Egypt. J. Phytopathol., Vol. **44**, No. 1, pp. 25-47 (2016)

Salicylic Acid and *Pseudomonas fluorescens* as Safe Control Means Against *Rhizoctonia solani* in Guar (*Cyamopsis tetragonoloba* (L.) Taub.)

M.F. Abdel-Monaim
Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.

Nineteen isolates of *Rhizoctonia solani* were found to be associated with root rot symptoms of guar plants collected from different fields located at New Valley Governorate, Egypt. All the obtained isolates were able to attack guar plants (cv. Local) causing damping-off and root rot diseases. *Rhizoctonia solani* isolate No. 8 (RG8) was the most virulent one in the pathogenicity tests. Salicylic acid (SA) and *Pseudomonas fluorescens* (PF) were examined individually or in combination *in vitro* and *in vivo* for controlling damping-off and root rot as well as growth promotion of guar plants. Activity of defence-related enzymes, including peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), pathogenesis related (PR) protein (chitinase and β 1,3 gluconase), were also studied in inoculated and non-inoculated guar plants treated with SA and *P. fluorescens* either individually or in combination, during the experimental period.

Keywords: Damping-off, defense, enzymes, growth parameters, guar, *Pseudomonas fluorescens*, *Rhizoctonia solani*, root rot, salicylic acid and yield components.

Guar or cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) belongs to the family Fabaceae (Leguminaceae) (Gillet, 1958), is a coarse, upright, bushy, a drought tolerant summer annual legume and it is cultivated as a feed crop for human and livestock consumption. Guar is commercially grown for its seeds as a source of natural polysaccharide (galactomannan), commercially known as guar gum. Guar gum has a number of usage in food (Khalil, 2001) and other industries, such as paper, textiles, oil well drilling and pharmaceuticals and a well-known traditional plant used in folklore medicine. It acts as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia anti-ulcer, anti-secretory, cytoprotective, hypoglycemic, hypolipidemic and anti-hyperglycemic effects (Mukhtar *et al*., 2006). In addition, guar beans are potentially high sources of additional phytochemicals (Wang and Morris, 2007).

Guar is known to suffer by many diseases which affecting its quality and yield resulting in severe economic losses to the country as it is an important cash crop with a great potential for foreign exchange (Mohamed *et al*., 2006 and Pareek and Varma, 2014). Among the different pathogens attacking the crop, *Rhizoctonia solani* is the most common fungus causing considerable yield losses (Matloob and Juber, 2013). The pathogen is causing damping-off disease in the seedling stage. At later stages of plant growth, the infected plants exhibit root-rot symptoms resulting in wilting of the host plant (Pareek and Varma, 2014).

Although application of fungicides is far the most effective method to control guar damping-off and root rot diseases, it can be involved in many problems due to health risk concerns and environmental pollution. Thus, there is a growing need to develop alternative approaches for the management of this pathogen. An acceptable approach that is being actively investigated involves the use of plant growth promoting rhizobacteria such *Pseudomonas fluorescens* and bio-active substances such salicylic acid in controlling soil borne fungi (Rajkumar *et al*., 2008 and Couillerot *et al*., 2009 and Abdel-Monaim, 2013).

Salicylic acid (SA) has been found to be active as antimicrobial agent and in various trials as disease resistance inducer. This has been reported for inducing resistance against several plant pathogens, i.e. TMV (Marrero *et al*., 1990), bacterial soft rot (El-Sayed, 1996), bacterial wilt (Abdel-Said *et al*., 1996) as well as soil borne fungi causing root rot and wilt diseases (Rajkumar *et al*., 2008 and Abdel-Moaniam, 2013). Moreover, few attempts proved the direct inhibitor effect of SA on the growth of phytopathogenic microorganisms (El-Mougy, 2002 and 2004).

On the other hand, the application of biological control using antagonistic microorganisms proved to be successful for controlling various plant diseases in many countries. Biological control is proposed to be an effective and non-hazardous strategy to reduce crop damage caused by plant pathogens. In recent years *P. fluorescens* has been extensively used for plant growth promotion and disease control (Tabarraei *et al*., 2011). Biological control of soil borne pathogens is often attributed to improved nutrition that boosts host defences or to direct inhibition of pathogen growth and activity. Amendment with certain abiotic factors (inducers) appears to stimulate the disease resistance by indirect stimulating indigenous populations of microorganism that are beneficial to plant growth and antagonistic to pathogens. For example chitin amendment of soil has been found to stimulate the growth of chitinolytic microorganisms (De Boer *et al*., 1999), increase the biocontrol activity and stimulate the expression of plant defence proteins (Abdel-Monaim, 2013). All these effects may culminate in enhancing plant protection .

Similarly, SA was tested in combination with biocontrol agents. Saikia *et al*. (2003) tested the efficiency of *P. fluorescens*, with or without SA amendment, in chickpea against Fusarium wilt infection. Application of *P. fluorescens* (pf4-92) with SA recorded highest protection of chickpea seedlings against wilting.

The present study is designed to evaluate the efficiency of SA and *P. fluorescens* when used individually or in combination, as safe control means against damping-off and root rot diseases in guar.

Materials and Methods

Isolation and identification of Rhizoctonia solani:

Rhizoctonia solani was isolated from guar plants, shown necrotic lesions on root and hypocotyls, collected from fields in New Valley Governorate. Small pieces of infected plant materials were surface sterilized by 0.5% sodium hypochlorite for 1 min., rinsed with sterile distilled water and plotted dry. The pieces were transferred into Petri plates (9-cm-diam) containing potato dextrose agar (PDA) medium

amended with 50 ml /L streptomycin sulphate. The plates were incubated at 25°C for 48-72 hrs. Fungal hyphae from the margin of developing colonies were transferred into PDA slants. *R. solani* isolates were identified on the basis of hyphal characteristics (Sneh *et al*., 1996). Identification was confirmed by Assiut University Mycological Centre (AUMC), Assiut Univ., Assiut, Egypt. The obtained isolates were maintained on PDA slants and kept in refrigerator at 5°C for further study.

Pathogenicity of Rhizoctonia solani isolates:

Pathogenicity tests of *R. solani* isolates were carried out at New Valley Agric. Res. Station, on local guar cultivar in pots containing soil infested with the tested isolates individually using homogenized culture technique according to Muthomi *et al*. (2007).

Preparation of the fungal inocula:

The inocula of the tested isolate (RG8) were prepared from one week old culture grown on 50 ml potato dextrose broth (PDB) medium in conical flasks (250 ml) and incubated at 25 ± 1°C. The content of flask was homogenized in a blender for one min. Sterilized plastic pots (25 cm in diam.) were filled with sterilized soil and mixed with fungal inocula at rate 100 ml homogenized culture per pot, seven days before planting. Five pots were used as replicates per isolate and another 5 pots with equal amount of sterile PDB medium, without fungal inoculation, were severed as check. Surface sterilized seeds were sown in pots at the rate of 5 seeds /pot. Percentage of damping-off was recorded 30 days after planting. Moreover, severity of root rot was determined 90 days after planting using a rating scale of 0 to 5 on the basis of root discoloration or leaf yellowing as follows: 0, neither root discoloration nor leaf yellowing; 1= from <0 to 25% root discoloration or one leaf yellowed; 2= from <25 to 50% root discoloration or more than one leaf yellowed; 3= from <50 to 75% root discoloration plus one leaf wilted; 4= up to 75% root discoloration or more than one leaf wilted; and 5, completely dead plants. For each replicate, a disease severity index (DSI) similar to that suggested by Liu *et al*. (1995) as follows:

$$DS= \Sigma d/(d\ max \times n) \times 100$$

Whereas: (d) is the disease rating possible, (d) max is the maximum disease rating and (n) is the total number of plants examined in each replicate.

Isolation and in vitro screening of P. fluorescens against R. solani pathogen:
Isolation of fluorescent pseudomonads:

Fluorescent pseudomonads were isolated from rhizosphere of guar grown in an agriculture field at El-Kharga, New Valley Governorate as detailed by Rajkumar *et al*. (2005). Fluorescence of the colonies under UV light was taken as the primary criterion for selection of the isolates.

Screening of fluorescent pseudomonads:

Pseudomonas florescence strains were screened for their *in vitro* antagonistic ability against *R. solani* by dual culture technique (Yoshida *et al*., 2001). A virulent strain of *R. solani* (RG8) was used in this study. Bacterial isolate was streaked at one side of Petri dish (1 cm away from the edge) containing PDA and incubated at 25±1°C for 24 hr. Then five mm mycelial plug taken from seven-day-old PDA cultures of *R. solani* were transferred on the opposite side of Petri dishes perpendicular to the bacterial streak 24hr after inoculation. Petri dishes inoculated

with fungal discs alone were served as check. Petri dishes were then incubated at 25±2°C until colony of check cover plate. Three replicates were used for each isolate. Observations check on width of inhibition zone and mycelial growth of test pathogen were recorded and percent inhibition of pathogen growth was calculated. One effective strain resulted from the above dual culture studies was selected and used in further studies.

Efficacy of salicylic acid and P. fluorescens against R. solani:
In vitro studies:

The effect of chemical inducers (SA) and plant growth promoting rhizobacteria (*P. fluorescens*) individually and/or in combination on *R. solani* was studied as follows:

Chemical inducer (SA):

The effect of SA on the growth of *R. solani* was evaluated on PDA medium. Twenty ml of PDA medium containing 1 mMSA was poured into the Petri dishes (9-cm-diam.) plates and inoculated with the pathogenic fungi as above mentioned.

Antagonistic biocontrol agents:

Pseudomonas fluorescens was streaked at opposite ends of PDA near the edge and incubated at 25±1°C for 24 hr., then a mycelial disc (5-mm-diam.) of the tested fungus was transferred onto the centre of each plate as above mentioned. Plates were incubated at 25±2°C for 7 days.

Combination between salicylic and P. fluorescens:

Flasks (250 ml) each containing 200 ml PDA medium amended with 1 mMSA then poured in 10 plates. These plates were inoculated with antagonistic isolate and pathogenic isolate as mentioned before. PDA plates inoculated only with discs of *R. solani* were kept as control (check). The inoculated plates were incubated at 25±1°C until colony of control grew to cover full plate. At this point, colony diameter was measured. Percentage of growth inhibition of the pathogen was calculated using the following formula:

$$\text{Inhibition (\%)} = (A-B)/A \times 100$$

Whereas: A= Colony diameter of the pathogen in check treatment and B= Colony diameter in treated plates.

In vivo studies:
Greenhouse experiments:

The effects of salicylic acid (SA) as inducer of plant resistance and *Pseudomonas fluorescens* (PF) as plant growth promoting rhizobacteria, individually or in combination, against guar damping-off and root rot diseases incited by *R. solani* were evaluated under greenhouse conditions. SA was prepared as solutions at 1 mM. Cell suspension of *P. fluorescens*, grown on nutrient broth medium for 3 days at 25±1°C, was adjusted to 2.5×10^8 cfu/ml (Abdel-Monaim, 2013). The combination between SA and *P. fluorescens* prepared by dissolving chemical inducers in suspension of *P. fluorescens*. Guar seeds soaked for 6 hr. in the following treatments: 1= SA (1 mM); 2= *P. fluorescens* (2.5×10^8 cfu/ml); 3= SA+*P. fluorescens*; and 4= check.

Plastic pots (25 cm in diam.) were filled with sterilized soil and mixed with *R. solani* (isolate RG 8) inocula at rate 100 ml homogenized culture per pot, seven days before planting, then sown by 5 seeds of each treatment. Five replicates were used for each treatment. Guar seeds soaked in water for 6 hr. and sown at the same rate were used as check treatment. Pots were irrigated as needed. All pots were examined after 30 and 90 days of sowing to calculate the percentage of damping-off and root rot severity, respectively.

Field experiment:

Field experiment was carried out at El-Kharga Res. Station Farm, New Valley Governorate and at Mallawy Res. Station Farm, Minia Governorate, during summer of 2014 growing season, to evaluate the efficiency of SA and *P. fluorescens*, individually or in combination, against damping-off and root rot diseases and their effect on growth and yield parameters under field conditions. The experiment was designed as complete randomized block with three replicates. The experimental unit area was 10.5 m^2 (3.5X3m). Each unit included 5 rows; each row was 3.5m in length and 60cm width. Guar seeds (cv. Local) were soaked in the tested treatments for 6 hr. as described before. Treated seeds were sown in hills 25 cm apart on one side of row, 2 seed per hill. Guar seeds soaked in water for 6 hr. and sown at the same rate were kept as check treatments. The normal cultural practices of growing guar were followed. Percentages of damping-off and root rot severity were calculated 30 and 90 days after sowing. At harvest time, plant height (cm), number of branches /plant, number of pods/ plant, guaran content (gm/plant) and, 100-seed weight (seed index) and total yield (kg/fed.) were measured. Guaran content (gm/plant) in seeds was recorded using the method of Anderson (1949).

Biochemical changes associated with salicylic acid and P. fluorescens treatments:

To observe the accumulation of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and pathogenesis related protein (Chitanase and β-1,3-glucanase) as well as content of each of phenolic compounds and lignin, 15 days old seedlings were injected with SA (1 mM), *P. fluorescens* (2.5×10^8 cfu/ml), SA+*P. fluorescens* and sterilized distilled water (SDW), 50 µl/plant. Two days after treatment, pot soils infested were inoculated with 100 ml of *R solani* (isolate RG8) homogenate suspension per pot. The following treatments were made (i) check- treated with SDW only; (ii) pathogen check-treated with *R. solani*; (iii) plants treated with SA; (iv) plants treated with *P. fluorescens*; (v) plants treated with SA+*P. fluorescens*; (vi) plants treated with SA and after 2 days inoculated with the pathogen (vii) plants treated with *P. fluorescens* and after 2 days inoculated with the pathogen (viii) plants treated with SA+*P. fluorescens* and after 2 days inoculated with the pathogen. The peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), chitanase and β-1,3-glucanase activities as well as content of phenolic compounds and lignin were estimated 0, 2, 4, 6, 8 and 10 days after inoculation.

One gram 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 and the homogenates were centrifuged at

8000 rpm at 4°C for 25 min. The supernatants (crude enzyme extract) were stored at −20°C or immediately used for determination PO, PPO, PAL, chitanase and β-1,3-glucanase enzymes activities and total protein. In the case of every enzyme under investigation, each treatment consisted of four replicates (3 plants/replicate) and two spectrophotometric readings using Milton Roy Spectrophotometer (Milton Roy spectronic1201) were recorded for each per replicate. The experiment for bioassays was repeated twice in time.

Peroxidase activity:

The enzyme activity of PO was determined by using the direct spectrophotometric method (Hammerschmidt *et al*., 1982) using guaiacol as common substrate for peroxidases. 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/mg protein (Urbanek *et al*., 1991).

Polyphenoloxidase activity:

The activity of PPO 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 protein per minute.

Phenylalanine ammonia-layse activity:

Phenylalanine ammonia-layse (PAL) activity was determined using the direct spectrophotometric method adapted by Cavalcanti *et al.* (2007). Two hundred microlitres 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 h, 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 protein.

Chitinase activity:

The chitinase activity was determined using the method described by Wirth and Wolf (1992). High polymeric carbomethyl-substituted chitin labelled covalently Remazol Brilliant Violet 5R (CM-Chitin-RBV. Comp. Loewe Biochemical) 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 N HCl. 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 unit/mg protein.

β-1,3-glucanase activity:

β-1,3-glucanase 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 375µl of dinitrosalicylic acid and heating for 5 min on boiling water, vortexed and its absorbance was measured at 500 nm. The enzyme activity was expressed as µg glucose released/min/mg protein.

Protein concentration:

Total protein content of the samples was quantified according to the method described by Bradford (1976).

Determination of phenolic compounds:

To assess phenolic content, 1 g fresh plant sample 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 725nm. Catechol was used as a standard. The amount of phenolic content was expressed as phenol equivalents in mg/ gm fresh tissue (Saikia *et al*., 2006).

Determination of lignin:

One gram plant tissue from each treatment was mixed with 10g of trichloroacetic acid (TCA) and incubated at 90°C. Delignification was stopped by cooling the reaction mixture after 240 min. of reaction time. The reaction vessel was immersed in cold water and 5 ml of cold acetone were added. Suspension was filtered and liquor was evaporated until dark, high consistency liquid without smell of acetone was obtained. Lignin was precipitated by pouring the liquid in 200 ml cold water. Lignin was filtered and washed with warm water several times. After that, lignin was air-dried overnight at 4°C then weight (Liken and Perdih, 1999).

Statistical analysis:

Analyses of variance were carried out using MSTATC (1991) program (Ver. 2.10). Least significant difference was employed to test for significant difference between treatments at $p \leq 0.05$ (Gomez and Gomez, 1984).

R e s u l t s

Isolation trails from rotted plants collected from different localities of New Valley Governorates yielded a fungus which was identified as *Rhizoctonia solani*.

Pathogenicity test using R. solani isolates:

Results illustrated in Fig. (1) show that all the 19 isolates tested were able to attack guar plants causing damping-off and root rot diseases with various degrees. *Rhizoctonia solani* isolates RG 7 and 8 caused the highest damping-off (50%) followed by isolates RG15 (45%). Isolates RG3, 10, 12, 13, 18 and 19 were the weak in this concern (5%).

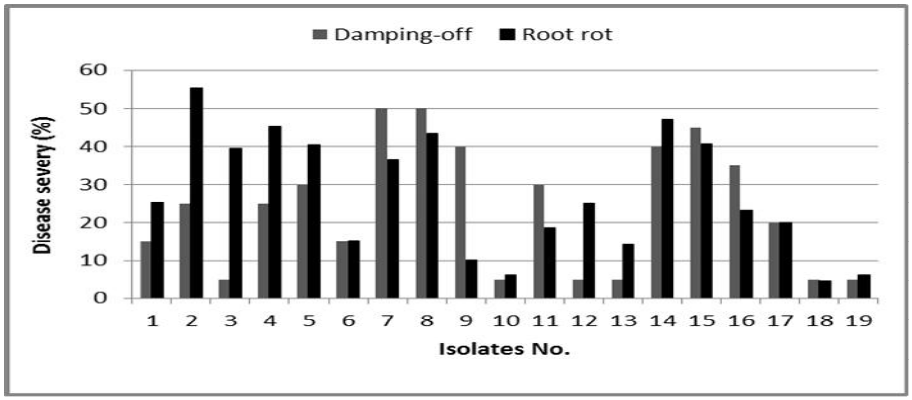

Fig. 1. Pathogenicity tests of *Rhizoctonia solani* isolates isolated from naturally diseased guar plants by damping-off and root rot.

Also, all *R. solani* isolates were able to attack guar plants in late stage of growth causing root rot symptoms with various degrees. *Rhizoctonia solani* isolate RG2 recorded the highest root rot severity (55.25%) followed by RG4, RG14 and RG8 (45.26 and 47.25, 43.36 %, respectively). While, *R. solani* isolates RG10, 18 and 19 recorded the lowest root rot severity (6.33, 4.56 and 6.35%, respectively). Generally, *R. solani* isolate RG8 followed by RG2 were more aggressive for attack guar plants than the other isolates.

Effect of SA and P. fluorescens on linear growth of R. solani (RG8):

Data in Fig. (2) reveal that SA and *P. fluorescens*, either individually or in combination have significantly reduced linear growth of tested isolate. In general, the combination between of SA and *P. fluorescens* was more effective than any of them individually tested. On the other hand, *P. fluorescens* was able to inhibit growth of *R. solani* isolate RG8 more than SA.

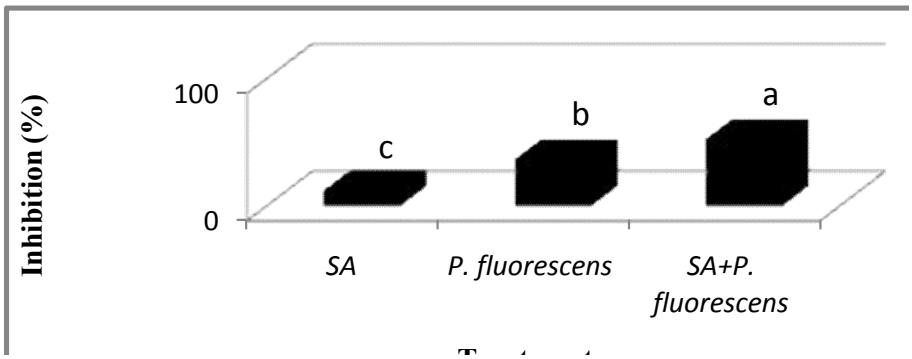

Fig. 2. *In vitro* effect of SA and *P. fluorescens* individually or in combination on the growth of *R. solani* (RG8). Different letters indicate significant differences among treatments within the same column according to least significant difference test (P ≤ 0.05).

Effect of salicylic acid and P. fluorescens on damping-off and root rot diseases caused by R. solani (RG8) in pots under greenhouse conditions:

Data shown in Fig. (3) clearly indicate that both treatment (SA and *P. fluorescens*) either applied individually or in combination significantly reduced damping-off and root rot diseases caused by *R. solani* isolate RG8 compared with the untreated control plants under greenhouse conditions. The combination between SA and *P. fluorescens* recorded the highest reduction in the disease severity of damping-off and root rot compared with used any of them alone. In fact, this treatment reduced damping-off disease from 50% in control to 5 % and reduced root rot severity from 36.35% in the control to 5.47%. On the other hand, treated seeds with *P. fluorescens* appeared more effective in reducing damping-off and root rot severity than SA treatment.

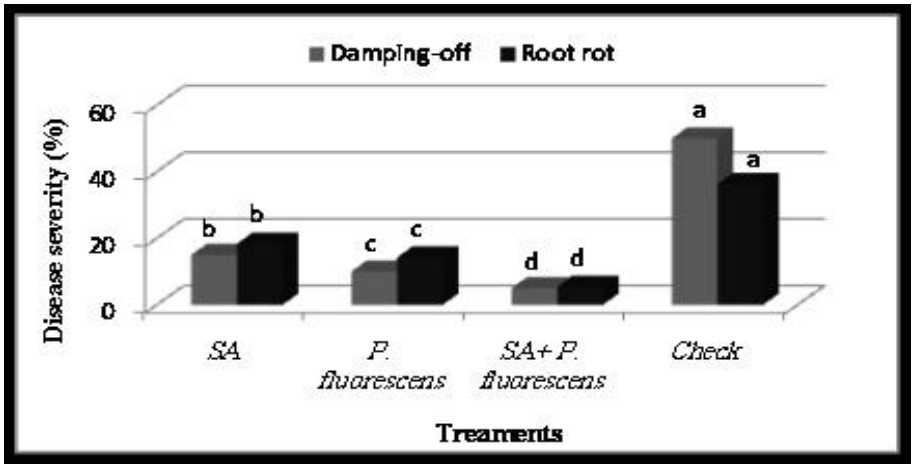

Fig. 3. Effect of salicylic acid (SA) and *P. fluorescens* (PF) individually or in combination, on damping-off and root rot caused by *R. solani* (RG8), under greenhouse conditions. Different letters indicate significant differences among treatments within the same column according to least significant difference test (P ≤ 0.05).

Effect of salicylic acid and P. fluorescens on damping-off and root rot diseases under field conditions:

Data presented in Figs. (4 and 5) show that all treatments with SA and *P. fluorescens* either alone or in combination had significantly protected guar plants against infection by *R. solani* as compared with the untreated check in both locations tested (El-Kharga Agric. Res. Station and Mallawy Agric. Res. Station) during summer season of 2014. The efficiency of combination between SA and *P. fluorescens* was more pronounced in controlling damping-off and root rot diseases than applied *P. fluorescens* or SA individually in both locations. Moreover, *P. fluorescens* was more effective for controlling damping-off and decreased root rot severity than SA in El-Kharga. In contrary, SA was more effective than *P. fluorescens* in Mallawy.

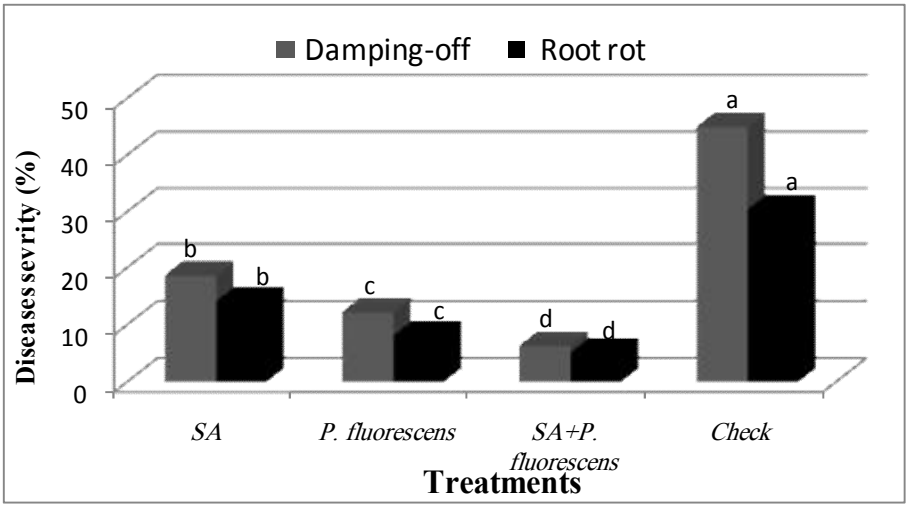

Fig. 4. Effect of salicylic acid (SA) and *P. fluorescens* (PF) individually or in combination, on the disease severity of damping-off and root rot of guar plants grown under naturally infested soil (open field conditions) in El-Kharga Agric. Res. Station, New Valley Governorate. Different letters indicate significant differences among treatments within the same column according to least significant difference test (P ≤ 0.05).

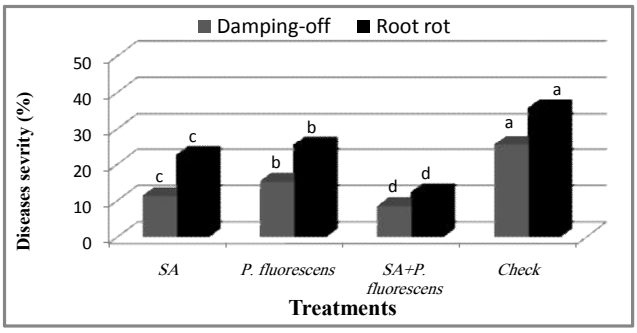

Fig. 5. Effect of salicylic acid (SA) and *P. fluorescens* (PF) individually or in combination, on the disease severity of damping-off and root rot of guar plants grown under naturally infested soil (open field conditions) in Mallawy Agric. Res. Station, El-Minia Governorate. Different letters indicate significant differences among treatments within the same column according to least significant difference test (P ≤ 0.05).

Effect of SA and P. fluorescens on guar growth and yield parameters:

All of the tested treatments *viz*. *P. fluorescens* and SA individually or in combination significantly increased growth and yield parameters in treated guar plants, *i.e.* plant height, number of branches /plant, number of pods /plant, Guaran content (gm/ plant), seed index (weight of 100 seeds) and total yield (Kg/fed) comparison with those of check treatment in both locations during growing season of 2014 (Table 1). In this respect, the treated with a combination of *P. fluorescens* and SA was most effective than application with any of the treatments alone. Moreover, using combined *P. fluorescens* and SA improved plant height from 113.4 and 105.3 cm in check treatment to 156.4 and 145.2 cm and increased number of branch/ plant from 8.3, 10.7 to 12.03, 16.3 and increased number of pods/ plant from 143.3, 122.3 in check to 220.7, 211.4 in both locations, respectively.

Table 1. Effect of SA and *P. fluorescens* individually or in combination on some growth parameters and yield of guar plants grown under field conditions in El-Kharga and Mallawy Agric. Res. Stations during 2014 growing season

Treatment	Plant height (cm)	No. of branches/ plant	No. of pods/plant	Guaran content (gm/plant)	Seed index (gm/100 seeds)	Total yield (Kg/ fed.)
	El-Kharga Agric. Res. Station					
Salicylic acid (SA)	129.7 c	9.5 b	170.9 c	7.4 c	3.3 c	1241.6 c
P. fluorescens (PF)	145.3 b	11.0 ab	190.5b	8.1 b	3.4 b	1452.1 b
SA+ PF	156.4 a	12.0 a	220.7 a	9.1 a	3.8 a	1852.4 a
Check	113.4 d	8.3 c	145.3 d	5.7 d	3.2 cd	996.3 d
	Mallawy Agric. Res. station					
Salicylic acid (SA)	130.5 b	13.2 c	180.5 c	6.1 c	3.3 b	1125.0 c
P. fluorescens (PF)	132.7 b	14.1 b	198.5 b	7.4 b	3.5 b	1325.1 b
SA+ PF	145.2 a	16.3 a	211.4 a	8.9 a	3.9 a	1623.2 a
Check	105.3 c	10.7 d	122.3 d	3.3 d	3.3 c	8524 d

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$).

Also, this treatment increased Guaran content (gm /plant) from 5.75, 3.35 in check to 9.15, 8.92 gm/ plant. Seed index and total yield (Kg/fed.) were increased from 3.25, 3.32 and 996.32, 852.36 in control to 3.8, 8.2 and 1852.3, 1623.2 Kg fed.$^{-1}$ in both locations, respectively. On the other hand, *P. fluorescens* recorded great increases in all growth and yield parameters in El-Kharga Res. Station than SA, but these results were reflected in Mallawy Agric. Res. Station where SA was more effective than *P. fluorescens* in this respect.

Biochemical changes associated with SA and P. fluorescens treatments:

Accumulation of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) enzymes, and pathogenesis related (PR) protein (chitinase and β-1,3 glucanase), phenolic compounds and lignin in plants suffering from infection by *R. solani* or healthy plants treated with *P. fluorescens* and SA individually or in combination were studied.

Peroxidase activity:

Data in Fig. (6) show that PO activity of inoculated and non-inoculated guar plants treated with SA, *P. fluorescens* and SA+ *P. fluorescens* was higher than that of untreated plants after all time of application. Inoculated plants recorded the highly PO activity than non-inoculated plants whether treated and untreated especially 4 days after any treatments except plants treated with SA and inoculated wilt *R. solani*. The combination between *P. fluorescens* and SA recorded the highly enzyme activity than *P. fluorescens* or SA individually either in inoculated or uninoculated plants. The highest levels PO were determined 8 days after treatment in all cases. The highest of PO activity was recorded in guar plants inoculated with the pathogen and treated with *P. fluorescens* and SA 10 day after application (2.46 enzyme unit mg^{-1} protein min^{-1}). In general, enzyme activity rapidly increased the activity of defence related components until 10 day after application then decreased progressively.

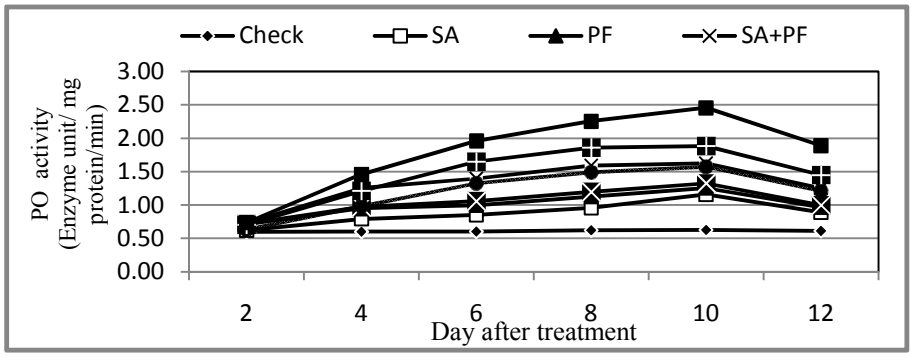

Fig. 6. Effect of SA and *P. fluorescens* (PF), individually and in combination, on activity of peroxidase (PO) in inoculated and non-inoculated guar plants. (The samples were collected from both inoculated and non-inoculated plants 2, 4, 6, 8, 10 and 12 days after treatment).

Polyphenol oxidase activity:

In general, a significant increase in the activity of PPO was observed in both non-inoculated and inoculated guar plants following treatment with SA, *P. fluorescens* and SA + *P. fluorescens* more than check treatment (Fig. 7). PPO accumulated more markedly in plants treated with SA, *P. fluorescens* and SA + *P. fluorescens*, especially in inoculated plants and treated with combined between *P. fluorescens* and SA. Also, PPO activity increase as days after application of treatment increased until 6^{th} day then decreased progressively. Maximum levels of PPO were recorded at 6^{th} days after application of treatments, respectively, in all cases. Guar plants treated with combination *P. fluorescens* and SA and inoculated with the pathogen recorded the highest level of PPO activity in 6^{th} day from application (2.31 enzyme unit mg^{-1} protein min^{-1}) followed by 8^{th} day of application of the same treatment (2.15 enzyme unit mg^{-1} protein min^{-1}). On the other hand, PPO activity in inoculated plants increases markedly than non-inoculated plants in all tested periods.

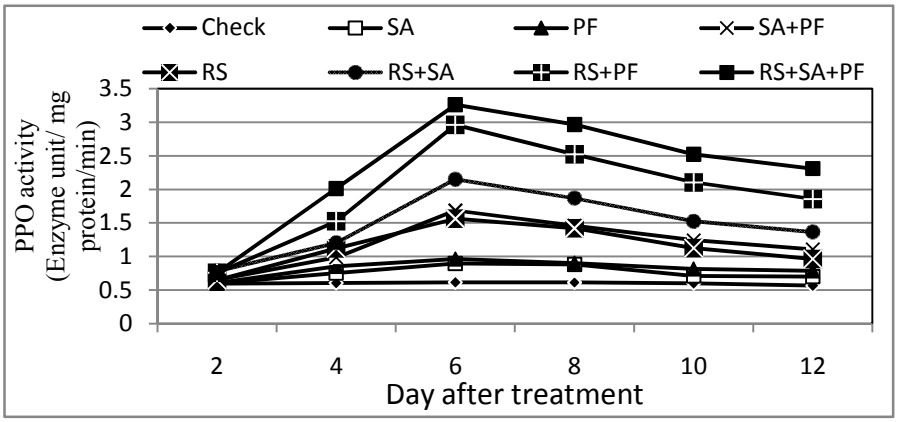

Fig. 7. Effect of SA and *P. fluorescens* (PF), individually or in combination, on activity of polyphenol oxidase (PPO) in inoculated and non-inoculated guar plants. (The samples were collected from both inoculated and non-inoculated plants 2, 4, 6, 8, 10 and 12 days after treatment).

Phenylalanine ammonia lyase activity:

Data in Fig. (8) show that the levels of PAL activity in inoculated plants were highly increased than in non-inoculated check plants until 8th day after inoculation then decreased approximately equal in activity in 10th day after inculcation. On the other hand, PAL activity was highly significant increase in inoculated plants and treated with *P. fluorescens* and SA individually or in combination than untreated inoculated plants. Also, the activity of PAL increased by increasing time after application until 8th days after application then the activity decreased. Focusly, the higher activities of PAL were determined in inoculated gaur plants 8th day after treatment with *P. fluorescens* + SA followed by *P. fluorescens* and SA (3.74, 3.55 and 2.89 enzyme unit mg $^{-1}$ protein min^{-1}, respectively). Generally, all plants treated with *P. fluoresces* or SA, either alone or in combination showed significant differences in the activities of PAL in guar plant extracts either inoculated or non-inoculated with *R. solani*. On the other hand, untreated and non-inoculated plants did not show any change in the pattern of PAL production.

Chitinase activity:

Data in Fig (9) indicate that all treatments have significantly increased the chitinase activity. Gaur plants treated with SA, *P. fluorescens* and SA+ *P. fluorescens* showed more activity of chitinase enzyme either inoculated or non-inoculated plants than check treatments. On the other hand, the combination between *P. fluorescens* and SA recorded the highest activity of chitinase enzyme in inoculated plants with *R. solani* after all tested periods of enzyme determination especially at 8 days after application (7.89 enzyme unit mg protein^{-1} min^{-1}). The enzyme activity was increased at 2, 4, 6 and 8 days after application and then decreased 10 days after application.

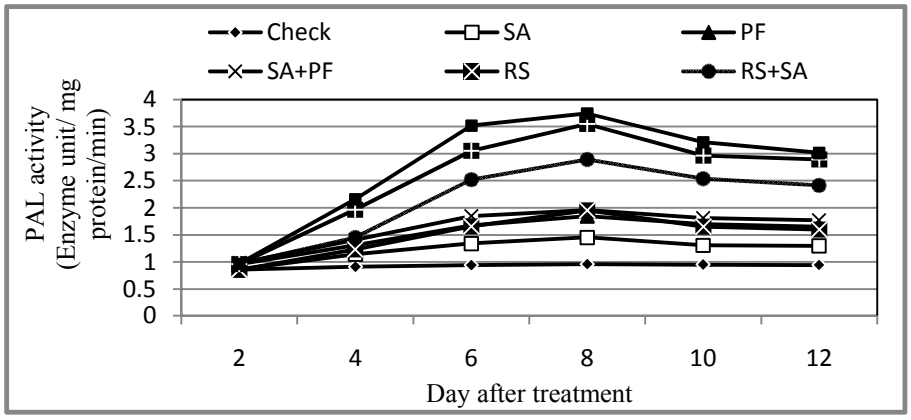

Fig. 8. Effect of SA and *P. fluorescens* (PF), individually or in combination, on activity of phenylalanine ammonia lyase (PAL) in inoculated and non-inoculated guar plants. (The samples were collected from both inoculated and non-inoculated plants 2, 4, 6, 8, 10 and 12 days after treatment).

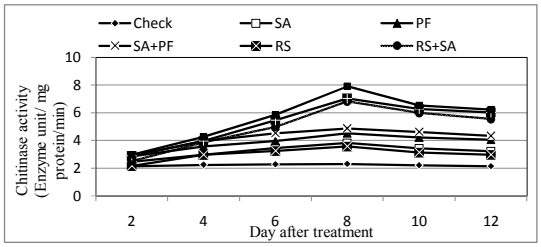

Fig. 9. Effect of SA and *P. fluorescens* (PF), individually or in combination, on activity of chitinase in inoculated and non-inoculated guar plants. (The samples were collected from both inoculated and non-inoculated plants 2, 4, 6, 8, 10 and 12 days after treatment).

β-1, 3 glucanase activity:

Data presented in Fig. (10) show that there was a great increase in β-1, 3 glucanase activity in all treatments, either in inoculated or non-inoculated plants, during the examination periods compared with the check. The maximum increase in β-1, 3 glucanase activity was recorded after 10 days in all treatment except in case of non- inoculated plants treated with SA, *P. fluorescens* individually or in combination, where recorded the maximum activity of β-1, 3 glucanase 8 days after treatment. The combination between SA and *P. fluorescens* were recorded the

highest increase of β-1, 3 glucanase activity in plants inoculated with *R. solani* during the examination periods followed by inoculated plants treated with *P. fluorescens* and SA, respectively. Generally, the increase in β-1, 3 glucanase activity was highly in plants inculcated with *R. solani* either treated or untreated more than non-inoculated plants.

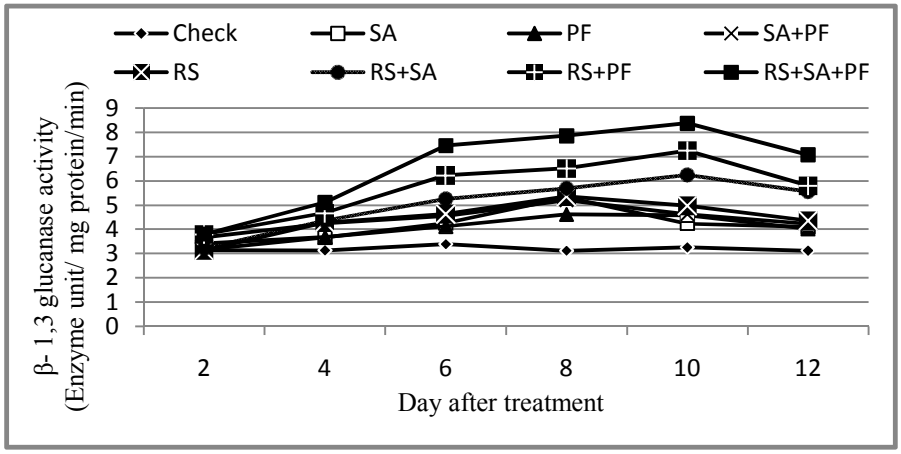

Fig. 10. Effect of SA and *P. fluorescens* (PF), individually or in combination, on activity of β-1, 3 glucanase in inoculated and non-inoculated soybean plants. (The samples were collected from both inoculated and non-inoculated plants 2, 4, 6, 8, 10 and 12 days after treatment).

Total phenol content:

The total phenols were measured in inoculated and non-inoculated guar plants treated and untreated with *P. fluorescens*, SA and *P. fluorescens* + SA (Fig. 11). All treatments show significant role to accumulation of phenolic compounds in inoculated and non-inoculated plants, whatever pre-treated guar plants challenge inoculated with the pathogen showed rapid increase in the accumulation of phenol compounds. Also, the accumulation of phenols in inoculated plants was highly increased than non-inoculated plants during all tested periods. On the other hand, the phenol contents were exhibited at 8th day after application in inoculated and treated plants with any treatments then decreased progressively thereafter. Maximum level of phenolic compounds were recorded at 8th after application of SA + *P. fluorescens* treatment (6.26 mg/gm fresh weight) followed by the same treatment at 6th after treatment (5.80 mg/gm fresh weight).

Lignin content:

Data in Fig. (12) indicate that lignin content increased in guar plants treated with *P. florescence*, SA, individually or in combination, either in inoculated or non-inoculated plants compared with check treatment. Plants inoculated with *R. solani* content highly level of lignin than non-inoculated control plants. The accumulation of lignin increased with increasing the determination periods after

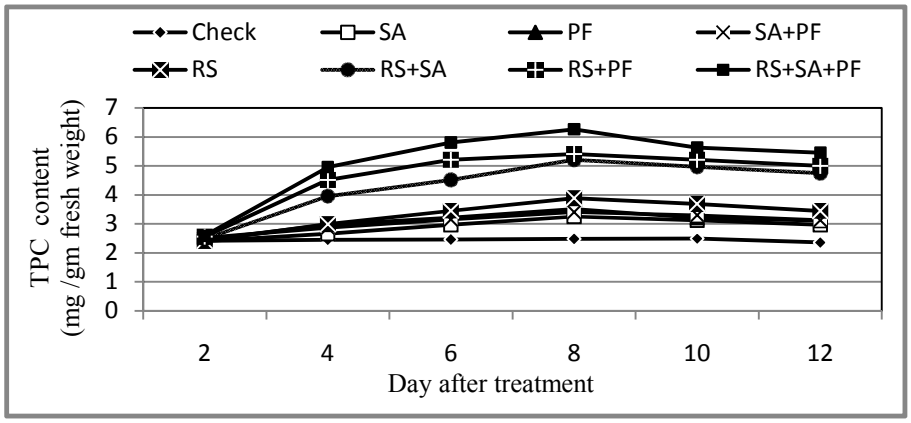

Fig. 11. Effect of SA and *P. fluorescens* (PF), individually or in combination, on total phenol content (TPC) in inoculated and non-inoculated guar plants. (The samples were collected from both inoculated and non-inoculated plants 2, 4, 6, 8, 10 and 12 days after treatment).

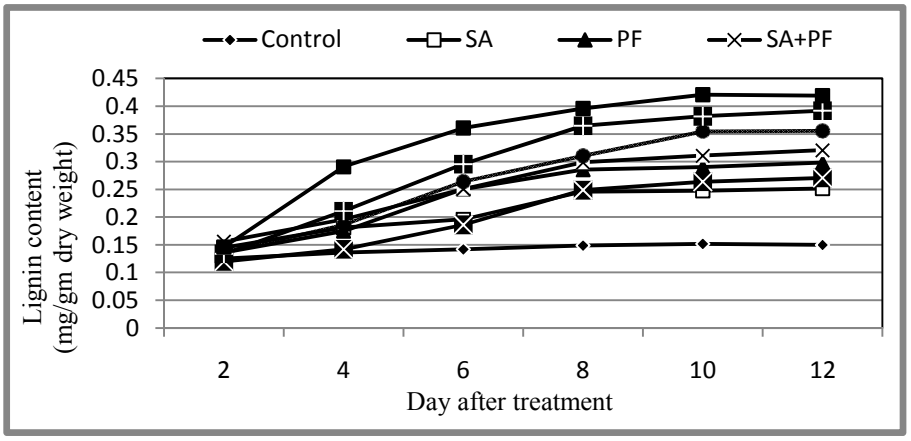

Fig. 12. Effect of SA and *P. fluorescens* (PF), individually or in combination, on lignin content in inoculated and non-inoculated guar plants. (The samples were collected from both inoculated and non-inoculated plants 2, 4, 6, 8, 10 and 12 days after treatment).

treatment application and/or inoculation with the pathogen until 10 days after treatment then became a slight increase at 12th day. Plant treated with *P. fluorescens* +SA recorded more highly lignin content than used of *P. fluorescens* or SA individually either in inoculated or non-inoculated plants. The highest lignin content was recorded by *P. fluorescens* + SA treatment in inoculated plants 10 days (0.42 mg /gm dry weight) after application followed by *P. fluorescens* + SA treatment after 12 days (0.42 mg /gm dry weight)**.**

Discussion

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* (A.B. Frank). Donk is an ecologically diverse soilborne fungus that causes root rot disease on guar plants. In this study, nineteen *R. solani* isolates were isolated from guar plants collected from different fields growing in New Valley Governorate. All these isolates were able to attack guar plants causing damping-off and root rot diseases. These results are in agreement with those reported by many researchers (Mohamed *et al*., 2006; Pareek and Varma, 2014 and Choudhary and Sindhu, 2015).

Damping-off and root rot diseases usually cannot be prevented by crop rotation or by the development of resistant crop varieties. In spite of promising results obtained by some chemical treatments in controlling damping-off and root rot, phytotoxicity and chemical residue are major problems leading to environmental pollution and human health hazards (Mandal *et al*., 2009). Thus, alternative control measures for the control of damping-off and root rot should be developed. Biological control and some chemical inducers are proposed to be an effective and non-hazardous strategy to reduce crop damage caused by plant pathogens. In recent years, *Pseudomonas florescence* and salicylic acid have been extensively used for plant growth promotion and disease control.

In the present study, it was planning to investigate the possibility of minimizing the infection with damping-off and root-rot diseases of guar using SA and *P. fluorescens* individually and/or in combinations as resistance inducer. The obtained data *in vitro* revealed that both SA and *P. fluorescens*, either individually or in combination, caused significant reduction in growth of *R. solani*. The combination between SA and *P. fluorescens* was more effective to inhibit the fungal growth than any of them individually. On the other hand, all treatments caused significant reduction to both damping-off and root rot diseases and increased the healthy survival plants either in pots or field experiments, compared with the check treatment. Also, these treatments improved plant growth (plant height and No. of branches/ plant) and yield components (No. of pods/plant, weight of 100 seeds and total yield/fed.) as well as guaran content (gm/plant) in both locations (El-Kharga and Mallawy Agric. Res. Stations) during growing season of 2014.The combination of SA and *P. fluorescens* recorded highly growth parameters and yield components more than that in the application of either of them alone. Similar results were reported by Abdel-Monaim (2013) and Choudhary and Sindhu (2015).

Induction of resistance by salicylic acid and *P. fluorescens* treatment is due to the accumulation of oxidative enzymes and pathogenesis-related proteins (PRS). These treatments cause an increase in the activity of peroxidase (PO), polyphenoloxidase (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, 2011). In this study, obtained data

indicated that guar plant treated with SA and *P. fluorescens*, individually or in combination, due to accumulation of PO, PPO, PAL and pathogenesis related protein (chitinase, β-1,3 glucanase) with recorded more increase of total phenol compounds and lignin in guar tissues either in inoculated or non-inoculated plants.

Several mechanisms have been suggested for disease control by *P. fluorescens* involving production of siderophores, HCN, ammonia, antibiotics, volatile compounds, production of hydrolytic enzymes, stimulation of phytoalexins or flavonoid-like compounds in roots, etc. or by competing with pathogens for nutrients or colonization space (Thomashow and Weller, 1996 and Sarhan and Shehata, 2014). In addition, fluorescent pseudomonads can trigger a plant-mediated resistance mechanism called induced systemic resistance (Pieterse *et al*., 2001). Biological control of soil borne pathogens is often attributed to improved nutrition that boosts host defences or to direct inhibition of pathogen growth and activity (Abdel-Monaim, 2010 and 2013).

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 reported to play an important role in expression of both local resistance controlled by major genes and systemic induced resistance developed after an initial pathogen attack (Hammerschmidt and Smith-Becker, 2000 and Saikia *et al*., 2003). Application of exogenous SA at a concentration of 1 to 5 mM has been well known to induce pathogenesis-related (PR) gene expression and acquired resistance against a variety of microbial pathogens (Meena *et al*., 2001). Low concentrations (10 to 100 µM) of SA have also shown to be sufficient for pathogen-induced defense gene expression, H_2O_2 accumulation and hypersensitive cell death in plant suspension cultures (Kauss and Jeblick, 1996). SA may not be a translocated primary signal for SAR, but it may only play a regulatory role in the expression of SAR genes (Seah *et al*., 1996).

In conclusion, the present study provides further evidence that may facilitate applying simple non-toxic chemicals as SA and *P. fluorescens* for controlling damping-off and root rot diseases in guar. Their low cost, low toxicity to the man and environmental pollution make them ideal seed soaking for diseases control under field conditions and increased seed yield and seed content from Guaran.

References

Abdel-Monaim, M.F. 2010. Integrated management of damping-off, root and/or stem rot diseases of chickpea with sowing date, host resistance and bioagents. *Egypt. J. Phytopathol*., **38**: 45-61.

Abdel-Monaim, M.F. 2011. Role of Riboflavin and Thiamine in Induced Resistance against Charcoal Rot Disease of Soybean. *Egypt. J. Phytopathol.,* **39**(1): 1-23.

Abdel-Monaim, M.F. 2013. Improvement biocontrol of damping-off and root rot/wilt of faba bean by salicylic acid and hydrogen peroxide. *Sci. J. Plant Pathol*., **2**(1): 14-24.

Abdel-Said, W.M.; Abdel-Ghfar, N.Y. and Shehata, S.A.M. 1996. Application of salicylic and aspirin for induction of resistance to tomato plants against bacterial wilt and its effect on endogenous hormones. *Ann. Agric . Sci . Ain Shames Univ*., **41**: 1007-1020.

Anderson, E. 1949. Endosperm mucilages of legumes: occurrence and composition. *Ind. Eng. Chem*., **41**: 2887-2890.

Bajoria, S.; Varshney A.K.; Pareek, R.P.; Mohan, M.K. and Ghosh, P. 2008. Screening and characterization of antifungal clusterbean (*Cyamopsis tetragonoloba*) rhizobacteria. *Biocontrol Sci. Technol*., **18**: 139-156.

Biles, C.L. and Martyn, R.D. 1993. Peroxidase, Polyphenoloxidase and Shikimate dehydrognase isozymes in relation to tissue type, maturity and pathogen induction of watermelon seedling. *Plant Physiol. Biochem*., **31:** 499-506.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of Microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem*., **72**: 248-250.

Cavalcanti, F.R.; Resendea, M.L.V.; Carvalhoc, C.P.S.; Silveirab, J.A.G. and Oliveira, J.T.A. 2007. An aqueous suspension of *Crinipellis perniciosa* mycelium activates tomato defense responses against *Xanthomonas vesicatoria*. *Crop Prot*., **6**: 729-738.

Choudhary, S.R. and Sindhu, S. 2015. Suppression of *Rhizoctonia solani* root rot disease of cluster bean (*Cyamopsis tetragonoloba*) and plant growth promotion by rhizosphere bacteria. *Plant Pathol. J.,* **14**(2): 48-57.

Couillerot, O.; Prigent-Combaret, C.; Caballero-Mellado, J. and Moenne-Loccoz, Y. 2009. *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. *Letters Applied Microbiol.,* **48**: 505–512.

De Boer, W., Gerards, S.; Klein Gunnewiek, P. J. and Modderman, R. 1999. Response of the chitinolytic microbial community to chitin amendments of dune soils. *Biol Fertil Soils*, 29:170-7.

El-Mougy, N.S. 2002. In vitro studies on antimicrobial activity of salicylic acid and acetylsalicylic acid as pesticidal alternatives against some soilborne plant pathogens. *Egypt. J. Phytopathol.***, 30**: 41-55.

El-Mougy, N.S. 2004. Preliminary Evaluation of salicylic acid and acetylsalicylic acid efficacy for controlling root rot disease of lupine under greenhouse conditions. *Egypt. J. Phytopathol*., **32**(1-2): 11-21.

El-Sayed, W.M. 1996. Induction resistance to bacterial soft rot disease of potato tubers by application of acetylsalicylic acid (Aspirin). *Ann. Agric. Sci., Ain Shams Univ*., **41:** 993-1006.

Frisvad, J.C. and Samson, R.A. 1991. Filamentous fungi in foods and feeds: ecology, spoilage and mycotoxin production. Pages: 31-68. In: Handbook of Applied Mycology: Vol. 3: Foods and Feeds. Arora, D.K.; Mukerjii, K.G. and Marth, E.H. (eds*).* Marcel Dekker, NY, USA.

Gauillard, F.; Richard-Forget, F. and Nicolas, J. 1993. New spectrophotometric assay for polyphenol oxidase activity. *Anal. Biochem.,* **215**: 59-65.

Gillet, J.B. 1958. Indigofera (Microcharis) in tropical Africa with related genera Cyamopsis and Rhynchotropis, Kew bulletin additional series; 1, 165 p.

Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research. A. Lviley-Interscience Publication, New York, 678 p.

Hammerschmidt, R. and Smith-Becker, J.A. 2000. The role of salicylic acid in disease resistance. Pp. 37–53. In: Mechanisms Of Resistance To Plant Diseases. Slusarenko, A.; Fraser, R.S.S. and Van Loon, L.C. (eds.). Kluwer Academic Publisher.

Hammerschmidt, R.; Nuckles, E.M. and Kuc, J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol.*, **20**: 73-82.

Kauss, H. and Jeblick, W. 1996. Influence of salicylic acid on the induction of competence for H_2O_2 elicitation: compari-son of ergosterol with other elicitors. *Pl. Physiol*., **111:** 755–763.

Khalil, M.M. 2001. Biochemical and technological studies on the production of isolated guar protein. *Nahrung*, **45**(1): 21-24.

Liken, M. and Perdith, A. 1999. Fractionation of spruce trichloroacetic lignin. *Acta Chin. Slov.,* **46**(1): 87-97.

Liu, L.; Kloepper, J.W. and Tuzun, S. 1995. Introduction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathology*, **85**: 695-698.

Mandal, S.; Mallick, N. and Mitra, A. 2009. Salicylic acid- induced resistance to *Fusarium oxysporum* f.sp. lycopersici in tomato. *Plant Physiol. Biochem.,* **47**: 642-649

Marrero, N.; Fernandez, T.; Caballero, O.; Rivero, M. and Lopez, M. 1990. Induction of antiviral factors in kidney beans var. Bolita 42. *Ciencias- de- la Agricultura*, **43**: 17-21.

Matloob, A. A. H. and Juber K. S. 2013. Biological control of bean root rot disease caused by *Rhizoctonia solani* under green house and field conditions. *Agric. Biol. J. N. Am*., **4**(5): 512-519.

Meena, B.; Marimuthu, T. and Velazhahan, R. 2001. Salicylic acid induced resistance in Groundnut against late leaf spot caused by *Cercosporidium personatum*. *J. Mycol. Pl. Pathol.*, **31**: 139–145.

Mohamed, I.A.I.; Bauiomy M.A.M. and Ibrahim A.S.A. 2006. Efficacy of different natural products as safe management of guar damping-off disease in Egypt. *Egypt. J. Phytopathol.,* **34**(1): 1-15.

MSTATC. 1991. A software program for the design, management and analysis of agronomic research experiments. *Michigan State University*, 400p.

Mukhtar, H.M.; Ansari, S.H.; Bhat, Z.A. and Naved, T. 2006. Antihyperglycemic activity of *Cyamopsis tetragonoloba* beans on blood glucose levels in alloxan-induced diabetic rats. *Pharm. Biol*., **44**(1): 10- 13.

Muthomi, J.W.; Oteino, P.E.; Chemining, Wa G.N.; Nderitu, J.H. and Wagacha, J.M. 2007. Effect of legume root rot pathogens and fungicide seed treatment on nodulation and biomass accumulation. *J. Biological Sci.*, **7**(7): 1163-1170.

Pan, S.Q.; Ye, X.S. and Kuc. J. 1991. Association of β-1,3- glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco induced by stem injection with *Pernospora tabacina* or leaf inoculation with tobacco mosaic virus. *Physiol. Molec. Pl. Pathol*., **39:** 25-39.

Pareek, V. and Varma, R. 2014. Phytopathological effects and disease transmission in cluster bean seeds grown in Rajasthan. *Indian J. Plant Sci*., **3(2):** 26-30.

Pieterse, C.M.J.; Van Pelt, J.A.; Van Wees, S. C.M.; Ton, J.; Leon-Kloosterziel, K. M., Keurentjes, J.J.B.; Verhagen, B.W.M.; Knoester, M.; Van der Sluis, I.; Bakker, P.A.H.M. and Van Loon, L.C. 2001. Rhizobacteria-mediated induced systemic resistance: triggering, signalling and expression. *Eur. J. Plant Pathol*., **107:** 51-61.

Rajkumar, M., Lee, W.H. and Lee, K.J. 2005. Screening of bacterial antagonists for biological control of Phytophthora blight of pepper. *J. Basic Microbiol*., **45**: 55–63.

Rajkumar, M.; Lee, K.J. and Freitas, H. 2008. Effects of chitin and salicylic acid on biological control activity of *Pseudomonas* spp. against damping-off of pepper. *South African J. Botany,* **74**: 268–273.

Saikia, R.; Singh, T.; Kumar, R.; Srivastava, J.; Srivastava, A.K.; Singh, K. and Arora, D.K. 2003. Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* in chickpea. *Microbiol. Res*., **158:** 203-213.

Saikia, R.; Yadav, M.; Varghese, S.; Singh, B.P.; Gogoi, D.K.; Kuma, R. and Arora, K.D. 2006. Role of riboflavin in induced resistance against Fusarium wilt and charcoal rot diseases of chickpea. *Plant Pathol. J*., **22(4):** 339-347.

Sarhan, E.A.D. and and Shehata, H.S.H. 2014. Potential plant growth-promoting activity of *Pseudomonas* spp. and *Bacillus* spp. as biocontrol agents against damping-off in Alfalfa. *Plant Pathol. J.,* **13**(1): 8-17.

Seah, S.; Sivasithamparam, K. and Turner, D.W. 1996. The effect of salicylic acid and resistance in wheat (*Triticum aestivam*) seedling roots against the take all fungus. *Gaeumannomyces graminis* var. *tritici*. *Aust. J. Bot.*, **44**: 499-507.

Sneh, B.; Burpee, L. and Ogoshi, A. 1998. Identification of *Rhizoctonia* species. The APS, St. Paul, Minesota, USA.

Tabarraei, M.; Amini, J. and Harighi, B. 2011. Effects of fluorescent Pseudomonads for control of damping-off disease of cantaloupe caused by *Phytophthora drechsleri*. *Australian J. Crop Sci.,* **5(11):**1427-1433.

Thomashow, L.S. and Weller, D.M. 1996. Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. Pages: 187-235. In: Plant–Microbe Interactions. Vol. 1. Stacey, G. and Keen, N.T. (eds.). Chapman & Hall, NY, USA.

Urbanek, H.; Kuzniak-Gebarowska, E. and Herka, H. 1991. Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturonase. *Acta Physiol. Plant,* **13:** 43-50.

Wang, M.L. and Morris J.B. 2007. Flavonoid content in seeds of guar germplasm using HPLC. Plant Genetic Resources. *Characterization and Utilization*, **5**(2): 96- 99.

Wirth, S.J. and Wolf, G.A. 1992. Micro-plate calorimetric assay for endo-acting cellulase, xylanase, chitinase, β-1, 3-glucanase and amylase extracted from forest soil horizons. *Soil. Biol. Biochem.,* **24**: 511-519.

Yoshida, S.; Hiradate, S.; Tsukamoto, T.; Hatakeda, K. and Shirata, A. 2001. Antimicrobial activity of culture filtrate of *Bacillus amyloliquefaciens* RC-2 isolated from mulberry leaves. *Phytopathology*, **91**: 181-187.

(Received 29/02/2016;
in revised form 30/03/2016)

حامض السالسليك وبكتريا *Pseudomonas fluorescens* كوسائل آمنة لمكافحة فطر *Rhizoctonia solani* فى الجوار

منتصر فوزى عبد المنعم
معهد بحوث أمراض النباتات - مركز البحوث الزراعية- الجيزة- مصر.

تسعة عشر عزلة من الفطر *Rhizoctonia solani* وجدت مصاحبة لأعراض عفن الجذور فى نباتات الجوار التى تم جمعها من أماكن مختلفة فى محافظة الوادى الجديد وقد وجد أن جميع هذه العزلات لها المقدرة على إصابة نباتات الجوار محدثة أمراض سقوط البادرات وأعفان الجذور وكانت العزلة رقم 8 (RG8) أكثرها قدرة مرضية. تم دراسة تأثير المعاملة بكلا من حامض السالسليك وبكتيريا *Pseudomonas fluorescens* إما منفردة او مجتمعة معا على انتشار أمراض سقوط البادرات وأعفان الجذور وصفات النمو تحت ظروف الصوبة والحقل.

تمت دراسة نشاط الانزيمات المرتبطة بالمقاومة والتى من أهمها إنزيمات البيركسيديز والبولى فينول أوكسيديز والفنيل الانين امونيا ليز والبروتينات المرتبطة بالمرضية انزيم الشيتينبز وبيتا جلاكونيز والفينولات الكلية ونسبة اللجنين سواء فى النباتات المعدية بالفطر الممرض أو الغير معدية.