damping-off and root rot diseases had evaluated in infested soil with the three fungi each alone. Pre- and post-emergence damping off were recorded at 15 and 30 days after sowing

respectively, while root rot was estimated at 90 days after sowing. Data in Table (2) show that Rizolex-T50 presented the highest reduction in disease parameters under all pathogenic fungi. On the other side, Bion came next to fungicide followed by humic acid then salicylic acid.

Table (2): Effect of chemical inducers on damping-off and root rot of cluster bean under artificially infested soil.

Treatments	*F. oxysporum*			*M. phaseolina*			*R. solani*		
	Damping-off		Root rot	Damping-off		Root rot	Damping-off		Root rot
	Pre-	Post-		Pre-	Post-		Pre-	Post-	
Humic acid	0.0	6.7 c	10.0 c	5.7 c	7.3 c	3.7 c	11.3 c	8.0 c	4.0 c
Bion	0.0	4.7 d	8.3 d	3.3 d	5.0 d	2.3 d	8.7 d	6.3 d	3.7 d
Salicylic acid	0.0	8.3 b	12.3 b	7.7 b	9.7 b	4.3 b	14.0 b	11.0 b	4.3 b
Rizolex-T50	0.0	2.3 e	6.7 e	1.7 e	3.0 e	1.0 e	7.3 e	3.7 e	2.3 e
Control	0.0	26.7 a	30.3 a	23.3 a	26.7 a	8.7 a	36.7 a	26.7 a	6.3 a

Means within each column followed by different letter significantly differ.

Biochemical changes associated with fungal infection as affected by the tested inducer resistance agents and the fungicide Rizolex-T50:

Plant defense enzymes:

The activity of chitinase and β-1,3-gluctronase enzymes:

Data in Table (3) reveal that all the tested inducer resistance chemicals and the fungicide Rizolex-T50 stimulated the activity of chitinase and β-1,3-gluctronase enzymes in cluster bean plants grown in artificially infested soil compared with control treatment. In general, the highest figures of the increase in the activity of both enzymes occurred under Bion (3.875. 3.895 and 4.352 mg/g fwt/min) for chitinase and (5.279, 4.471 and 5.352 mg/g fwt/min) for β1,3 glucanase when cluster bean was planted in soil infested with the three tested fungi, *i.e., F. oxysporum, M. phaseolina* and *R. solani* respectively. Meanwhile, the lowest values were resulted from those treated with the fungicide Rizolex-T50, being 2.671, 2.752 and 2.683 mg/g fwt/min for chitinase activity, 3.465, 3.475 and being 5.578 for β1,3 glucanase activity, respectively.

Table (3): Enzymatic activity (mg/g fwt/min) of chitinase and β-1,3-gluctronase enzymes in cluster bean plant grown in artificially infested soil with the tested fungi as a response of inducer resistance agents and the fungicide Rizolex-T50.

Treatments	Chitinase activity (mg g fwt^{-1} min^{-1})			β1,3 glucanase activity (mg g fwt^{-1} min^{-1})		
	F. oxysporum	*M. phaseolina*	*R. solani*	*F. oxysporum*	*M. phaseolina*	*R. solani*
Humic acid	3.818 c	3.820 c	4.261 c	5.173 c	4.782 b	5.176 c
Bion	3.875 a	3.895 a	4.352 a	5.279 a	4.871 a	5.352 a
Salicylic acid	3.853 b	3.872 b	4.321 b	5.243 b	4.787 b	5.283 b
Rizolex-T50	2.671 d	2.752 d	2.683 d	3.465 d	3.475 c	3.578 d
Control	2.531 e	2.673 e	2.651 e	3.352 e	3.378 d	3.415 e

Means within each column followed by different letter significantly differ.

The activity of three oxidative reductive enzymes:

Results shown in Table (4) clear that the activity of the three oxidative reductive enzymes *i.e.*, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase in cluster bean plants grown in the soil infested with the tested fungi was increased greatly with the application of all the tested resistance inducers and the fungicide Rizolex-T50 compared with the control. The highest values in the activity of the three enzymes in the infected cluster bean tissues were recorded due to the application of three inducer resistance chemicals *i.e.*, Bion, salicylic acid and humic acid, respectively. While the fungicide Rizolex-T50 came late. Control treatment recorded the lowest activity to the three enzymes.

Total phenols and lignin content in cluster bean plants:

It is clear from Table (5) that seed soaking with inducer resistance agents and the fungicide Rizolex-T50 resulted considerable increase to the total content of phenolic compounds and lignin (mg/ g dry weight of the leaves) in cluster bean plants grown in the infested soil with the

three tested fungi compared to the control. Comparing with control, the highest values of phenolic compounds in the infected cluster bean plant were occurred, with Bion, salicylic acid and humic acid, respectively followed by Rizolex-T50.

Table (4): Enzymatic activity (mg/g fwt/min) of three oxidative reductive enzymes in cluster bean plants grown in soil infested with the tested fungi as a response of inducer resistance agents and the fungicide Rizolex-T50.

Treatments	Peroxidase activity (mg/g fwt/min)			Polyphenol oxidase activity (mg/g fwt/min)			Phenylaanine ammonia-layse activity (mg/g fwt/min)		
	F. oxysporum	*M. phaseolina*	*R. solani*	*F. oxysporum*	*M. phaseolina*	*R. solani*	*F. oxysporum*	*M. phaseolina*	*R. solani*
Humic acid	0.815 c	1.053 b	1.116 c	0.317 b	0.738 b	0.821 b	1.539 b	1.837 c	1.883 c
Bion	0.933 a	1.122 a	1.482 a	0.363 a	0.763 a	0.835 a	1.652 a	1.893 a	1.936 a
Salicylic acid	0.841 b	1.068 b	1.276 b	0.284 c	0.712 c	0.782 c	1.546 b	1.871 b	1.913 b
Rizolex-T50	0.515 d	0.526 c	0.547 d	0.245 d	0.253 d	0.248 d	0.955 c	0.967 d	0.965 d
Control	0.412 e	0.473 d	0.433 e	0.232 e	0.253 d	0.249 d	0.825 d	0.932 e	0.957 e

Means within each column followed by different letter significantly differ.

Table (5): Effect of the tested inducer resistance chemicals on total phenols content and total lignin in cluster bean plants grown in infested soil with the tested fungi under greenhouse conditions.

Treatments	Total phenol compounds (mg/g fwt)			Total lignin (mg/g dwt)		
	F. oxysporum	*M. phaseolina*	*R. solani*	*F. oxysporum*	*M. phaseolina*	*R. solani*
Humic acid	3.269 b	3.317 c	3.372 c	0.252 b	0.317 b	0.339 c
Bion	3.386 a	3.422 a	3.463 a	0.269 a	0.336 a	0.351 a
Salicylic acid	3.274 b	3.368 b	3.382 b	0.251 b	0.315 b	0.346 b
Rizolex-T50	2.392 c	2.536 d	2.562 d	0.147 c	0.186 c	0.193 d
Control	2.015 d	2.136 e	2.133 e	0.108 d	0.121 d	0.127 e

Means within each column followed by different letter significantly differ.

Field experiments:

Photosynthesis pigments:

Data presented in Table (6) show that pre-treatment of cluster bean seeds with the tested inducer resistance chemicals and fungicide Rizolex-T50 before sowing in the field caused considerable increase in the photosynthetic pigments (chlorophyll-a, chlorophyll-b and total chlorophyll) in the leaves compared with plants grown without any treatment. In this respect, the highest values of the increase in the photosynthetic pigments occurred due to using Bion followed by salicylic acid then humic acid while, fungicide Rizolex-T50 came the end, being 1.704, 1.688, 1.679, 1.674, mg/g fw, respectively in Sharkia governorate and 1.708, 1.698, 1.689 and 1.688 in Dakahliya governorate. It is worthy to mention that chlorophyll A was a higher than chlorophyll B in all treatments.

Seed quality:

Cluster bean seed quality was estimated as seed nitrogen% then confirmed as seed protein%. As shown in Table (7), treating seeds with the tested inducer resistance agents and the fungicide Rizolex-T50 before sowing in the field increased significantly total nitrogen%, in turn total protein% compared with control (plants grown without any treatment). The highest values of both characters occurred from the treatment with Bion, salicylic acid and humic acid, being (4.25, 4.23, 4.22 and mg/g dw) total nitrogen in Sharkia governorate and (4.30, 4.24, 4.23 mg/g dw), in Dakahliya governorate, respectively. Meanwhile, the lowest increase in total nitrogen and total protein, was achieved by the control treatment recorded, being (3.62 and 22.62 mg/g dw) in Sharkia governorate and (3.66 and 22.87 mg/g dw) in Dakahliya governorate, respectively.

Table (6): Photosynthetic pigments in cluster bean leaves grown in natural field infection at Sharkia and Dakahliya governorates as affected by inducer resistance agents and the fungicide.

Treatments	Chlorophyll-a (mg/g fw)			Chlorophyll-b (mg/g fw)			Total chlorophyll (mg/g fw)		
	Sharkia	Dakahliya	Mean	Sharkia	Dakahliya	Mean	Sharkia	Dakahliya	Mean
Humic acid	0.942 b	0.950 b	0.946 b	0.737 c	0.739 c	0.738 d	1.679 c	1.689 c	1.684 c
Bion	0.947 a	0.953 a	0.950 a	0.757 a	0.755 a	0.756 a	1.704 a	1.708 a	1.706 a
Salicylic acid	0.943 b	0.948 b	0.946 b	0.745 b	0.750 b	0.748 b	1.688 b	1.698 b	1.693 b
Rizolex-T50	0.935 c	0.940 c	0.938 c	0.739 c	0.748 b	0.744 c	1.674 c	1.688 c	1.681 c
Control	0.850 d	0.870 d	0.860 d	0.702 d	0.701 d	0.702 e	1.552 d	1.571 d	1.562 d

Means within each column followed by different letter significantly differ.

Table (7): Effect of seed treatment with the tested inducer resistance chemicals and the fungicide Rizolex-T50 on total nitrogen and total protein of cluster bean grown under natural field infection at Sharkia and Dakahliya governorates.

Treatments	Total nitrogen (mg/g dw)			Total protein (mg/g dw)		
	Sharkia	Dakahliya	Mean	Sharkia	Dakahliya	Mean
Humic acid	4.22 b	4.24 b	4.23 b	26.37 c	26.5 b	26.44 b
Bion	4.25 a	4.30 a	4.28 a	26.56 a	26.87 a	26.72 a
Salicylic acid	4.23 ab	4.23 b	4.23 b	26.43 b	26.43 c	26.43 b
Rizolex-T50	4.21 b	4.21 c	4.21 c	26.31 d	26.31 d	26.31 c
Control	3.62 c	3.66 d	3.64 d	22.62 e	22.87 e	22.75 d

Means within each column followed by different letter significantly differ.

DISCUSSION

It has been found that the three tested inducer resistance agents' *viz.*, bion, humic acid and salicylic acid resulted in significant reduction in cluster bean damping-off and root rot under artificially infested soil with the three tested fungi compared with control treatment. Bion agent was the superior in its efficiency followed by humic then salicylic acid. These effects may be due to, Bion (BTH) is an acquired systemic resistance elicitor, which reduces many fungal diseases (Oostendorp *et al.*, 2001 and Zyton and Hassan, 2017). This protection is known to be related to the induction of the phenol pathway, but the particular metabolites involved have not been determined yet. This suggests fungal growth impairment by both direct toxic effect as well as plant cell wall reinforcement. Also, Humic acid increased the activity of chitinase enzyme which causes a degradation of fungal cell wall (Abdel- Kaream *et al*. 2007).

Salicylic acid (SA) is a phenolic compound that affects a variety of biochemical and molecular events associated with induction of disease resistance. SA has been shown to play an important role in expression of both local resistances controlled by major genes and systemic induced resistance developed after an initial pathogen attack (Hammerschmidt and Smith-Becker, 2000 and Saikia *et al*., 2003).

SA as resistant inducer plays an essential role in the defense response to pathogen attack, improved plant growth, photosynthesis and chlorophyll content of pea, but it decreased plant injuries (Popova *et al.*, 2008).

Abdel-Kareem (2007), EL-Mohamedy, and Ahmed (2009); and Tabarraei *et al*. (2011) mentioned that induce resistance by humic acid and bion (BTH) due to increase basic nutrients, such as nitrogen, phosphorus, potassium, calcium, sulfur and magnesium are crucial elements in many processes in the development of the plant and the formation of the yield. But besides these elements, microelements play a large role in the quality of final product.

It has been found that all the tested inducer resistance chemicals agents and the fungicide Rizolex-T50 resulted a marketable increase in the activity of chitinase and β-1,3-gluctronase enzymes in cluster bean plants grown in the infested soil with the three tested fungi compared with the control. In general, the highest figures of the increase in the activity of both enzymes in the infected cluster bean tissues was occurred from the treatment with, Bion followed salicylic acid then humic acid, Punja and Zang (1993) reported that chitinases are

enzymes that hydrolyze the N-acetylglucosamine polymer chitin, and they occur in diverse plant tissues over a broad range of crop species. The enzymes may be expressed constitutively at low levels but are dramatically enhanced by numerous abiotic agents (ethylene, salicylic acid, salt solutions, ozone, UV light) and by biotic factors (fungi, bacteria, virus, viroid, fungal cell wall components, and oligosaccharides). Different classes of plant chitinases are distinguishable by molecular, biochemical, and physicochemical criteria. In turn, plant chitinases may differ in substrate-binding characteristics, localization within the cell, and specific activities. Because chitin is a structural component of the cell wall of many phytopathogenic fungi, extensive research has been conducted to determine whether chitinases have a role in defense against fungal diseases.

The importance's of the structurally functional organization of peroxidases, promoting the concentrated polymerization of the phenolic compounds with participation of ROS on the mycelium surface of pathogenic fungus. So, induction resistance by salicylic acid and bioagents may be due to the accumulation of oxidative-reductive enzymes and pathogenesis-related proteins (PRS). These treatments cause an increase in the activity of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL), chitinase, β-1,3 glucanase, the increase in such enzymes activity was correlated with increased lignin and phenolic compounds (Abdel-Monaim, 2018 and Sarhan *et al*., 2018). In our study, resistance of cluster bean plants treated with the inducer resistance chemicals to damping-off and root-rot may be due to accumulation of PO, PPO, PAL and pathogenesis related protein (chitinase, β-1, 3 glucanase) with add to increase of total phenol compounds and lignin in guar tissues either in inoculated or non-inoculated plants.

In connection with role of peroxidase in a plant morphogenetic processes and lignin synthesis special interest represents their ties with processes of plant tissue defense from pathogens and phytopathogen (wound response). The important feature of a lignin is that only few of a lot of number of parasitic microorganisms (for example, fungus destroying wood) can cleave it. Therefore, lignification coats of cells serve as a barrier on a way of distribution of an infection. The induced anionic peroxidase or transgenic plants with constitutively high enzyme activity become toxic for pathogen or phytopathogens (Behle *et al*., 2002).

The accumulation of lignin is one of the important plant defense mechanisms against pathogens and wound. The artificial inhibition of lignification can lead to disorder of the immune response that has been shown on an example of wheat infection by stripe rust (*Puccinia striiformis* f.sp. *tritici*) (Moldenhauer *et al*., 2006). First of all, it is possible to note high stability of vessels of a conductive tissue to pathogens. Besides, initiation of morphogenetic processes in culture of the plant cells also leads to enhancement of their stability to pathogenic fungi (Troshina *et al*., 2000). The intensive generation of the active oxygen species and the subsequent lignification of their cell walls with participation of anionic pathogen-induced peroxidase and calluses were found (Troshina *et al*., 2004). Shehata *et al*. (2016) showed that humic acid increased chlorophyll and carotenoid contents of head lettuce and cucumber.

Treating cluster bean seeds with the tested inducer resistance chemicals and fungicide Rizolex-T50 before sowing in a field has a back history of the natural infection by root-rot and wilt diseases caused considerable increase in the photosynthetic pigments (chlorophyll-a, chlorophyll-b and total chlorophyll) of these plants compared with plants grown without any treatment.

Photosynthetic pigments in plants comprise chlorophylls *a* and *b* and these pigments mainly capture light in the antenna complex via photosystem II, with consequent electron transport (Candan and Tarhan, 2003). Other pigments such as carotenoids are also found in plants and are considered as accessory components in the photosynthetic complex by providing photoprotection and stability of proteins present in the photosystem II (Simkin et al. 2008). Recently, studies indicated that *Phaseolus vulgaris* suffers significant pigment loss when it is exposed to pathogen infection (Berova *et al*., 2007). Lobato *et al.* (2010) mentioned that stomatal conductance, transpiration rate, photosynthesis rate and photosynthetic water use efficiency were reduced by the presence of the pathogen. Linear relationships between carotenoids and chlorophyll were the main cause for the lower photosynthesis rates.

From the previous results and discussion, it may be suggested that using bion or humic acid or salicylic acid as seed soaking treatment could be applied for controlling damping-off and root rot diseases in cluster bean and enhancing physiological status as well as seed quality.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest

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