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INDUCTION OF SALICYLIC ACID, CHITINASE AND PHENOLS AGAINST STEM ROT DISEASE IN CHICKPEA PLANTS BY *Pseudomonas* spp.

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ABSTRACT

Two strains of *Pseudomonas* (*P. fluorescens* and *P. aeruginosa*) were used as only seed treatment or seed treatment followed by soil drench to study their effect against chickpea stem rot disease caused by *Sclerotinia sclerotiorum* (Lib) under greenhouse conditions.

Application of *P. aeruginosa* and *P. fluorescens* significantly reduced disease incidence compared with untreated-infected control. However, seed treatment followed by soil drench was more effective and recorded the maximum reduction in disease incidence (85.72 and 71.42% reduction respectively). Also, *P. aeruginosa* was superior compared with fungicide treatment (Rhizolex-T).

Both *P. fluorescens* and *P. aeruginosa* induced the synthesis of salicylic acid (SA), total phenols and chitinase in treated-infected plants with varied amounts at different growth stages of chickpea plants. These increases were much higher and reached to several-fold increase, especially when *P. fluorescens* and *P. aeruginosa* were applied as seed treatment followed by soil drench. Besides the systemic resistance effect, the two strains also increased fresh and dry weight and nodulation in treated-infected chickpea plants compared with untreated-infected control.

Keywords: chickpea, induced resistance, *S. sclerotiorum*, *Pseudomonas* spp., salicylic acid, chitinase, phenols, nodulation.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important food legume with high protein content. The major constraints to chickpea production are susceptibility to diseases (Pande *et al.*, 2005).

Chickpea stem rot disease caused by *Sclerotinia sclerotiorum* (Lib) de Bary, usually causes considerable yield losses under favourable conditions in various chickpea-growing areas (Sharma *et al*., 1999 and Bardin and Huang, 2001). Omar *et al*. (1992) and Mazen (1995) indicated that chickpea stem rot disease is common in most cultivated area in Egypt.

Because of the limitation in the use of fungicides as well as to minimize pollution hazards, use of biological control is likely to be the best alternative to conventional chemical control methods (Sharma *et al.*, 1999). The biological control usually caused by several mechanisms, one of them is induced resistance (Van Loon *et al.*, 1998).

In recent years, strains of *Pseudomonas* spp. have been used extensively for plant growth promotion and induced systemic resistance against a variety of pathogens in several crops including chickpea (Srivastava *et al.*, 2001; Saikia *et al.*, 2003 and Singh *et al.*, 2003), rice (Saikia *et al.*, 2006), cucumber (Chen *et al.*, 1999) and tobacco (Zhang *et al.*, 2004).

It has become apparent that *Pseudomonas* spp. mediated induced resistance is associated with marked metabolic changes, culminating in number of physical and biochemical responses designed to limit pathogen penetration and development in host tissues. These responses including increase in level of salicylic acid (Chen *et al.*, 1999; Zhang *et al.*, 2002 and Saikia *et al.*, 2003). Increase in chitinase activity (Srivastava *et al.*, 2001; Radjacommare *et al*., 2004 and Saikia *et al.*, 2005) and accumulation of phenolic compounds (Pathak *et al*., 2004 and Balamuralikrishnan *et al*., 2005).

Besides the systemic resistance effect, *Pseudomonas* spp. can also promote plant growth and increase nodule formation especially when they are co-inoculated with rhizobia (Lucas Garcia *et al*., 2004; Radjacommare *et al*., 2004 and Zhang *et al.*, 2004).

The objectives of this study were to evaluate the capacity of *P. fluorescens* and *P. aeruginosa* to protect chickpea plants against *S. sclerotiorum* and to assessed metabolic changes in chickpea plants including salicylic acid, chitinase and phenols, due to application of *Pseudomonas* strains as well as to verify the effect on growth of chickpea plants and nodulation.

MATERIALS AND METHODS

- Source of *Pseudomonas* and rhizobia strains:

Two *Pseudomonas* strains, *P. fluorescens* and *P. aeruginosa* and rhizobia strain (*Mesorhizobium ciceri*) were provided from Dept. of Microbiology, Soil, Water and Environment Res. Inst., ARC, Giza.

- Preparation of *Pseudomonas* and rhizobia strains:

Rhizobia strain (*Mesorhizobium ciceri*) was cultured in yeast extract mannitol broth in 250 ml flasks, and incubated at 25^{o}C for 7 days.

P. fluorescens and *P. aeruginosa* were cultured individually in nutrient broth in 250 ml flasks, and incubated at 28^{o}C for 24 h. Then strain of rhizobia adjusted to provide 10^{7} cfu/ml, while both *Pseudomonas* strains were adjusted to provide 10^{9} cfu/ml.

- Seed treatment with strains of *Pseudomonas*:

Surface sterilized seeds of chickpea (Giza 2 cv.) were soaked in cell suspension of *P. fluorescens* and *P. aeruginosa* individually (10^{9} cfu/ml) mixed with 1% carboxymethyl cellulose for 6 h. Then the seeds were allowed to air-dry.

- Preparation of fungal inoculum:

Glass bottles (500 ml in volume) contain corn meal-sand medium (3:1 w/w) were autoclaved at 121^{o}C for 30 min. The sterilized bottles were then inoculated with discs (5 mm) of 6 days-old culture of *S. sclerotiorum* and incubated at 20^{o}C for 18 days (until the whole medium was covered with sclerotia).

Soil infestation was carried out by mixing fungal inoculum with sterilized potted-soil at the rate of 3% (w/w). The infested soil was watered for 7 days to enhance growth and distribution of the fungal inoculum.

Pots (30 cm-diam.) containing infested soil were sown with chickpea seeds (Giza 2 cv.) with different treatments, including seeds treated with *P. fluorescens*, seed treated with *P. aeruginosa*, (as mentioned previously) seed treated with fungicide (Rhizolex T, 3 g/kg seed). Untreated seeds were sown in infested and uninfested soil as a control. Then, cell suspension of rhizobia (10^7 cfu/ml) was added to the soil at the rate of 10 ml/pot.

After 15 days of sowing, pots which containing seeds treated with *P. fluorescens* or *P. aeruginosa* were divided into two groups, one of them inoculated with cell suspension of *P. fluorescens* and *P. aeruginosa* individually, (10^9 cfu/ml) at the rate of 20 ml/pot according to the method of Zhang *et al*. (2004). Three replicates were used for each treatment, and eight seeds were sown in each pot.

Number of rhizobia per plant was recorded 50 days after sowing. All plants were examined during growth stages and disease incidence was recorded after 65 days. Then fresh and dry weights were recorded.

- Metabolic changes associated with induced resistance in chickpea plants by *P. fluorescens* and *P. aeruginosa*:

Samples of chickpea plants (shoots), untreated (healthy or infected) and treated infected were collected after 10, 20, 30 and 40 days of sowing to assay level of salicylic acid, chitinase activity and total phenols content.

A. Salicylic acid:

Assay of salicylic acid was carried out in the Central Laboratory of Biotechnology, Plant Pathology Research Inst., ARC, Giza. One gram of each sample tissues was extracted and homogenized with acetonitrile : 0.1% phosphoric acid (15:85 v/v). The extract was filtered through a Whattman filter paper No. 1 and micro filter (0.45 µm) and stored in vials. HPLC analysis was used to determine salicylic acid in the extracts. The analysis was performed on a model "HP1050" HPLC equipped with UV detector. Separations and determinations were performed on RP18 (ODS) column (4.6 x 250 mm). The mobile phase was the same one which used in the extraction. UV detector was 254 nm and flow rate was 1.5 ml/min according to Gertz (1990).

B. Chitinase activity:

Enzyme extraction was carried according to the method of Tuzun *et al*. (1989) by homogenizing the samples with 0.2 M trais HCl buffer pH 7.8 containing 14 mM B-mercaptoethanol at the rate of 1 : 3 (v/v). The homogenate was filtered and then centrifuged at 3000 rpm for 15 minutes at 6^{o}C. the supernatant was stored at – 20^{o}C. Determination of chitinase activity was carried out according to the colourimetric method of Monreal and Reese (1969) at 540 nm, using 1% colloidal chitin substrate prepared from chitin powder according to the method of Ried and Ogryd-Ziak (1981).

Chitinase activity was expressed as mM N-acetyl glucoseamine equivalent, released from colloidal chitin per gram fresh weight tissue/60 minutes.

C. Total phenols:

Total phenols were spectrophotometrically determined at 520 nm using Folin Denis reagent described by Snell and Snell (1953) as mg calecol/g fresh weight of plants.

RESULTS

Data presented in Table (1) showed that all treatments significantly reduced disease incidence caused by *Sclerotinia sclerotiorum* compared with untreated infected control.

Table 1: Effect of application of *P. fluorescens* and *P. aeruginosa* as seed treatment or seed treatment followed by soil drench on chickpea stem rot disease caused by *Sclerotinia sclerotiorum*, under greenhouse conditions.

Treatment	Disease incidence %	Reduction % relative to control
- Seed treatment:		
P. fluorescens	33.33	42.86
P. aeruginosa	29.17	49.99
- Seed treatment+soil drench:		
P. fluorescens	16.67	71.42
P. aeruginosa	8.33	85.72
- Fungicide (Rhizolex T)	12.50	78.57
Control	58.33	--
L.S.D. 5%	**16.07**	

Application of *P. fluorescens* or *P. aeruginosa* as seed treatment followed by soil drench were more effective. This treatment provided a good protection to chickpea plants against *S. sclerotiorum* during growth stages and recorded the maximum reduction in disease incidence (71.42 and 85.72% reduction, respectively), compared with application of only seed treatment with *P. fluorescens* or *P. aeruginosa* which resulted in 42.86 and 49.99% reduction, respectively.

Moreover, obtained results of *P. aeruginosa* (as seed treatment followed by soil drench) were better than that of the traditional fungicide (Rhizolex T).

Data presented in Table (2) and Fig (1) showed that considerable increase in salicylic acid content in chickpea plants induced by *P. fluorescens* or *P. aeruginosa* and infected with *S. sclerotiorum* compared with untreated healthy plants during examination periods.

Table 2: Salicylic acid concentration in chickpea plants as affected by application of *P. fluorescens* and *P. aeruginosa* as seed treatment or seed treatment followed by soil drench.

Treatment	Salicylic acid (ng/g fresh weight) after days:			
	10	20	30	40
- Seed treatment:				
P. fluorescens	969	570	230	114
P. aeruginosa	1116	597	374	126
- Seed treatment+soil drench:				
P. fluorescens	949	2036	882	348
P. aeruginosa	1114	2305	978	624
Control 1 (infected)	374	199	87	61
Control 2 (healthy)	22	29	28	24

Moreover, accumulation of salicylic acid was much higher in treated infected plants compared with untreated infected control. Application of *P. fluorescens* and *P. aeruginosa* as seed treatment recorded maximum increase in salicylic acid content after 10 days (up to 2.5 fold increase exhibited after 10 days). Thereafter, the concentration sharply decreased. Whereas, application of *P. fluorescens* and *P. aeruginosa* as seed treatment followed by soil drench resulted in additional increase in salicylic acid content. Maximum increase was found during 20 days after planting (up to 10-11 fold increase was exhibited after 20 days than untreated-infected control), then decreased progressively.

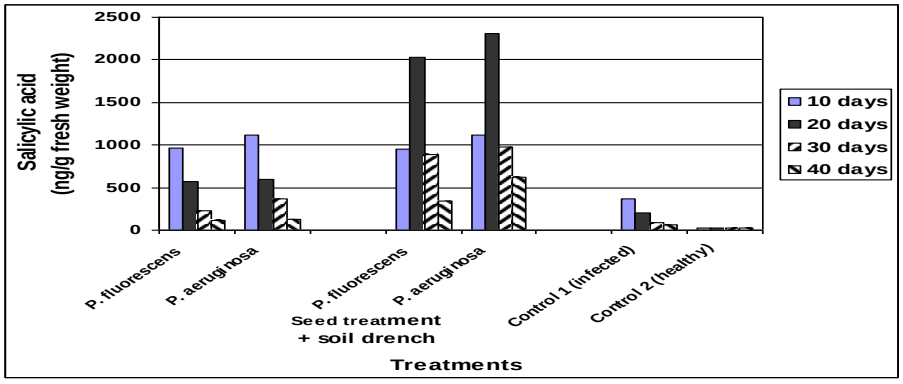

Fig. 1: Induced salicylic acid in chickpea plants when *P. fluorescens* and *P. aeruginosa* applied as seed treatment or seed treatment followed by soil drench.

Generally, it could be concluded that application of *P. fluorescens* and *P. aeruginosa* as seed treatment followed by soil drench was more effective and exhibited maximum accumulation of salicylic acid for a long time. *P. aeruginosa* was superior indicating by the highest increase of salicylic acid during examination period.

Data presented in Table (3) showed chitinase activity in chickpea plants treated with *P. fluorescens* or *P. aeruginosa* as seed treatment or seed treatment followed by soil drench. All treatments showed a pronounced increase in chitinase activity during examination period compared with untreated-infected control. Maximum increase in chitinase activity was recorded after 30 days, thereafter it decreased slightly. At the same time, chitinase activity remained at low level in healthy untreated plants and no such increase in activity was recorded.

Application of *P. fluorescens* and *P. aeruginosa* as seed treatment followed by soil drench resulted in maximum increase in chitinase activity during 20, 30 and 40 days after sowing compared with application of seed treatment only. Up to 3-4 fold increase in chitinase activity after 20 days, 2.5-3 fold increase after 30 days and 2-3 fold increase after 40 days relative to untreated infected control.

Meanwhile, seed treatment showed slight increase in chitinase activity during examination periods. *P. aeruginosa* was more effective for induction and accumulation of chitinase in treated-infected chickpea plants.

Table 3: Changes in chitinase activity in chickpea plants treated with *P. fluorescens* or *P. aeruginosa* as seed treatment or seed treatment followed by soil drench.

Treatment	** Chitinase activity after days:			
	10	20	30	40
Seed treatment:				
P. fluorescens	1.07	1.62	2.63	2.33
P. aeruginosa	1.39	2.74	2.88	2.54
Seed treatment+soil drench:				
P. fluorescens	1.12	4.29	5.97	4.30
P. aeruginosa	1.32	4.86	7.13	6.75
Control 1 (infected)	0.95	1.18	2.00	2.20
Control 2 (healthy)	0.64	0.55	0.57	0.61

**** Chitinase activity expressed as mM N-acetyl glucose amine equivalent released/g fresh weight/60 minutes.**

Data presented in Table (4) showed that infection with *S.sclerotiorum* led to a gradual increase in total phenols content in infected control during examination periods as compared with untreated-healthy control. At the same time, application of *P. fluorescens* and *P. aeruginosa* as seed treatment or seed treatment followed by soil drench resulted in a pronounced increase in total phenols content compared with untreated-infected control, during time course of examination.

Table 4: Effect of application of *P. fluorescens* and *P. aeruginosa* as seed treatment or seed treatment followed by soil drench on total phenols content in chickpea plants.

Treatment	Total phenols (mg/g fresh weight) after days:			
	10	20	30	40
- Seed treatment:				
P. fluorescens	5.95	6.21	7.65	7.20
P. aeruginosa	5.39	5.80	6.69	6.32
- Seed treatment+soil drench:				
P. fluorescens	5.52	7.64	12.15	11.76
P. aeruginosa	5.13	6.79	10.13	8.62
Control 1 (infected)	3.83	4.11	4.36	5.52
Control 2 (healthy)	2.20	3.32	3.48	2.91

Maximum increase in total phenols content was recorded after 30 days. This increase reached more than 1.5 fold in seed treatment, while it was more than 2.5 fold increase in total phenols content when *P. aeruginosa* and *P. fluorescens* were used as seed treatment followed by soil drench. *P. fluorescens* recorded the highest increase in total phenols content compared with *P. aeruginosa*.

Data presented in Table (5) showed that all treatments significantly increased fresh and dry weights of chickpea plants compared with untreated-infected control. In case of fresh weight, *P. fluorescens* followed by *P. aeurginosa* (as seed treatment followed by soil drench) were the most effective treatments judged by the maximum increase in root and shoot fresh weights followed by seed treatment with fungicide and *P. fluorescens* as seed treatment. While, *P. aeurginosa* as seed treatment recorded the lowest root and shoot fresh weights. The same trend was found in root dry weight. Whereas, maximum increase in shoot dry weight was recorded by *P. fluorescens* and *P. aeurginosa* (as seed treatment followed by soil drench) followed by *P. fluorescens* (as seed treatment), and fungicide, respectively. While, *P. aeurginosa* as seed treatment recorded the lowest increase.

Concerning the number of rhizobia, obtained data revealed that all treatments significantly increased number of rhizobia/plant compared with untreated-infected control. Moreover, this increase was more than 3-6 folds according to the method of application.

P. aeruginosa and *P. fluorescens* as seed treatment followed by soil drench were the most effective; they recorded the highest number of rhizobia 32.66 and 25.67 rhizobium per plant, respectively, followed by seed treatment which recorded 20.00 and 19.33 rhizobium per plant. While seed treated with fungicide (Rhizolex T) recorded the lowest number of rhizobia.

Table 5: Effect of application of *P. fluorescens* and *P. aeruginosa* as seed treatment or seed treatment followed by soil drench on fresh weight, dry weight and nodulation of chickpea plants.

Treatment	Fresh weight (g)		Dry weight (g)		No. of rhizobia per plant
	Root	Shoot	Root	Shoot	
- Seed treatment:					
P. fluorescens	2.90	7.33	1.03	2.44	19.33
P. aeruginosa	2.03	5.43	0.88	1.50	20.0
- Seed treatment+soil drench:					
P. fluorescens	5.33	9.97	1.88	3.00	25.67
P. aeruginosa	4.15	8.50	1.50	2.53	32.66
- Fungicide (Rhizolex T)	2.97	7.45	1.10	1.90	17.0
Control	1.37	2.60	0.47	0.67	5.33
L.S.D. 5%	**1.17**	**1.59**	**0.24**	**0.31**	**4.62**

DISCUSSION

Obtained data indicated that application of *P. fluorescens* and *P. aeruginosa* reduced chickpea stem rot disease incidence compared with untreated-infected control. However, application of bacteria, as seed treatment followed by soil drench, were more effective indicated by the maximum reduction in disease incidence compared with seed treatment only. Moreover, *P. aeruginosa* was superior than fungicide treatment (Rhizolex T). Several investigations indicating that seed treatment with *Pseudomonas* spp. followed by soil drench after planting can strengthen the induced systemic resistance and growth promotion effects (Van Loon *et al.*, 1998; Srivastava *et al*., 2001; Radjacommare *et al*., 2004 and Zhang *et al*., 2004).

Control of chickpea soil borne diseases has been demonstrated by various *Pseudomonas* spp. Srivastava *et al*. (2001) found that *P. fluorescens* induced resistance against charcoal rot disease in chickpea caused by *M. phaseolina*. *P. aeruginosa* an isolate from chickpea plants rhizosphere significantly reduced the incidence of Fusarium wilt disease caused by *F. oxysporum* f.sp. *ciceris* (Anjaiah and Koedam 2003). Also, Saikia *et al*. (2003) tested different strains of *P. fluorescens* against Fusarium wilt disease of chickpea, they found that all strains systemically induced resistance against Fusarium wilt and reduced the wilt disease by 26-50% compared to the control. Singh *et al*. (2003) indicated that application of *P. fluorescens* and *P. aeruginosa* as seed treatment protected chickpea plants against *Sclerotium rolfsii* infection. *P. aeruginosa* gave the best protection to the seedlings.

Application of *P. fluorescens* and *P. aeruginosa* as seed treatment only or seed treatment followed by soil drench resulted in increase in salicylic acid level in treated-infected chickpea plants compared with untreated-infected control. Meanwhile, this increase was not found in healthy plants. During 10

days, seed treatment with *Pseudomonas* recorded maximum increase in salicylic acid level. This increase reached to 2.5 fold, while seed treatment with *Psuedomonas* followed by soil drench was more effective. Additional increase in salicylic acid level was found in this treatment after 20 days (up to 10-11 folds increase than untreated-infected control), then the levels of salicylic acid decreased but it was still higher compared with other treatments. *P. aeruginosa* induced comparatively more salicylic acid in chickpea plants than *P. fluorescens*. This result indicating that sensitization of plants by PGPR was dependent on different strains (Zhang *et al*., 2002).

Different investigation indicating that induced resistance against plant diseases using *Pseudomonas* spp. was associated with increase levels of salicylic acid in plant tissues. Root colonization of tobacco plants with *P. fluorescens* CHAO as well as leaf infection with TMV, caused up to five fold increase in salicylic acid in the leaves (Van Loon *et al*., 1998). Chen *et al*. (1999) reported an increase in salicylic acid levels in cucumber roots after treatment with two strains of *Pseudomonas* spp. Saikia *et al*. (2003) found that isolates of *P. fluorescens* induced resistance against Fusarium wilt of chickpea and increased salicylic acid in root tissues; PF4-92 induced comparatively more salicylic acid than other isolates. 1700 to 2000 eta g SA g^{-1} fresh root was detected from the application site of root. Whereas, the amount of SA at distant site ranged between 400-500 eta g. Both *P. fluorescens* and *P. aeruginosa* protected chickpea plants against *S. rolfsii* when applied as seed treatment. The two strains induced synthesis of salicylic acid with varied amounts at different growth stages of chickpea plants. Salicylic acid induced frequently during only the first 3 weeks of growth and *P. aeruginosa* was more effective (Singh *et al*., 2003). Also, Saikia *et al*. (2006) showed that treating rice seedlings with two strains of *P. aeruginosa* (Pa-RsG 18 and Pa-RsG 28) resulted in more salicylic acid accumulation in root tissues of bacterized site than in distant root on the opposite site of the root system. Moreover, salicylic acid level in Pa-RsG18-treated root tissues was higher than in Pa-RsG 28-treated root. Many studies have indicated that salicylic acid (SA) play an important role in plant defense response against pathogen attack and is essential for the development of systemic acquired resistance (Ryals, 1994; Van Loon *et al*., 1998 abd Zhang *et al*., 2002).

Increased salicylic acid level in plant may inhibit activity of catalase and ascorbic peroxidase, which then leads to increased levels of H_2O_2. The elevated H_2O_2 levels activate PR gene expression and increased the rate of polymerization of phenolic compounds into lignin like substances and making the plants more resistant to pathogen attack (Chen *et al.*, 1993 and Zhang *et al*., 2002).

Obtained results showed considerable increase in chitinase activity in chickpea plants induced with *P. fluorescens* or *P. aeruginosa* during examination periods compared with untreated-infected control. Giri *et al*. (1998) found a positive relationship between induction of chitinase in chickpea plants and resistance to *Fusarium oxysporum*.

Application of *P. fluorescens* or *P. aeruginosa* as seed treatment combined with soil drench resulted in maximum increase in chitinase activity compared with application of seed treatment only. This increase reached to 3

- 4 fold, 2.5 - 3 fold and 2 - 3 fold increase during 20, 30 and 40 days and was associated with the highest reduction in disease incidence. Radjacommare *et al*. (2004) also indicated that chitinases were induced in response to invasion by pathogen have greater antifungal activity to the pathogen in suppressing symptoms. Chitinases have been suggested to play a major role in defense responses of plants to the pathogen attack by lyse hyphal tips of fungi or are involved in release of oligosaccharide signal molecules (elicitors) that can activate plant defense mechanisms Ryan (1988). More generally, stimulation of such enzyme activities has been associated with incompatible plant pathogen interaction (Daulagala *and* Allan 2003).

Increase in chitinase activity associated with induced resistance against different fungal diseases have been reported on some crops. Srivastava *et al*. (2001) reported that treatment chickpea seedling with *P. fluorescens* followed by soil drench induced systemic resistance against *M. phaseolina* and increased level of chitinase by 6.6 up to 4 days post inoculation. Viswanathan and Samiyappan (2001) indicated that application of *Pseudomonas* spp. as seed treatment followed by soil treatment induced higher accumulation of chitinase in sugarcane stalk tissues. Inoculation with *Colletotrichum falcatum* in the stalk tissues showed multifold increase in chitinase activity in the *Pseudomonas*-treated canes, whereas in the *Pseudomonas*-untreated canes, the increase was less. Higher chitinase activity was found in Chinese cabbage seedling treated with *P. syringae* pv. *phaseolicola* and infected with *Botrytis cinerea*. Maximum increase in chitinase activity was recorded after 31 days of inoculation (Daulagala and Allan, 2003). Radjacommare *et al*. (2004) reported that treating rice plants with *P. fluorescens* (seed + soil + foliar) induced systemic resistance against *R. solani*. *Pseudomonase*-treated plants showed two fold increases in chitinase activity compared with untreated control. Also, Saikia *et al*. (2005) indicated that treatment chickpea seedling with *P. aeruginosa* induced resistance against Fusarium wilt of chickpea and increased chitinase activity than the untreated-infected control.

Time course of phenol content showed a gradual increase in total phenolic contents in chickpea plants induced with *P. fluorescens* and *P. aeruginosa* and infected by *S. sclerotiorum*. This increase was much higher compared with untreated-infected control. Maximum increase was recorded after 30 days of planting. Moreover, application of *P. fluorescens* and *P. aeruginosa* as seed treatment followed by soil drench recorded the highest increase in total phenols content (up to 2.5 fold increase), which was associated with the maximum reduction in disease incidence. Compared with seed treatment only which showed 1.5 fold increase in total phenolic content and the lowest reduction in disease incidence. Obtained data were similar to that obtained by Singh *et al*. (2003). They indicated that application of *P. fluorescens* and *P. aeruginosa* induced resistance against *S. rolfsii* and induced the synthesis of total phenolics with various amounts at different growth stages of chickpea plants. The maximum amount of total phenolics was recorded in all the aerial parts of 4 weeks-old plants.

Bazzalo *et al*. (1985) showed a positive relationship between accumulation of phenolic compound in sunflower stem and resistance to Sclerotinia stem rot.

They also indicated that those phenolic compounds have fungitoxic effects *in vitro*.

The importance of phenolic compounds in host-parasite interaction is that they act as hydrogen donors/acceptors in oxidation reduction and their involvement in resistance by toxic to microorganisms (Farkas and Kiraly, 1962 and Gupta *et al.*, 1992). Many studies reported that the enhanced induction of total phenols might have contribution for induced systemic resistance triggered by the various biotic inducers specially *Pseudomonas* spp. (Meena *et al*., 2000; Ramamoorthy *et al.*, 2002; Sivakumar and Sharma, 2003; Pathak *et al*., 2004 and Balamuralikrishnan *et al*., 2005).

With regard to fresh and dry weights of chickpea plants, the present results showed that induction of resistance was accompanied with increase in fresh and dry weights of chickpea plants induced by *P. fluorescens* and *P. aeruginosa* compared with untreated-infected control. However, application of both bacteria as seed treatment followed by soil drench recorded the highest increase in fresh and dry weights compared with seed treatment only. Obtained data were in line with those reported by Zhang *et al*. (2004), they found that PGPR strains increased fresh and dry weights of tobacco plants when applied as seed treatment followed by root drenches 2-3 weeks after planting compared with seed treatment only.

Plant growth promotion is another beneficial effect of *Pseudomonas* spp. and research on *Pseudomonas* spp. was initially focused on this effect (Saikia *et al*., 2003; Lucas Garcia *et al*., 2004; Bhatia *et al*., 2005 and Saikia *et al*., 2006).

The mechanisms by which these bacteria affect the plants involve the production of sidrophore, hydrocyanic acid (HCN), phytohormons (indole-3-acetic acid (IAA)), gibberellin (GA_3) and cytokinin and other associated activities which include phosphate solubilization in soil resulting in plant growth promotion (Peng *et al*., 1992; Zhang *et al*., 2004 and Bhatia *et al*., 2005).

Significant increase in number of rhizobia in chickpea plants induced by *P. fluorescens* and *P. aeruginosa* and infected with *S. sclerotiorum* compared with untreated-infected plants. Maximum number of rhizobia was found when *P. fluorescens* and *P. aeruginosa* were applied as seed treatment followed by soil drench. *P. fluorescens* was superior. Obtained results were in agreement with those reported by Zhang *et al*. (1996), Rao *et al*. (1999) and Lucas Garcia *et al*. (2004). They indicated that application of *Pseudomonas* spp. as seed treatment or soil drench or in combination increased number of rhizobia compared with untreated control.

All phytohormons are implicated in nodule formation in one way or another (Hirsch *et al*., 1997).

Common mechanisms used by rhizobacteria to alter nodule formation include the release of phytohormons such as auxins, gibberellins, cytokinins and ethylene, or the alternation of endogen levels in plants (Hirsch *et al*., 1997; and Zhang *et al*., 1997). The effects of some phytohormons are indirect, as they stimulate root growth, providing further sites for infection and nodulation (Zhang *et al*., 2004).

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إستحثاث تكوين حامض السليسليك وإنزيم الشيتينيز والفينولات ضد مرض عفن الساق فى نباتات الحمص بواسطة بكتريا السيدوموناس

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تم إستخدام نوعين من بكتريا السيدوموناس هما سيدوموناس إيروجينوزا وسيدوموناس فلوريسنس كمعاملة بذرة فقط أو معاملة بذرة يليها معاملة التربة وذلك لدراسة تأثيرهما ضد مرض عفن الساق فى نباتات الحمص المتسبب عن الفطر سكليروتينيا سكليروتيورم تحت ظروف الصوبة.

أدى إستخدام كل من عزلتى البكتريا سيدوموناس إيروجينوزا وسيدوموناس فلوريسنس إلى خفض نسبة الإصابة بالمرض معنوياً وذلك مقارنة بالكنترول الغير معامل. كما وجد أن معاملة البذرة بالبكتريا يليها معاملة التربة كانت أكثر فعالية حيث سجلت هذه المعاملة إقصى إنخفاض فى شدة الإصابة (٨٥,٧٢ ، ٧١,٤٢% إنخفاض على التوالى) وتفوقت المعاملة بعزلة البكتريا سيدوموناس إيروجينوزا فى تأثيرها مقارنة بالمعاملة بمبيد الريزولكس تى.

وجد أن المعاملة بكل من سيدوموناس إيروجينوزا وسيدوموناس فلوريسنس أدى إلى إستحثاث تكوين كل من حامض السليسيلك وإنزيم الشيتينيز والفينولات فى نباتات الحمص المعاملة بالبكتريا مقارنة بالكنترول الغير معامل والمعدى بالفطر وذلك خلال مراحل نمو النبات المختلفة ، وكانت الزيادة عالية جداً فى حالة إستخدام كل من عزلتى البكتريا فى صورة معاملة بذرة يليها معاملة التربة.

بالإضافة إلى المقاومة الجهازية فقد أدى إستخدام كل من عزلتى البكتريا إلى زيادة ملحوظة فى الوزن الرطب والجاف وزيادة عدد الريزوبيا فى نباتات الحمص المعاملة بالبكتريا مقارنة بالكنترول الغير معامل.