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INFLUENCE OF RESISTANCE INDUCERS ON SOYBEAN DAMPING-OFF, NODULATION AND N_2-FIXATION

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ABSTRACT

The effect of K_2HPO_4, salicylic acid, H_2O_2, cobalt sulfate, Ethepone, ascorbic acid, and gibberelic acid on the growth of *Bradyrhizobium japonicum* 3407 strain was in vitro evaluated *B. japonicum* showed no sensitivity to any chemical inducers on liquid culture.

Theses materials were evaluated individually with *B. japonicum* as inducers for resistance agents for reducing damping-off disease caused by *R.solanii*, or *S. rolfsii* or *M.phaseolina* in soybean plants under greenhouse conditions.

In non-inoculated plants with *B. japonicum* infestation of soil with *R solani*, or *S. rolfsii* or *M.phaseolina* drastically increased damping-off disease and reduced plant growth parameters compared to non-infested ones

In plants inoculated with the *B .japonicum* and infested with *R solani*, or *S. rolfsii* or *M. phaseolina*, the detrimental effect cause by pathogenic fungi infestation was less detected in salicylic acid followed by K_2HPO_4, cobalt sulfate and ascorbic acid in reducing damping-off. Concerning the effect of these inducer agents on nodulation and growth of soybean plants in infested soil with aforementioned pathogens, the results indicate that the cobalt sulfate or salicylic acid or K_2HPO_4 and co-inoculation of *B. japonicum* gave the best result in terms of number and dry weight of nodules, dry weight of shoot and its N-content compared to control (pathogen + *B. japonicum*) at 75 days from sowing.

Results in field experiments indicated that seed treatments with some of these inducers which are the most effective in greenhouse, significantly reduced pre-and post-emergence damping-off in presence of *B. japonicum* as seed inoculated treatment compared to control (*B. japonicum* only) under field conditions. Salicylic acid followed by cobalt sulfate, K_2HPO_4 and ascorbic acid they recorded the lowest percentage of damping-off and the highest percentage of survived plants in two successive seasons. Also use of chemical inducers as seed soaking resulted in an increase in crop parameters on soybean compared to control (*B. japonicum* only). Seed treatment with SA followed by cobalt sulfate, K_2HPO_4 and ascorbic acid recorded the highest biological yield. Seed weight of treated plot with K_2HPO_4, SA or cobalt sulfate and ascorbic acid were the highest values. Seed and straw protein percentage significantly increased by SA, ascorbic acid and cobalt sulphate as compared with untreated treatment.

Application of inducers as seed treatment had a positive effect on the total number of rhizospher microflora especially with ascorbic acid and SA at 75 day old soybean plant.

INTRODUCTION

Soybean (*Glycin max* L.) is an important leguminous crop in Egypt and other countries. Plants may be severely attacked by many soil borne pathogenic fungi causing serious damages (Sinclair and Backman, 1989).

Damping off is considered as one of the most important diseases which cause limited yield. Several attempts to control damping-off disease were done. However, fungicides are considered one of several factors involving in environmental pollution inspite of their satisfactory results in the control of plant diseases.

All fungicidal treatments are likely to be toxic to rhizobia to some degree (Abou –Neama, 1983). On the other hand, the application of biological control using antagonistic microorganisms proved to be some successful for controlling various plants diseases, but it is still not easy in field applications. It can serve, however, as a better control measure under greenhouse conditions (Sivan and Chet, 1986). Recently, the induced resistance has been emerge as a new strategy for disease control in many crops (Kuc, 1987). Resistance against plant pathogens can be induced by several chemical as salicylic acid or acetylsalicylic acid (Floryszek and Wieczorek, 1993). Ethephon compound (2-chloroethyl phosphonic acid) as well as phosphate is commonly used for induction of resistance against foliar diseases in various plants (Abd-El-Kareem *et al.*, 2001 and 2002).

Aly *et al.* (1988) tested ethyphone as seed soaking or spray treatments against powdery mildew caused by *Erysiphe graminis f.sp.hordei* and found that the most effective treatment was seed soaking in 1000 ppm. Aly (1989) reported that ethyphone seed treatment could induce resistance in broad bean plants against chocolate spot fungus (*Botrytis fabae*) and *stemphylium* blight fungus. Treatment with 800 and 1000 ppm were effective against chocolate spot fungus. Treatment with 1000 and 1200 ppm was more effective against *Stemphylium blight.* Sallem *et al.* (1992) found that soaking of faba bean seed in 800 ppm ethephone resulted in a significant reduction in the severity of chocolate spot and rust and a noticeable increase in seed/plant. Harfoush and Salama (1992) reported that soaking cucumber seeds for 24hrs in 0.5 ppm of $CoSO_4$ was highly effective against powdery mildew. Abd-El-Kareem (1998) used three concentrations of cobalt i.e., (0.25, 0.5 and 1.0ppm)against powdery mildew of cucumber as seed or foliar treatment and found that seed treatment with all concentrations caused a highest reduction in disease incidence. Mazen (2004) found that faba bean seed treatment with salicylic acid (SA 7mM) followed by Ethephone (800 ppm), Co^{++} (1 ppm) was the most effective as a biotic inducers against *R. Solani.* El-Mougy (2004) found that Acetylsalicylic acid as seed dressing or soil drench showed superior effect on lupine root rot incidence followed by SA and rhizolex.

Solutions of KH_2PO_4, K_2HPO_4, Na_3PO_4 and Na_2PO_4 sprayed on the undersides of the first and second true leaves of cucumber plants induced systemic resistance in leaves 3 and 4 to anthracnose caused by *C. lagenarium*.(Gottstein and Kuc, 1989).

The main objective of this research is to study the efficacy of different inducers in soil inoculated with *Bradyrhizobium japonicum* on the incidence of damping-off in soybean under greenhouse and field conditions, also to study their effect on nodulation, vegetative characters and yield.

MATERIALS AND METHODS

Pathogenic isolates of *Rhizoctoni solani* Kuhn, *Scelortium rolfsii* Sacc., and *Macrophomina phaseolina* (Maubl.) Ashby were isolated from infected soybean seedlings. The preliminary studies proved that these isolates were pathogenic to soybean and caused damping-off disease to the variety Giza35 (*Glycine max* L.).

Rhizobium strain of soybean (*Bradyrhizobium Japonicum* 3407) was supplied by the Unit of Biofertilizers, Department of Microbiology, SWERI, ARC, Giza, used as symbiotic organism. The strain was grown and maintained on yeast extract manitol agar medium (Vincent, 1970).

Soybean seeds (*Glycin max* L. Merr) cv. Giza 35 were kindly provided by Legume Crops Research Section, Field Crops Research Institute (FCRI), ARC, Giza, Egypt. Soil was collected from the Agric. Station of Itay El-Baroud, Behira Governorate. The soil is as clay loam in texture (36.0 clay, 47.6 silt, 16.4 sand, pH 7.6 and EC 3.2 dSm^{-1}). Soil sample was air dried, crushed and sieved through a 2 mm screen and thoroughly mixed. Mechanical and chemical properties were determined according to Piper (1950); Richard (1954) and Page *et al.* (1982). Chemical inducers used in the present investigation are shown in Table (1).

Table (1): Compounds proposed as resistance inducers along with their composition and concentration used in the present study

Compound	Chemical composition	Concentration
1. Potassium phosphate	K_2HPO_4	5, 10 and 15 mM
2. Salicylic acid	Hydroxy benzoic acid	2, 5 and 7.5 mM
3. Hydrogen peroxide	H_2O_2	1, 2.5 and 5 ppm
4. Cobalt sulphate	Co^{++} ions as ($CoSO_4.7H_2O$)	0.5, 1.0 and 1.5 ppm
5. Ethephone	2-choloroethyl phosphonic acid	100, 200 and 300 ppm
6.Ascorbic acid(vitamin C)	L. Ascorbic acid	100, 150 and 200 ppm
7. Gibberellic acid	GA_3	100, 150 and 200 ppm

Effect of inducers on growth of *B. Japonicum* in vitro:

The three different concentrations of each inducer mentioned before were prepared in sterilized distilled water. Discs of sterilized filter paper 5 mm in diameter were immersed in different solutions of inducers and arranged on yeast extract manitol agar medium (YEMA) which were previously atomized with a suspension of *Bradyrhizobium Japonicum* 3407 strain at 8 x 10^8 cell/ ml. Four Petri dishes were used for each concentration and incubated at 28 ° C for 5 days.

Greenhouse experiments

Efficacy evaluation of different resistance inducers on controlling damping-off disease:

A pot experiment was carried out under greenhouse at the unit of Biofertilizers, Agric. Microbiol. (SWERI), Giza, Egypt. Plottery pots (30 cm) were filled with sterilized clay soil at the rate of 10kg/pot. Soil in pots was supplemented with NPK fertilizer regimes of ammonium sulfate (20.6% N) at a rate of 20kg N/fed, super phosphate (15.5% P_2O_5) at a rate of 100kg/fed and potassium sulphate (48% K_2O) at a rate of 50kg K_2O/fed. The soil was infested, 15 days before sowing, with each of the three tested pathogens alone (*R. solani, S. rolfsii and M. phaseolina*) using sorghum grain sand medium of 15 days old at respective inoculum rate of 4 g per 100 g soil. The fungi inocula were prepared by growing on autoclaved sorghum grain sand medium (3:1 w/w) in 500 ml bottles and incubated at 25 °C for two weeks.

Soybean seeds (cv. Giza-35) were soaked, before sowing, in solution of seven chemical inducers i.e., K_2HPO_4 at concentrations (5, 10 and 15 mM.), salicylic acid (SA) at (2, 5 and 7.5 mM), H_2O_2 (1, 2.5 and 5 ppm), cobalt sulphate (0.5, 1.0 and 1.5 ppm), Ascorbic acid (100, 150 and 200 ppm), Gibberelic acid (GA_3) (100, 150 and 200 ppm), and ethephone (100, 200 and 300 ppm) for half hour. Seed were soaked in tap water were used as control. (Table1). After being wetted, the seeds were spread out in a thin layer and sown in infested soil with pathogenic fungi that mentioned above at a rate of eight seeds/pot, then 15 ml of cell suspension($6x10^8$ cell/ml) of ***B. Japonicum***(3407) were pippeted on soil 7 days after sowing. The experiment comprises 62 treatments arranged as three for pre and post emergence stage and other three for nodules, all the treatments were arranged in complete randomized design.

Pre, post–emergence damping-off and survived plants were recorded 15, 30 and 75 days after sowing. At 75 days after sowing (DAS) plants were gently uprooted without tearing of root system as possible, washed and nodules were separated, counted and plant weight were determined as dry weight after drying at 70 °C. Total nitrogen was estimated using micro-Kjeldahl method (Piper, 1950).

Extraction and assay of phenol-oxidizing enzymes:

Plant tissues obtained from the different treatments at 20 days after sowing, were cut into small portions, and rapidly ground with 0.1M sodium phosphate buffer at pH 7.1 (2 ml buffer (g of fresh tissue) in a mortar. These triturated tissues were strained through four layers of cheese-cloth and the filtrates were centrifuged at 3000 r.p.m for 20 min. at 6 °C (Maxwell and Bateman, 1967). The supernatant fluids were used for enzymes assays.

Peroxidase:

Peroxidase enzyme activity was determined colormetrically by measuring the oxidation of pyrogallol to purprgallin in the presence of H_2O_2 at 425 nm. The reaction mixture contained 0.5 ml of 0.1 M sodium phosphate buffer solution at pH=7.0, 0.3 ml sample extract, 0.3 ml 0.05 M pyrogallol, and 0.1 ml of 1.0% H_2O_2, then completed with distilled water up to 3 ml. The activity was expressed as absorbance change per minute (Abs/min) (Allam and Hollis, 1972)

Polyphenoloxidase:

The activity of polyphenoloxidase was measured by the colorimetric method of Maxwell and Bateman (1967). The reaction mixture contained 1.0

ml enzyme extract, 1.0 ml of 0.2 Sodium phosphate buffer at pH 7.0 and 1.0 ml of 10^3 N catechol brought to a final volume of 6.0 ml with distilled water.

The activity of polyphenoloxidase was expressed as the change in absorbance 1.0 ml of extract per min. at 495 nm.

Field experiment:

This study was carried out to investigate the efficiency of chemicals (Table1) as seed soaking on soybean damping-off disease as well as their effect on the N_2- fixation ability of *B. Japonicum* for soybeen plants. The most effective concentrations treatment in pot experiment, against damping-off disease were applied to study their efficiency either in the presence or the absence of *Brandyrhizobium Japonicum* strain no (3704) against the occurrence of disease in addition to the determination of crop parameters. Four chemical inducers, i.e., cobalt sulfate (1ppm), Salicylic acid (7.5mM), K_2HPO_4 (10 mM). and Ascorbic acid (150 ppm) were used. The soybean (cv G 35) was used. All treatments were distributed in complete randomized block design and data were statistically analyzed. The field plot was (8.4 m^2), and three plots were replicated for each treatment.

The experiment was conducted in 2 successive seasons, 2004 and 2005, at Agricultural Reasearch Station of Itay El-Baroud, Behira Governorate. Soil ploughed twice and supper phosphate (15.5% P_2O_5) was basically incorporated into soil at a rate of 100 kg/fed. The inoculum of *Bradyrhizobium Japonicum* was applied as seed coating at the rate of 300 g/ 50kg seeds. Seed coating was made by mixing seeds with the Gamma irradiation sterilized vermiculate – Irish peat mixture-based inoculum of *B. Japonicum* and the Arabic gum solution (16%), as a sticking agent. After germination, the plots were supplied with ammonium sulfate (20.6%N) at rate of 20 Kg N/fed as a starter nitrogen dose and potassium sulphate (48% K_2O) fertilizer was applied at the rate of 50kg/fed.

At 75 days after sowing, plants were sampled and determined for shoot length, dry weight and its nitrogen content. Roots were separated, washed and free from adhering soil and examined for number and dry weight of nodules. At harvest yield parameters, as well as the number of pods, seed weight kg/plot and biological yield of pots, seed and straw yields and protein content percentage were estimated. After 20 and 45 days of planting, the percentages of pre and post- emergence damping-off were recorded.

Obtained data were subjected to analysis of variance (ANOVA) according to the procedure of Snedecor and Cochran (1980).

Determination of microbial population in soil:

Total count of bacteria, fungi and actinomycetes were determined according to Page (1982). The rhizosphere soybean soil area was randomly sampled at 75 days from sowing and at harvest to estimate the total of microorganisms.

RESULTS

In vitro test:

Inducers effect on growth of *Bradyrhizobium japonicum*:

Data in Table (2) indicate the effect of inducers on growth of *B. japonicum* strain 3407 on (YEMA) medium. Results revealed that good growth of *B. japonicum* under different concentrations of inducers was observed for all inducers.

Table (2): Effect of the inducer materials on the growth of *Bradyrhizobium japonicam* strain 3407 under laboratory conditions

Induces	Concentration		
	I	II	III
K_2HPO_4	+	+	+
Salicylic acid	+	+	+
H_2O_2	+	+	+
Cobalt sulfate	+	+	+
Ethephone	+	+	+
Ascorbic acid	+	+	+
Gibberelic acid	+	+	+
B.japonicum (control)	+	+	+

+ normal growth of *B.japonicum*

Effect of soybean seed treatment with chemical inducers on the pre and post-emergence damping-off caused by *R. solani, S. rolfsii* and *M. phaseolina*

Data presented in Table (3) show that a variation was recorded among the chemical inducers and within their concentration on the pre and post-emergence damping-off caused by *R. solani, S. rolfsii* and *M. phaseolina*. In general, salicylic acid, K_2HPO_4 and cobalt sulfate gave a protection against the aforementioned pathogenic fungi, judged by the low percentage of pre and post-emergence damping-off and high percentage of survived plants compared with (Pathogen + *B. japonicum*) treatment. Other treatments can be ranked in the descending order as follows ethephone, GA_3, H_2O_2 and ascorbic acid.

Concerning the used concentrations, only salicylic acid showed a positive correlation between the concentration and the effect on soil borne pathogenic fungi. Also, data show that increasing inducers concentration until the second concentration led to an increase of the effect on these pathogenic fungi, this positive correlation was observed in K_2HPO_4, $CoSO_4$ and ascorbic acid inducers dealing with pre-emergence damping-off. Concerning with post-emergence damping-off, K_2HPO_4, salicylic acid and H_2O_2 showed a positive correlation between the concentration and the effect on the pathogenic fungi. Other chemical inducers exhibited unclear correlationship between their concentration and the infection percentage.

Peroxidase and polyphenol oxidase activity in soybean plant treated with some chemical inducers as soaking seed

Data in Table (4) indicate that cobalt sulfate (1.0ppm), salicylic acid (7.5 mM), ascorbic acid (150 ppm) and K_2HPO_4 (10 mM) caused the highest activity in the peroxidase and polyphenol oxidase.

Table (3): Effect of soaking soybean seeds in chemical inducers on soybean damping-off disease

Inducers	concentration	Pre%				Post %				Survival %			
		R. solani	*S. rolfsii*	*M. phaseolina*	*Mean*	*R. solani*	*S. rolfsii*	*M. phaseolina*	*Mean*	*R. solani*	*S. rolfsii*	*M. phaseolina*	*Mean*
Potassium phosphate	5mM	45.8	41.7	41.7		12.5	16.7	16.7		41.7	41.7	41.7	
	10mM	29.2	20.8	16.7		8.3	8.3	4.2		62.5	70.8	79.2	
	15mM	33.3	33.3	29.2	32.4	12.5	12.5	4.2	10.7	54.2	54.2	66.6	57.0
Salicylic acid	2mm	41.7	45.8	37.5		12.5	16.7	12.5		45.8	37.5	50.0	
	5mM	37.5	37.5	29.2		8.3	8.3	8.3		54.2	54.2	62.5	
	7.5mM	16.7	20.8	16.7	31.5	4.2	4.2	4.2	8.8	79.2	75.0	79.2	59.7
Hydrogen peroxide	1ppm	58.3	58.3	50.0		16.7	16.7	16.7		25.0	25.0	33.3	
	2.5ppm	54.2	50.0	50.0		12.5	12.5	16.7		33.3	37.5	33.3	
	5ppm	54.2	58.3	45.8	52.8	16.7	12.5	12.5	14.8	29.2	29.2	41.7	32.0
Cobalt sulphate	0.5ppm	50.0	45.8	41.7		12.5	16.7	8.3		37.5	37.5	50.0	
	1.0ppm	29.2	25.0	20.8		8.3	8.3	4.2		62.5	66.7	75.0	
	1.5ppm	37.5	33.3	29.2	34.7	12.5	12.5	8.3	10.2	50.0	54.2	62.5	55.6
Ethephone	100ppm	62.5	54.2	50.0		16.7	16.7	12.5		20.8	29.2	37.5	
	200ppm	58.3	54.2	50.0		12.5	16.7	16.7		29.2	29.2	29.2	
	300ppm	58.3	50.0	50.0	54.2	16.7	12.5	12.5	15.3	25.0	37.5	25.0	29.2
Ascorbic acid	100ppm	45.8	50.0	41.7		16.7	16.7	12.5		37.5	33.3	45.8	
	150ppm	33.3	29.2	25.0		12.5	8.3	8.3		54.2	62.5	66.7	
	200ppm	37.5	37.5	33.3	37.0	16.7	16.7	16.7	13.9	45.8	45.8	50.0	49.1
Gibberellic acid	100ppm	58.3	50.0	50.0		16.7	16.7	16.7		25.5	33.3	33.3	
	150ppm	54.2	54.2	45.8		20.8	20.8	12.5		25.0	25.0	41.7	
	200ppm	50.0	50.0	41.7	50.5	29.2	25.0	20.8	19.9	20.8	25.0	37.5	29.6
Fungus		62.5	58.3	50.5		20.8	16.7	12.5	5.6	16.7	25.0	37.5	
Fungus+ *B. japonicum*		45.8	37.5	37.5	40.3	8.3	4.2	4.2		45.8	58.3	58.3	54.1
B. japonicum		8.3	8.3	8.3		4.2	4.2	4.2		87.5	87.5	87.5	
Soil free		12.5	12.5	12.5		8.3	8.3	8.3		79.2	79.2	79.2	
L.S.D at 5%		7.0	8.7	7.1		7.0	6.6	5.9		8.3	8.3	10.2	

On the other hand H_2O_2 and Ethephone exhibited lower activity in peroxidase and polyphenol oxidase.

Table (4): Activity of peroxidase and polyphenol oxidase enzymes of soybean G35 v. as affected by seven inducer chemicals as seed soaking under greenhouse

Treatment		Concentration	Peroxidase			Polyphenoloxidase		
			R. solani	*S. rolfsii*	*M. phaseolina*	*R. solani*	*S. rolfsii*	*M. phaseolina*
Potassium phosphate	+ *B. japonicum*	5.0	1.21	1.11	1.17	0.15	0.12	0.14
		10.0	1.50	1.49	1.22	0.29	0.31	0.41
		15.0mM	1.29	1.20	1.16	0.19	0.13	0.15
Salicylic acid		2.0	1.30	1.41	1.21	0.23	0.30	0.28
		5.0	1.83	1.69	1.56	0.70	0.67	0.59
		7.5mM	1.90	1.80	1.75	0.78	0.75	0.76
Hydrogen peroxide		1.0	0.79	1.03	0.98	0.09	0.10	0.08
		2.5	1.11	1.31	1.13	0.19	0.24	0.20
		5 ppm	1.15	1.72	1.15	0.21	0.27	0.18
Cobalt sulfate		0.5	0.99	1.07	1.20	0.31	0.27	0.37
		1.0	1.92	1.77	1.81	0.67	0.50	0.71
		1.5ppm	1.25	1.12	1.30	0.67	0.47	0.56
Ethephone		100	0.79	1.01	0.90	0.10	0.12	0.10
		200	1.05	1.30	1.21	0.11	0.18	0.15
		300ppm	1.38	1.45	1.25	0.18	0.22	0.14
Ascorbic acid		100	1.01	0.98	1.11	0.15	0.10	0.18
		150	1.62	1.51	1.62	0.50	0.53	0.59
		200ppm	1.38	1.31	1.20	0.25	0.23	0.20
Gibberelic acid		100	1.17	1.20	1.12	0.15	0.17	0.13
		150	1.31	1.41	1.38	0.18	0.26	0.25
		200ppm	1.25	1.33	1.25	0.16	0.18	0.17
Pathogen			0.76	0.65	0.55	0.13	0.17	0.14
Path.+ *B. japonicum*			0.64	0.72	0.58	0.17	0.21	0.15
B. japonicum			0.81	0.83	0.75	0.32	0.40	0.29
Untreated soil			0.55	0.61	0.45	0.10	0.15	0.12

Effect of seed soaking with chemical inducers on nodulation with *B.japonicum* and plant growth parameters after 75 days from sowing in soil infested with pathogenic fungi

Table (5) indicate that the infection with *R.solani, S. rolfsii and M. phaseolina* had a drastic effect on the nodulation of the tested soybean cultivar (Giza35), there were no nodules formed on roots from infested soil with *R.solani*,or *S. rolfsii*. However, the number of nodules per plant decreased significantly in case of infection with *M. phaseolina*, which gave the lower value in dry weight as nodules compared with the control (*Bradyrhizobium. japonicum*) treatment, which resulted in the highest number of nodules after 75 days from sowing. Results also, indicate that the infection with tested pathogens individually significantly decreased dry weight of shoot. *Rhizoctonia solani* and *R. rolfsii* was the most effective causing higher reduction followed by *M. phaseolina* compared with dry shoot of control plant (*B. japonicum*). Also these pathogenic fungi caused significant decrease in N_2-content of shoot/plant.

Table (5): Effect of seed soaking with chemical inducers on plant nodulation and some growth parameters after 75 days from sowing in soil infested with *R. solani, S. rolfsii and M. phaseolina* (in greenhouse)

Treatments	No. of nodules/plant				Dry weight of nodules (mg/plant)				Dry weight of shoot (mg/plant)				N_2-content of shoot (mg/plant)			
	R. solani	*S. rolfsii*	*M. phaseolina*	*Mean*	*R. solani*	*S. rolfsii*	*M. phaseolina*	*Mean*	*R. solani*	*S. rolfsii*	*M. phaseolina*	*Mean*	*R. solani*	*S. rolfsii*	*M. phaseolina*	*Mean*
K_2HPO_4	48	43	60	50.3	264	238	271	257.7	8.5	8.1	8.7	8.43	255	235	260	250.0
Salicylic acid	58	45	57	53.3	300	269	306	291.7	9.9	9.7	10.2	9.93	290	291	300	293.3
H_2O_2	42	43	48	44.3	220	200	214	211.3	6.5	8.1	6.5	7.03	245	240	246	243.7
Cobalt sulphate	56	57	50	54.3	319	269	306	307.0	10.3	10.3	10.9	10.5	300	250	310	286.7
Ascorbic acid	45	50	45	46.7	309	282	299	296.7	9.3	8.6	9.3	9.07	290	292	298	293.7
GA3	20	25	39	28.0	190	177	128	165.0	8.3	8.4	8.4	8.37	200	204	217	207.0
Ethephone	40	40	43	41.0	223	205	200	209.3	8.0	5.91	8.2	7.37	228	230	225	227.7
Pathogen	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	1.8	2.7	2.27	80	66	89	78.3
Path + *B.j*	0.0	0.0	7.0	2.3	0.0	0.0	30	10.0	4.4	3.8	4.2	4.13	110	99	121	110.0
B. japonicum	40	40	40	40.0	197	197	197	197.0	7.8	7.8	7.8	7.8	226	236	226	226.0
Untreated	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1	4.1	4.1	4.1	123	123	123	123.0
L.S.D at 5%	4.21	4.68	3.54		9.02	18.0	12.8		0.59	0.58	1.00		8.58	8.03	6.36	

Seed treatment with chemical inducers in presence of *B. japonicum* improved nodulation. Cobalt sulphate followed by salicylic acid(SA) and K_2HPO_4 increased nodules number from 40(*B. japonicum*) to 54.3, 533.3 and 50.3, respectively. On the other hand, GA_3 treatment showed lower effect. The same trend of nodules dry weight was obtained with cobalt sulfate. Ascorbic acid, SA and K_2HPO_4. Concerning dry weight of shoot, all treatments with chemical inducers to seeds previously inoculated with *B. japonicum* and sown in infested soil with pathogenic fungi resulted in dry weight of shoot. Cobalt sulfate, SA, Ascorbic acid and K_2HPO_4 gave increase in shoot dry weight compared with (pathogen + *B. japonicum*). Other treatments, i.e., ethephone and H_2O_2 gave the least amount of dry weight. Similar results were observed due to N_2-content of shoot per plant. While, ascorbic acid was the most effective and increased N_2-content of shoot followed by SA and cobalt sulphate.

Effect of chemical inducers used for the control of damping-off disease on growth and nodulation in two successive seasons and their combined analysis at 75 days after sowing (2004 and 2005)

Data in Table (6) show a significant increase in stem length of soybean in response to the use of chemical inducers as seed soaking over the control (*B. japonicum* only) except for the use of with ascorbic acid. The most effective inducer was SA followed by cobalt sulphate and K_2HPO_4 in 2004. In 2005 growing season the data showed a similar trend to that obtained in 2004. Minor differences, however, were observed; cobalt sulfate was the most effective inducer. Mean plant length for both seasons ranged from 69.3 to 81.9 cm.

Mean of dry weight for both seasons ranged from 23.5 to 28.7 g/plant, respectively. Dry weight of shoot resulting from cobalt sulphate and SA was greater than of other chemical inducers. Also, number of nodules per plant in the induced plants increased significantly over the control plants (pre treatment plants) with priority to SA treated plants while, recorded the highest number of nodules plant in both seasons. The SA followed by cobalt sulphate gave the best results followed by K_2HPO_4 and ascorbic acid over both tested seasons. Concerning dry weight of nodules means of dry weight of nodules higher in both tested seasons and ranged from 520 to 625 mg/plant.

Microbial density in the rhizospher:

Data in Table (7) showed that ascorbic acid and SA had the highest effect on number of different microorganisms recorded being 7.5×10^7, 6.7×10^6 and 1.4×10^5 for bacteria, actinomycetes and fungi, respectively, 75 day after sowing of ascorbic acid treated seeds. The corresponding numbers due to salicylic acid treatments under the same conditions were 5.7×10^7, 4.0×10^6 and 1.8×10^5. Similar trend was noticed at harvest stage but with marked decline in densities of various rhizosphere groups. In contrast the application of K_2HPO_4 was the lowest in numbers count of bacteria, actinomycetes and fungi in both growth of 75 and harvest stages.

Table (6): Effect of chemical inducers used for control of damping-off disease on growth and nodulation status in two successive seasons and their combined analysis at 75 days after sowing (2004and 2005)

Treatments	Plant height (cm plant^{-1}) (shoot length cm)			D.W. of shoot) (shoot dry weight (g)			No. of nodules (no/plant^{-1})			Dry weight of nodules (mg/plant^{-1})			N-content of shoot (mg)/plant (content/plant)		
	2004	2005	Combined analysis	2004	2005	Combined analysis	2004	2005	Combined analysis	2004	2005	Combined analysis	2004	2005	Combined analysis
K_2HPO_4	70.2	70.7	70.5	23.7	24.5	24.1	80	75	77.5	561	536	548	641	640	640.5
Salicylic acid	83.3	80.2	81.8	27.0	28.3	27.7	94	96	95.0	591	600	596	704	700	702.0
Ascorbic acid	69.5	69.3	69.4	23.2	23.8	23.5	78	72	75.0	540	501	520	550	595	572.5
Cobalt sulphate	80.0	81.9	81.0	28.9	28.5	28.7	86	90	88.0	640	610	625	650	690	670.0
B. japonicum	62.1	64.7	69.4	21.7	23.3	22.5	76	69	72.5	500	480	490	480	530	505.3
Control	55.3	50.2	52.8	14.8	15.9	15.4	15	13	14	107	98	102	320	301	310.5
L.S.D at 5%	2.84	1.55	1.51	3.49	2.59	2.00	4.21	8.82	4.58	25.4	16.13	14.09	40.4	26.6	22.5
T x S			2.14			n.s			n.s			19.92			31.8

Table (7): Effect of the inducers on the microbial densities in the rhizosphere under field conditions in 2004 and 2005 seasons

Induces	2004 (S1)						2005 (S2)					
	Bacteria $X10^7$		tinomycetes 10^6		Fungi $x\ 10^3$		Bacteria $X10^7$		tinomycetes 10^6		Fungi $x\ 10^3$	
	*75	**Har	*75	**Har	*75	**Har	*75	*Har	*75	**Har	75	**Har
K_2HPO_4	3.4	2.2	2.5	1.5	1.1	0.6	9.0	1.8	2.3	1.3	1.1	0.6
Salicylic acid	5.7	3.8	4.0	2.4	1.8	1.0	5.0	3.1	3.6	2.0	1.6	0.8
Cobalt sulfate	2.6	2.4	2.5	1.5	1.1	0.8	2.2	2.1	2.1	1.2	1.0	0.6
Ascorbic acid	7.5	5.2	6.7	4.5	1.4	1.2	7.0	4.8	6.2	4.1	1.2	1.0
B. japonicum	3.6	1.6	1.8	2.0	1.6	1.0	3.1	1.5	1.6	1.8	1.0	1.0

*75 days after planting
**Harvest stage for planting

Number before planting	(S1)	(S2)
Bacteria	1.5×10^6	1×10^6
Actinomycetes	1.4×10^5	1×10^5
Fungi	0.8×10^4	2.8×10^4

Effect of chemical inducers on pre-and post-emergence damping-off disease in both successive seasons(2004 and 2005) and their combined analysis

Results of the field experiments are presented in Table (8). In 2004 growing season, seed soaking treatment by chemical inducers in presence of *B. japonicum* decreased pre and post-emergence damping-off compared with untreated seed (*B. japonicum* only). Chemical inducers differed in their effect. Salicylic acid followed by cobalt sulphate and K_2HPO_4 were the highest effective as seedling emergence was increased, where the survived plant average were 82.5, 82.2 and 77.9%, respectively. On the other hand, ascorbic acid was the least effective. Results obtained during 2005 growing season were almost the same as in 2004. Minor differences, however, were observed. Cobalt sulphate was more effective in controlling seedling disease in 2005 than 2004. However, in case of ascorbic acid, the effect was reduced in 2005 than 2004 seasons.

Also, results demonstrated significant differences among tested chemical inducers in the percentage increases of survival in 2004 growing season. Cobalt sulphate followed by SA, K_2HPO_4 and ascorbic acid were found to have the most effect in increasing survival percentage. In general, similar results were obtained in the second season 2005. The average of pre-damping-off in 2005 was slightly high than recorded in 2004. SA followed by cobalt sulfate, K_2HPO_4 gave the highest percentages of survival 80.9 79.8 and 72.9%, respectively. The effect of ascorbic acid was the least treatment.

Table (8): Effect chemical inducers on pre-and post-emergence damping-off disease in two successive seasons and their combined analysis (2004and 2005)

Treatments	Pre %			Post %			Survival %		
	2004	2005	Combined analysis	2004	2005	Combined analysis	2004	2005	Combined analysis
K_2HPO_4	15.4	18.4	16.9	6.7	8.7	7.7	77.9	72.9	75.4
Salicylic acid	12.9	14.2	13.6	4.9	4.9	4.9	82.2	80.9	81.6
Cobalt sulfate	19.3	22.6	21.0	9.5	9.6	9.6	71.2	67.8	69.5
Ascorbic acid	13.7	13.9	13.8	3.8	6.3	5.1	82.5	79.8	81.2
B. japonicum	20.5	23.7	22.1	9.6	10.2	9.9	69.9	66.1	68.0
Control	28.2	33.7	31.0	12.7	14.7	13.7	59.1	51.6	55.4
L.S.D at 5%	1.45	2.96	1.54	0.43	0.74	0.57	1.45	3.49	1.77
*T x **S			2.18			0.40			2.50

*T: Treatment **S: season

Effect of chemical inducers on biological yield and crop parameters (2004 and 2005)

Results in Table (9) indicate that the chemical induced plants recorded significantly higher values of biological yield and crop parameters comparison with untreated plants (*B. japonicum* only). Also, differences were positively significant among chemical inducers treatments in their effect on biological yield for plot ($8.4m^2$). The highest biological in 2004 growing season was obtained from plots treated with SA followed by those K_2HPO_4, cobalt sulfate and ascorbic acid in comparison with control (*B. japonicum* only). The season 2005 recorded a similar trend to that obtained in 2004. Minor differences, however, were observed. Cobalt sulphate was more effective in increasing biological yield in 2005 than in 2004. However, in case of ascorbic acid, the effect was lower than 2004. Concerning pod number of plant, some improvements were observed with chemical inducers as seed soaking in all yield components. In 2004 SA, cobalt sulphate and K_2HPO_4 with exception for ascorbic acid had significantly increased pod number of plant 9 (13.3%), 11.4 (19.4%) and 5(8.5%), respectively, over that in the control (*B. japonicum* only). In 2005 growing season, data showed a similar trend to that obtained in 2004. On the other hand, chemical inducers as seed soaking with exception of ascorbic acid affected significantly seed weight yield. The highest yield was due to K_2HPO_4 followed by SA or cobalt sulphate. The increases of seed yield over the untreated plot (*B. japonicum* only), due to the application of K_2HPO_4, SA or cobalt were 29.9, 29.3% and 29.3, respectively, over those obtained by the control. On the other hand, owing the differences among these inducers for seed protein percentage, the mean seed protein percentage ranged over years between 32 and 36.5%. Cobalt sulphate, ascorbic acid or SA significantly increased seed protein percentage by 5.5, 5.1 and 4.1%, respectively. However, the lowest treatment in increasing this value was K_2HPO_4 (0.6%). Concerning straw protein percentage, the mean straw protein ranged over years between 18.1 and 27.1%. SA and ascorbic acid were the most effective treatments.

Table (9): Effect of the tested chemical inducers on biological yield and crop parameters under field condition in both successive seasons (2004-2005)

Treatments	Biological yield (kg/plot)			Pod number/plant			Seed weight (kg/plot)			Seed protein %			Straw protein %		
	2004	2005	Combined analysis	2004	2005	Combined analysis	2004	2005	Combined analysis	2004	2005	Combined analysis	2004	2005	Combined analysis
K_2HPO_4	6.88	7.04	6.96	63.7	68.1	65.9	2.39	2.38	2.39	34.7	34.9	34.8	24.5	25.7	25.1
Salycilic acid	7.44	7.24	7.34	67.7	70.3	69.0	2.40	2.35	2.38	35.9	36.1	36.0	27.1	27.0	27.1
Cobalt sulfate	6.65	7.36	7.00	60.5	63.1	61.8	2.39	2.36	2.38	36.7	36.3	36.5	25.3	26.5	25.9
Ascorbic acid	6.20	5.96	6.08	70.1	72.5	71.3	1.99	1.80	1.90	36.9	35.8	36.4	26.9	27.2	27.1
B. japonicum	5.88	6.05	5.97	58.7	60.1	59.4	1.78	1.90	1.84	34.5	34.7	34.6	24.8	24.8	24.8
Control	4.70	5.20	4.95	48.3	53.7	51.0	1.20	1.24	1.22	31.9	32.1	32.0	18.3	17.8	18.1
L.S.D at 5%	0.31	0.26	0.19	3.9	3.3	0.10	0.27	0.27	0.18	1.79	0.95	0.95	1.2	0.86	0.70
*T x **S			0.27			2.4			0.25			n.s			1.0

*T: Treatment

**S: season

DISCUSSION

Soybean (*Glycine max* (L.) merr) is one of the most important food crops in Egypt and many other parts of the world. Soybean production in Egypt has been reduced greatly due to the attack by damping-off disease caused by a number of pathogenic fungi. Methods of planting and cultural practices can provide considerable control, but these procedures are still not sufficient to fully damage of root disease and to achieve high yields in this cropping system. Nowadays, the potential of certain chemicals as inducers to control soil-borne plant pathogens when introduced into the rhizosphere as seed dressing or soil drench has been well documented (El-Mougy, 2004). Greatest success with this strategy will likely depend on kind of abiotic chemical selected for their rhizosphere compliance on the host plant target for production as well as their ability to protect against the target soil-borne pathogens. The present study investigated the possibility of minimizing damping-off disease of soybean using seven chemical compounds as resistance inducers in presence of *Bradyrhizobium. Japonicum* strain 3407. Results obtained indicate that soybean treatment with these inducers reduced pre and post emergence damping-off and increase survival plants compared with untreated seed treatment (pathogen + *B. Japonicum*). Salicylic acid followed by K_2HPO_4 , Cobalt sulfate and ascorbic acid were the most effective treatments, they recorded the highest percentage of survived plants under field conditions. Cobalt sulfate (1.0ppm) followed by SA (7.5 mM), K_2HPO_4 (10 Mm) and ascorbic acid (150ppm) in presence of *B. Japonicum* were the most effective in controlling damping-off during both 2004 and 2005 seasons. The role of chemical inducers in induced resistance has been reported in different researches. Application of SA stimulated biosynthesis of different families of P-Rproteins (Raskin, 1992) and increasing the activities of chitinase, peroxidase and B-1.3-glucanase (Tilak *et al.*, 2002). Reuveni *et al.* (1995) indicate that treatment with phosphate associated with systemic resistance to different diseases, (El-Wakil and Metwally (2000) found that seed treatment with ascorbic acid, salicylic acid, decreased pre-and post-emergence damping-off of peanut under greenhouse.

Seed treatment with ethephone, H_2O_2 and GA3 showed no significant effect compared with the control (Pathogen + B. *Japonicum*). Application of Co^{++} as suppressor of ethylene biosynthesis was found to be effective in reducing dowing mildew as seed or foliar treatment. The efficiency of Co^{++}as an inducer of SAR was reported by Aly *et al.* (1993) seed treatment with ethephone is not agreement with results obtained by Ibrahim (1993), this may be attributed to the low concentration. Also, seed treatment with H_2O_2 is not in agreement with the results obtained by Sallem *et al.* (1992) and Abodou *et al.* (1999).

Data of number and dry weight of nodule plant grown in infested soil with pathogenic fungi and inoculated with *B. Japonicum* gave the lowest number of nodules with average reduction of 100, 100 and 82.6% after 75 days of sowing when compared with non-infested ones (*B. Japonicum* only),. Such reduction in nodule numbers could be explained by the detrimental effect on the pathogens with the survival of rhizobia in the rhizosphere and nodule formation. These results are confirmed by the observations recorded by Ghobrial *et al.* (1996), Mousa (1969) and Eisa (1998) who noted that the percentage of *Fusarium* spp and *Selerotium* spp., capable of rapidly killing the root of alfalfa plants might be due to impaired of metabolism of the plant than direct antagonism of the fungi to *Rhizobium*. When the pathogen was controlled by soaking seeds in chemical inducers and inoculated with *B. Japonicum*.

The negative effect caused by *R.solani*, S. *rolfsii* and *M. phaseolina* infestation on nodule formation was less detected particularly in SA or Cobalt sulfate treatments, the average increasing was (45, 12.5 and 42.5%), (40.0, 42.5 and 25%) after 75 days respectively, compared with non-infested ones. Also, it was found that higher dry weight of nodules were recorded dry plants grown under *B. Japonicum* + Cobalt sulfate followed by ascorbic in infested soil with each of pathogen, the average value of these treatments were (319.296 and 306) for cobalt, (309, 282 and 299) for Ascorbic acid, (300, 269 and 306) for SA and (264, 238 and 271 mg/plant) for K_2HPO_4 after 75 days for sowing. Infestation of soil with pathogenic fungi of soybean plants resulted in variable decrease in plant growth parameters. However, the detrimental effect due to pathogen infestation was less detected in treatment after 75 days of planting, in the control treatment, *R. solani*, S. *rolfsii* and *M. phaseolina* drastically reduced plant growth compared to non-infested ones. The decreases in plant growth parameters caused by aforementioned pathogens were (43.9 and 35%), (56.1 and 46.3%) and (34.1 and 27.6%), respectively, for shoot dry weight and N_2-content. Increasing in shoot dry weight due to (pathogen + *B. Japonicum* + cobalt, Pathogen + *B. Japonicum* + AS and Pathogen + *B. Japonicum* + Ascorbic acid were (134.1, 171.4 and 109.5%), (125, 155.3 and 142.9%) and (111.4, 126.3 and 121.4%), respectively. Biological yield and growth parameters were highly significant decrease in control treatment.

Further studies are needed to study the use of selected combination of ***B. Japonicum*** and chemical inducers that might be one of the possible approach to increase the degree and consistency of damping-off control.

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المقاومة المستحثة لمرض موت البادرات فى نباتات فول الصويا باستخدام بعض المستحثات وتأثيرها على التعقد وتثبيت النيتروجين

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تم اختبار بعض العوامل المستحثه مثل فوسفات البوتاسيوم ثنائية القاعدية، حمض الساليسليك ، فوق اكسيد الهيدروجين ،سلفات الكوبلت، حمض الاسكوربيك ، حمض الجبريليك ، الاثيفون على نمو بكتريا البرادى رايزوبيوم الخاصة بمحصول فول الصويا وذلك على البيئة السائلة ووجد أن هذه المركبات لم يكن لها أى تأثير على نمو هذه البكتريا.

تم تقييم هذه المواد كعوامل للمقاومة المستحثه لمرض موت البادرات فى نباتات فول الصويا تحت ظروف الصوبة فكانت ا لنتائج المتحصل عليها كالآتى :-

بصفة عامة النباتات التى لم تلقح أو الملقحة بالبرادى رايزوبيوم 3407 بدون معاملة البذور بالمواد المستحثة والتي تمت زراعتها في تربة ملوثة بفطريات الرايزوكتونيا سولانى، أوسكليروشيوم رولفزاى أو ماكروفومينا فاسيولينا المسببة للمرض قد تأثرت بشدة حينما زادت النسبة المئوية لمرض موت البادرات خاصة فى المعاملات التى لم تلقح بالبكتيريا وأيضا قللت من كفاءة اللقاح البكتيرى المستخدم وذلك مقارنة بنفس تلك المعاملات والتى عوملت بالمواد المستحثة. وكانت أفضل هذه المواد كفاءة (مقاومة) هى حمض الساليسيليك وفوسفات البوتاسيوم ثنائية القاعدية وسلفات الكوبالت حيث أظهرت النتائج زيادة فى النشاط الانزيمى لكلا من من البيراوكسيديز والبولى فينول اكسيديز في النباتات المعاملة بهذه المواد.
بالنسبة للنتائج المتحصل عليها تحت الظروف الحقلية (عند عمر ٧٥ يوما من الزراعة) كانت كالاتى:أظهرت هذه المستحثات تأثيرا ايجابيا على التعقيد والمحتوى النيتروجينى وأيضا الوزن الجاف للنباتات وكانت أفضل هذه المواد هى بحمض الساليسليك وسلفات الكوبلت وفوسفات البوتاسيوم ثنائية القاعدية وكان نفس الاتجاة فى النتائج ملحوظا عند الحصاد حيث أدى استخدام هذه المواد الى الزيادة المعنوية للمحصول (كجم/فدان) وأيضا النسبة المئوية لمحتوى البذور والقش من النتروجين وأدى استخدام بعض المواد المستحثة كمعاملة البذرة الى الزيادة ألمعنوية فى الاعداد الكلية للمحتوى الميكروبى لمنطقة الريزوسفير لنباتات فول الصويا المنزرعة فى الحقل وذلك فى مرحلة النمو الخضرى.