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# Integrated Management of Damping-off, Root and/or Stem Rot Diseases of Chickpea with Sowing Date, Host Resistance and Bioagents

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**Eleven** fungal isolates were isolated from naturally infected chickpea roots collected from different locations in New Valley governorate. The isolated fungi were purified and identified as *Rhizoctonia solani* (5 isolates), *Fusarium solani* (4 isolates) and *Sclerotinia sclerotiorum* (2 isolates). The isolated fungi proved their pathogenicity on cv. Giza 3. Response of chickpea cvs. Giza 1, 2, 3, 4, 88, 195 and 531 to infection by the tested fungi was significantly varied. Giza 1 was the most resistant one followed by Giza 531, while the other tested cvs. were highly susceptible. Seven biocontrol agents, namely *Bacillus subtilis, B. megaterium, B. cereus, Trichoderma viride, T. harzianum, Aspergillus* sp., *Penicillium* sp. isolated from chickpea rhizosphere, were tested for their antagonistic action against the tested pathogens. *B. subtilis* isolate BSM1, *B. megaterium* isolate TVM5, *T. viride* isolate TVM2 and *T. harzianum* isolate THM4 were the most antagonistic ones to the tested fungi *in vitro*, while the other isolates were moderate or weak antagonists. The most antagonistic isolates as well as the commercial biocide Rhizo-N were applied as seed treatment for controlling damping-off, root and/or stem rot diseases caused by the tested fungi under greenhouse conditions. The obtained data showed that all tested antagonistic isolates were able to cause significant reduction of damping-off, root and/or stem rot diseases in chickpea plants. *T. viride* (isolate TVM2) and *B. megaterium* (isolate BMM5) proved to be the most effective isolates for controlling the diseases. Under field conditions, these antagonistic isolates were used as seed treatment of resistant cultivar Giza 1 and sown in three sowing dates to be work of an integrated control program to reduce spread of damping-off, root and/or stem rot diseases under field conditions. The obtained data indicated that all the tested antagonistic isolates significantly reduced damping-off, root and/or stem rot. *T. viride* (isolate TVM2) and *B. megaterium* (isolate BMM5) recorded the highest reduction of damping –off, root and/or stem rot in all sowing dates. Sowing of treated seeds with bioagents in first of November gave the highest protection against root diseases in chickpea. The reduction in damping-off, root and/or stem rot severity was significantly reflected on the produced seed yield. In this respect, seeds previously treated with *T. viride* (TVM2) produced the highest seed yield in all sowing dates followed by seed treated with *B. megaterium* (TVM5). First of November was the best sowing date to reduce these diseases and to increase seed yield/fed. On the other hand, the antagonistic isolates isolated from chickpea rhizosphere, were most active than the commercial biocide Rhizo-N in reducing chickpea root diseases and increase of seed yield in greenhouse and field conditions.

**Keywords:** *Bacillus* spp., chickpea, *Fusarium solani*, resistant cultivar, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, sowing date, and *Trichoderma* spp.

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop in the world after beans (*Phaseolus vulgaris* L.) (Anonymous, 2005). In addition to its importance as a source of human food and animal feed, it also helps in the management of soil fertility, particularly in dry lands. As soil-borne fungi, *Fusarium eumartii, F. oxysporum* f.sp. *ciceris, F. solani, Pythium ultimum, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum* and *Verticillium albo-atrum* are reported to be the most pathogenic fungi in chickpea causing damping -off, root and/or stem rot and wilt diseases (Nene and Reddy, 1987). In Egypt, many authors reported that chickpea is attacked with many soil borne fungi, *i.e. Fusarium* spp., *Rhizoctonia solani, Sclerotinia sclerotiorum, Pythium* spp., *Macrophomina phaseolina* causing damping -off, root and stem rot diseases (Rahhal *et al.*, 2000 and Khalil, 2007).

Different fungicides and soil fumigants are currently used to control soil borne plant pathogens. However, many of these compounds proved to be quite toxic to the environment and to the ground water. Methyl bromide is a good example for a very efficient soil fumigant that has a great impact on the environment and has been recently phased out due to the public concern and international agreements.

The use of antagonistic microorganisms against *R. solani, F. solani* and *S. sclerotiorum* has been investigated as one of the alternative control methods. Both *Trichoderma* spp. and *Bacillus* spp. are wide spread throughout the world and have been recognized as the most successful biocontrol agents for soil borne pathogens. Several modes of action have been described, including competition for nutrients, antibiosis, induced resistance, mycoparasitism, plant growth promotion and rhizosphere colonization capability (Hassanein *et al.*, 2006; Siddiqui and Akhtar, 2007 and Bailey *et al.*, 2008).

Also, cultural practices, such as planting date proved to be very effective in reducing fungal attack to plants, but they are insufficient under high disease pressure, especially when weather conditions are particularly conductive to disease development (Khalil, 2007). The use of resistant cultivars appears to be the most practical and economically efficient measure for management of root diseases of chickpea and is also a key component in IDM programs (Jiménez-Díaz *et al.*, 1998).

The objective of this research was to identify the benefits of integrating several control measures including choice of sowing date, partially resistant cultivars, and biological control, which previously were shown to be useful in the management of damping-off, root rot and/or stem rot of chickpeas when used individually. Studies were conducted during two consecutive years in naturally infested field micro-plots in research farm of El-Kharga Agric. Res. Station, New Valley governorate that have been used in previous work to (i) select resistant chickpea cultivars (ii) select bacteria and fungi obtained from the chickpea rhizosphere with antagonistic activity against the tested fungi (iii) determine the ability of selected bacteria and fungi to suppress damping-off, root rot and stem rot diseases caused by a highly virulent isolates of *R. solani, F. solani* and *S. sclerotiorum* and determine suitable sowing date that gives the best control of root diseases and increase of seed yield.

An integrated control program using suitable sowing date, bioagents and resistant cultivar for controlling damping-off, root and stem rot and increasing seed yield was conducted under field conditions.

## Materials and Methods

*Isolation, purification and identification of fungi causing root and stem rot diseases of chickpea:*

Chickpea plants infected with root and stem rot were collected from different locations in New Valley governorate, Egypt. Infected roots and stems were washed with running tap water to remove any soil remains, then cut into small pieces before being dipped in sodium hypochlorite solution (2%) for two min. then passed through changes of distilled water, dried between sterilized filter paper, then placed on PDA medium with and without antibiotics. The plates were incubated at 25±1°C and scanned daily for fungal development.

Preliminary microscopic examination of the fungi isolated showed that they could be classified under three genera, *i.e. Fusarium, Rhizoctonia* and *Sclerotinia.* Fusarium isolates were purified by plating single conidial spores (Booth, 1985) while, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were purified using the hyphal tip technique (Dhingra and Sinclair, 1985). Representative isolates were maintained on PDA slants for further studies. Isolated fungi were identified according to their morphological features as described by Booth (1985); Dhingra and Sinclair (1985) and Barnett and Hunter (1986).

*Pathogenicity tests of the isolated fungi:*

Conical flasks, each containing 100 g barley grains and about 100 ml tap water, were autoclaved at 1.5 kg/cm$^2$ pressure for 30 min. They were inoculated with 0.7 cm diameter fungal discs from the fungal isolates and incubated at 25±1°C for two weeks. Soil and plastic pots (30 cm in diameter) were sterilized with 5% formalin solution and left in the open air for 3 weeks to evaporate remained formalin. Soil infestation was performed by mixing in about 100 g of inoculum with the soil in each pot (rate of 2%) and pots were then irrigated. Sterilized uninoculated barley grains were added to the soil at the same rate and used as control. Seven days after soil infestation, eight seeds of susceptible cultivar cv. Giza 3, were sown in each pot and pots were irrigated directly. Four replicated pots were used for each treatment. The percentage of damping –off was recorded one month after sowing. three months after planting, chickpea plants were pull-off from the soil, washed thoroughly to remove soil debris, root and stem rot were assessed according to disease index: 0 = roots or stems without discoloration (no infection), 1= 1-20%, 2= 21- 40%, 3= 41-75%, 4= 76-100% discoloration of root or stem mass and 5= completely dead plants including pre-or post emergence damping- off or old plants for each replicate. For each replicate a disease severity index (DSI) similar to that described by Liu *et al.* (1995) was calculated as follows:

$$DSI= \sum d/(d_{max} X\ n)X100$$

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

Re-isolation was carried out from the diseased plants to fulfil Koch's pustules and the developing fungi were compared with the original isolates.

*Evaluation of chickpea cultivars to infection with root and stem rot pathogens:*

Response of seven chickpea cultivars namely Giza 1, 2, 3, 4, 88, 195 and 531 (obtained from Legume Crop Research Department, Field Crop Research Institute, Agric. Res. Centre, Giza) to infection with the highly pathogenic isolates (*R. solani* isolate C1, *F. solani* isolate C6 and *S. sclerotiorum* isolate C11). The tested cultivars were grown in plastic pots containing sterilized soil and infested with the pathogenic fungal isolates individually. Four replicated pots were used for each treatment and each pot was planted with 8 seeds. The inocula of the tested fungi were prepared and applied similarly as mentioned under the pathogenicity test. Data were recorded for damping-off and root/stem rot after 30 and 90 days of sowing as above mentioned.

*Isolation, purification and identification of antagonistic organisms:*

Antagonistic microorganisms were isolated from soil rhizosphere samples of healthy chickpea plants producing area at New Valley governorate, Egypt. The used bioagents were isolated on selected medium according to the methods recommended by Turner *et al.* (1998). The isolated antagonists were purified by using single spores and/or single colonies techniques described by Landa *et al.* (2001). The fungal isolates were identified as *Trichoderma harzianum, T. viride, Aspergillus* sp. and *Penicillium* sp. on the basis of their morphological characters (Barnett and Hunter, 1986) and the bacterial isolates were identified as three *Bacillus* spp., *i.e. B. subtilis, B. megaterium* and *B. cereus*, according to the morphological and biochemical activities in standard tests (Sneath *et al.*, 1986).

*In vitro screening test for antagonistic effect:*

The tested isolates of antagonistic fungi were grown on PDA medium at 25±1°C for 6- days and used as inocula. Disks from each isolate of antagonistic fungi (7 mm in diameter) were inoculated on PDA medium in one side in Petri plate and the opposite was inoculated by pathogenic fungi (*R. solani, F. solani* and/or *S. sclerotiorum*) inocula (Larkin and Fravel, 1998). While in case of *Bacillus* spp. isolates, each isolate was streaked at one side on PDA medium in plates and incubated for 24 hrs. at 25±1°C, then one disc (7 mm in diameter) of any of the pathogenic isolates was placed on the opposite side (Kaur *et al.*, 2007). Four replicates were used for each treatment. The inoculated plates with pathogenic fungi only were used as control. After 7 days incubation at 25±1°C, linear growth of pathogenic isolates in all treatments was recorded. The decrease of percentage that occurred in linear growth of the pathogenic fungi was determined at the end of the experiment using formula suggested by Fokemma (1973) as follows:

$$\text{Reduction in linear growth} = [(R1 - R2)/R1] \times 100$$

Whereas: R1= the radius of normal growth in control plates.
R2= the radius of inhibited growth.

The highly antagonistic isolates of *B. subtilis* isolate 1 (BSM1), *B. megaterium* isolate 5 (BMM5), *T. viride* isolate 2 (TVM2) and *T. harzianum* isolate 4 (THM4) were selected and used in further studies.

*Preparation of formulated antagonistic fungi and bacteria:*

Antagonistic bacterial inocula used for treatment of chickpea seeds cv. Giza 3 were produced as described by Landa *et al.* (2001). Inocula of *B. subtilis* (BSM1) and *B. megaterium* (BSM5) were produced in 100 ml of PDB medium (pH 7) in 250-ml conical flasks, on an orbital shaker at 125 rpm and 28°C for 3 days. Bacterial cells were harvested by centrifugation (10,000 × *g* for 20 min) and washed twice with sterile 0.1 M MgSO4 solution. Bacterial concentration in the suspension was adjusted to approximately $5\times10^8$ cells per ml by measuring absorbance at 600 nm (*A*600) in a spectrophotometer and using standard curves for each bacterial isolate. Inocula of *T. viride* (TVM2) and *T. harzianum* (THM4) were prepared as described by Sallam *et al.* (2008) as follows: the tested isolates of *Trichoderma* spp. were grown in 1000 ml conical flasks, each containing 250 g vermiculate soil (El-Halal Company, El-Khatatpa, Egypt). 250 g wheat bran and 250 ml Czapek's medium and autoclaved for 20 min. at 121°C. on two consecutive days. After 25 days of incubation period, contents of flasks were transferred to plastic plates under sterilized conditions, left to air dry then mixed in a blender to become powder and kept in sterilized polyethylene bags at room temperature until the used colony forming units in any formula of *Trichoderma* spp. adjusted to $3\times10^7$ cfu/g.

*Effects of seed treatment with biocontrol agents on chickpea damping-off and root and /or stem rot diseases under greenhouse conditions:*

In this experiment, cultivar Giza 3, the highly susceptible cultivar to infect by the pathogenic fungal isolates was used to study the effect of bioagent isolates on damping-off and root and/or stem rot diseases in chickpea.

Before treatment with biocontrol agents, seeds were surface disinfested in 2% NaOCl for 3 min, washed three times in sterilized distilled water, and dried between sterilized filter paper layers. Seeds were treated at the time with a bacterial bioagents isolates (10 ml of bacterial biocontrol agent suspension in 0.1 M $MgSO_4$ and 0.5% carboxymethyl cellulose per 100 g of chickpea seeds) and fungal bioagents isolates (10 g of Trichoderma prepared inoculum and 10 ml of 0.5% carboxymethyl cellulose per 100 gm chickpea seeds) as well as seeds treated with bio commercial product Rhizo –N (*B. subtilis*- produced by El-Naser of Fertilizers and Biotic Fungicides Co., El-Sadat, Egypt) used as compression at 4 g/ kg seeds.

Chickpea treated seeds with bioagents were sown in sterilized soil infested with any of the tested fungi as mentioned before at 2% (w/w). Untreated chickpea seeds with biocontrol were sown in infested soil used as a control. Four replicates were used; each replicate consisted of three pots (8 seeds/ pot). Data were recorded for damping –off, root and/or stem rot after 30 and 90 days of sowing, respectively.

*Integrated control of root and stem rot in chickpea plants under field conditions:*

Field experiments were conducted at the Experimental Farm of El-Kharga Agric. Res. Station, New Valley governorate, Egypt in 2008-2009 and 2009-2010 growing seasons. The experimental layout was split plot design with four replications. The field plots (10.5 m$^2$) consisted of 6 rows of 3.5 m long and 0.60 m in between. Chickpea seeds cv. Giza 1 (resistant cultivar) were treated with fungal and bacterial bioagents isolates as abovementioned as well as seeds treated with the bio commercial product Rhizo-N were used as compression and grown in holes at the rate of 2 seeds/hole with 10 cm apart between holes (35 holes/row). Untreated seeds were sown as a control treatment. All treatments were sown in three sowing dates 1st October, 1st November, 1st December in seasons 2008/2009 and 2009/2010. All the agricultural practices were applied as usual. Data were recorded as damping-off, root and/or stem rot after 30 and 90 days. At the end of experiment, seed yield was harvested, weighed and calculated as kg/fed.

*Statistical analysis:-*

In all experiments the least significant difference (LSD) at 0.05 confidences was determined according to Gomez and Gomez (1984).

## Results

*Isolation, identification of the causal fungi and pathogenicity tests:*

Eleven isolates were obtained from naturally infected chickpea plants collected from different locations in New Valley governorate. The isolated fungi were consisting of isolates belonging to the genera *Rhizoctonia*, *Fusarium* and *Sclerotinia* as shown by preliminary microscopic examination. The isolated *Fusarium* isolates were identified as *F. solani*, the isolated *Rhizoctonia* isolates were identified as *R. solani* and *Sclerotinia* isolates were identified as *S. sclerotiorum*. Data in Table (1) show that the highest percentage of pre-emergence damping-off was recorded by *R. solani* isolate C1 (50%) followed by *R. solani* isolate C3 then *F. solani* isolate C6 and *S. sclerotiorum* isolate C11, being 40.6, 34.4 and 40.6%, respectively. While moderate pre-emergence damping-off (18.8-31.3%) was occurred by *R. solani* isolates C2, C4 and C5, *F. solani* isolates C7 and C8 and *S. sclerotiorum* isolate C10. Whereas, *F. solani* isolate C9 showed weak ability to cause pre-emergence damping-off (9.4%). On the other hand, *R. solani* isolates of C1 and C3 and *F. solani* isolate C6 caused the highest percentage of post emergence damping-off (21.9%) followed by *R. solani* isolate C5 (15.6%). While, the other isolates caused 3.1–12.5% post-emergence damping-off.

*F. solani* isolate C6 caused the highest root rot severity (31.0%) followed by *S. sclerotiorum* isolate C11 (29.4%). *R. solani* isolate C4 and *F. solani* isolate C9 were found too weak to cause root rot symptoms (9.2 and 9.9%, respectively).

It could be noted from these results that *R. solani* isolate C1, *F. solani* isolate C6 and *S. sclerotiorum* isolate C11 were the highest pathogenic isolates for causing damping-off and root/stem rot in chickpea plants.

*Evaluation of chickpea cultivars to infection with damping-off, root and/or stem rot pathogens:*

Response of chickpea cultivars to *R. solani*, *F. solani* and *S. sclerotiorum* infection was significantly varied (Table 2). Cultivar Giza 1 was resistant to infection by any of the tested pathogenic fungi followed by cv. Giza 531, compared to the other cultivars. The percentages of damping-off and root rot caused by *R. solani* were 18.8, 11.2% and 21.9, 12.6% in both cultivars, respectively. While, *F. solani* caused 15.6 and 21.9% damping-off as well as 14.0, 15.4% root rot in case

**Table 1. Pathogenicity tests of fungal isolates obtained from naturally diseased chickpea plants (cv. Giza 3)**

| Isolate | Damping-off (%) | | | Dead plants (%) [c] |
|---|---|---|---|---|
| | Pre-emergence [a] | Post-emergence [b] | Total | |
| *Rhizoctonia solani* | | | | |
| C1 | 50.0 [d] | 21.9 | 71.9 | 25.0 |
| C2 | 31.3 | 15.6 | 46.9 | 18.3 |
| C3 | 40.6 | 21.9 | 62.5 | 17.4 |
| C4 | 18.8 | 9.4 | 28.2 | 9.2 |
| C5 | 25.0 | 15.6 | 40.6 | 13.6 |
| Mean | 33.14 | 16.88 | 50.02 | 16.70 |
| *Fusarium solani* | | | | |
| C6 | 34.4 | 21.9 | 56.3 | 31.0 |
| C7 | 25.0 | 6.3 | 31.3 | 18.4 |
| C8 | 25.0 | 9.4 | 34.4 | 16.3 |
| C9 | 9.4 | 3.1 | 12.5 | 9.9 |
| Mean | 23.45 | 10.18 | 33.63 | 18.9 |
| *Sclerotinia sclerotiorum* | | | | |
| C10 | 25.0 | 9.4 | 34.4 | 16.3 |
| C11 | 40.6 | 12.5 | 53.1 | 29.4 |
| Mean | 31.3 | 12.5 | 43.8 | 22.85 |
| LSD at 0.05 | 2.4 | 2.0 | 3.6 | 1.6 |

[a], [b], [c] Assessed 15, 30, 90 days after sowing, respectively. [c] Dead plants (%) due to infection by root rot and/or stem rot. [d] Values are means of 4 replicates.

**Table 2. Varietal response of seven chickpea cultivars towards the tested fungi, greenhouse experiment**

| Cultivar | *R. solani* isolate C1 | | *F. solani* isolate C6 | | *S. sclerotiorum* isolate C11 | | Mean | |
|---|---|---|---|---|---|---|---|---|
| | Damping-off (%)[a] | Root rot | Damping -off (%) | Root rot (%) | Damping -off (%) | Stem rot (%) | Damping -off (%) | Dead plants (%)[c] |
| Giza 1 | 18.8 [d] | 11.2 | 15.6 | 14 | 12.5 | 14.0 | 15.63 | 13.07 |
| Giza 2 | 46.9 | 25.3 | 40.6 | 30.0 | 50.0 | 28.0 | 45.83 | 27.77 |
| Giza 3 | 59.4 | 29.0 | 50.0 | 35.0 | 56.3 | 25.2 | 55.23 | 29.73 |
| Giza 4 | 50.0 | 23.8 | 21.9 | 19.1 | 50.0 | 26.0 | 40.63 | 22.97 |
| Giza 88 | 62.5 | 18.8 | 25.0 | 17.3 | 46.9 | 26.3 | 44.80 | 20.80 |
| Giza 195 | 50.0 | 22.9 | 43.8 | 24.9 | 50.0 | 23.1 | 47.93 | 23.63 |
| Giza 531 | 21.9 | 12.6 | 21.9 | 15.4 | 15.6 | 15.5 | 19.80 | 14.50 |
| Mean | 44.21 | 20.51 | 31.26 | 22.24 | 40.19 | 22.59 | 38.55 | 21.78 |

| LSD at 0.05 for: | | Damping-off | Dead plants |
|---|---|---|---|
| Cultivars (A) | = | 3.54 | 2.37 |
| Fungi (B) | = | 2.62 | 2.26 |
| Interaction (AxB) | = | 5.13 | 4.42 |

[a] Assessed 30 days after sowing and [b] assessed 90 days after sowing. [c] Dead plants (%) due to infection by root rot and/or stem rot. [d] Values are means of 4 replicates.

of cvs. Giza 1 and Giza 531, respectively. *S. sclerotiorum* caused 12.5 and 15.6% damping-off and 14.0, 15.5% stem rot in both tested cultivars, respectively. On the other hand, the other tested cultivars were highly susceptible or susceptible towards infection by *R. solani*, *F. solani* and *S. sclerotiorum*. Cv. Giza 3 showed the highest susceptibility to infection by all the tested fungi. Also, the obtained data showed that *R. solani* and *S. sclerotiorum* were more pathogenic than *F. solani*.

*Selection of antagonistic organisms for ability to inhibit in vitro growth of pathogenic fungi*:

Antagonistic effect of 8 bacterial isolates and 9 fungal isolates isolated from the rhizosphere of chickpea plants was tested against *R. solani*, *F. solani* and *S. sclerotiorum* in dual culture under *in vitro* conditions.

*A) Antagonistic bacteria:*

Data in Table (3) show that all antagonistic bacterial isolates were able to inhibit the growth of *R. solani*, *F. solani* and *S. sclerotiorum*, some were stronger than others. *B. subtilis* isolate BSM1 and *B. megaterium* isolate BMM5 were found to be the most potent antagonistic bacteria to all pathogenic fungi. However, the other antagonistic bacterial isolates were moderate or weak. *B subtilis* isolate BSM1 inhibited the growth of *R. solani*, *F. solani* and *S. sclerotiorum* by 40.2, 53.5 and 50.1%, respectively. While, *B. megaterium* isolate BMM5 inhibited growth of these fungi by 48.4, 55.7 and 52.4%, respectively.

**Table 3. *In vitro* antagonistic action between the bacterial isolates and the tested fungi**

| | Bacterial isolate | Inhibition (%) | | | |
|---|---|---|---|---|---|
| | | *R. solani* | *F. solani* | *S. sclerotiorum* | Mean |
| 1 | *Bacillus-subtilis* (BSM1) | 40.2 [a] | 53.5 | 50.1 | 47.93 |
| 2 | *B. subtilis* (BSM2) | 32.4 | 22.9 | 35.1 | 30.13 |
| 3 | *B. subtilis* (BSM3) | 24.3 | 28.4 | 33.0 | 28.57 |
| 4 | *B. megaterium* (BMM4) | 33.2 | 43.2 | 39.2 | 38.53 |
| 5 | *B. megaterium* (BMM5) | 48.4 | 55.7 | 52.4 | 52.17 |
| 6 | *B. cereus* (BCM6) | 8.5 | 12.4 | 10.4 | 10.43 |
| 7 | *B. cereus* (BCM7) | 10.3 | 21.2 | 25.1 | 18.87 |
| 8 | *B. cereus* (BCM8) | 28.3 | 29.4 | 33.0 | 30.23 |
| LSD at 0.05 | | 1.7 | 1.8 | 1.4 | - |

[a] Values are means of 4 replicates.

*B) Antagonistic fungi:*

Data in Table (4) show that all the tested fungal species, *i. e. Trichoderma* sp., *Aspergillus* sp. and *Penicillium* sp. were found to be antagonistic to the pathogenic fungi, based on the reduction in growth area of pathogens. *Trichoderma* spp. accounted more than 50% of the rhizosphere fungi. *T. viride* isolate TVM2 and *T. harzianum* isolate THM4 were found to be the most potent antagonistic fungi to all tested pathogens. *Trichoderma viride* isolate TVM2 reduced the growth of *R. solani*, *F. solani* and *S. sclerotiorum* with 68.2, 75.1 and 70.4%, respectively.

**Table 4.** ***In vitro*** **antagonistic action between the fungal isolates and the tested fungi**

| | Fungal isolate | | Inhibition (%) | | | |
|---|---|---|---|---|---|---|
| | | | *R. solani* | *F. solani* | *S. sclerotiorum* | Mean |
| 1 | *Trichoderma viride* | (TVM1) | 40.1 [a] | 50.1 | 55.2 | 48.47 |
| 2 | *T. viride* | (TVM2) | 68.2 | 75.1 | 70.4 | 71.23 |
| 3 | *T. viride* | (TVM3) | 58.1 | 65.0 | 52.2 | 58.43 |
| 4 | *T. harzianum* | (TVM4) | 62.4 | 73.5 | 66.8 | 67.57 |
| 5 | *T. harzianum* | (TVM5) | 42.7 | 50.1 | 54.0 | 48.93 |
| 6 | *Aspergillus* sp | (AM7) | 30.0 | 35.4 | 33.4 | 32.93 |
| 7 | *Aspergillus* sp | (AM8) | 40.5 | 49.4 | 47.1 | 45.67 |
| 8 | *Penicillium* sp | (PM9) | 25.4 | 33.4 | 35.0 | 31.27 |
| 9 | *Penicillium* sp | (PM10) | 28.3 | 21.4 | 30.4 | 26.70 |
| LSD at 0.05 | | | 2.0 | 2.2 | 1.9 | - |

[a] Values are means of 4 replicates.

Also, *T. harzianum* isolate THM4 was able to reduce growth of *R. solani*, *F. solani* and *S. sclerotiorum* with 62.4, 73.5 and 66.8%, respectively.

The highest antagonistic fungal isolates, *i.e. T. viride* (isolate TVM2) and *T. harzianum* (isolate THM4) and, among the bacterial isolates, *B. subtilis* (isolate BSM1) and *B. megaterium* (isolate BMM5) were selected to study their role in biological control of damping-off, root and stem rot diseases in chickpea plants under greenhouse and field conditions.

*Effects of seed treatment with biocontrol agents on chickpea damping-off and root and /or stem rot diseases under greenhouse conditions:*

Data in Table (5) show that all the antagonistic isolates were able to cause significant reduction to damping-off and root and/or stem rot diseases. All antagonistic isolates isolated from chickpea rhizosphere gave high protection against all the tested pathogens than the commercial biocide product (Rhizo-N). Treatment chickpea seeds with *T. viride* isolate TVM1 was the most effective to reduce damping-off, and root and/or stem rot diseases in case of soil infested with *R. solani*, *F solani* and *S. sclerotiorum*. Seed treatment with *B. megaterium* isolate BMM2 came next *T. viride*. *B. subtilis* isolate BSM1 gave the lowest protection against *R. solani* and *F. solani*, while *T. harzianum* isolate THM4 recorded the lowest protection against infection by *S. sclerotiorum*.

*Integrated control of damping-off, root and/or stem rot diseases in chickpea plants under field conditions*:

Data of the previous experiments, *i.e.* the *in vitro* antagonism and in the greenhouse experiment indicated that *B. subtilis* isolate BSM1, *B. megaterium* isolate BMM5, *T. viride* isolate TVM2 and *T. harzianum* isolate THM4 were the most effective antagonistic microorganisms against the root and stem rot pathogens. These isolates were used in the integrated disease management programs to control root and stem rot diseases in chickpea plants.

**Table 5. Effect of treatment chickpea seeds (cv. Giza 3) with various bioagents on damping-off and root rot diseases caused by the tested fungi, greenhouse experiment**

| Tested bioagent | *R. solani* | | *F. solani* | | *S. sclerotiorum* | |
|---|---|---|---|---|---|---|
| | Damping-off (%) | Root rot (%) | Damping-off (%) | Root rot (%) | Damping-off (%) | Stem rot (%) |
| *Bacillus subtilis* | 22.6[a] | 14.0 | 18.6 | 13.1 | 17.4 | 12.0 |
| *B. megaterium* | 19.2 | 10.3 | 16.8 | 10.4 | 13.2 | 12.9 |
| *Trichoderma viride* | 16.4 | 9.0 | 14.0 | 9.8 | 12.8 | 9.8 |
| *T. harzianum* | 20.0 | 12.0 | 17.4 | 10.8 | 19.4 | 13.2 |
| Rhizo-N | 24.2 | 15.1 | 20.2 | 14.7 | 22.4 | 14.0 |
| Control | 56.3 | 33.5 | 50.0 | 32.2 | 53.1 | 25.4 |
| Mean | 26.45 | 15.65 | 22.83 | 15.17 | 23.05 | 14.55 |

| LSD at 0.05 for: | | Damping -off | Dead plants[b] |
|---|---|---|---|
| Tested bioagents (A) | = | 0.637 | 0.637 |
| Isolates (B) | = | 0.581 | 0.581 |
| Interaction (AxB) | = | 1.304 | 1.304 |

[a] Values are means of 4 replicates.
[b] Dead plants (%) due to infection by root rot and/or stem rot.

Data presented in Table (6) clearly show that all tested biological agents significantly decreased damping-off, root and stem rot compared to the untreated plants (control) at all sowing dates. The efficacy of bioagents significantly affected by sowing dates. In this regard, sowing at 1st November in both seasons, gave the highest effect in reducing damping-off, root and/or stem rot. In addition, *B. megaterium* (isolate BMM5) and *T. viride* (isolate TVM2) were the most effective for controlling damping-off and root and/or stem rot in both seasons. In sowing date of 1st November, *B. megaterium* and *T. viride* reduced damping-off from 17.95% in control to 3.75 and 6.2% (average of both seasons), respectively and reduced root and/or stem rot from 10.45% to 2.7 and 2.55%, respectively.

Seed treated with Rhizo –N gave the lowest protestant against damping-off, root and stem rot diseases compared with the other bioagents. In control treatment, sowing dates showed significant effect on damping-off and root /stem rot diseases in chickpea plants. The highest average of damping-off on chickpea plants occurred in case of sowing at 1st October in both seasons (21.95%) while sowing at 1st December recorded the lowest damping-off (15.85%). Also, the same trend was obtained in case of root and stem rot (16.0% in sowing at 1st October and 9.4% in sowing at 1st December).

On the other hand, the reduction in both damping-off and root and/or stem rot was significantly reflected on the produced seed yield which was affected significantly with sowing dates. In this respect, seeds previously treated with

**Table 6. Effect of treatment chickpea seeds (cv. Giza 3) with bioagents on damping-off, root and stem rot and seed yield during 2008-2009 and 2009-2010 growing seasons under field conditions at New Valley governorate**

| Sowing date | Tested Bioagent | Damping-off (%) | | | Dead plants[a] (%) | | | Total seed yield (Kg fed$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2008-09 | 2009-10 | Mean | 2008-09 | 2009-10 | Mean | 2008-09 | 2009-10 | Mean |
| 1st October | BSM1 | 16.4[b] | 15.2 | 15.80 | 7.9 | 10.1 | 9.00 | 535.51 | 511.41 | 523.46 |
| | BMM5 | 11.6 | 11.4 | 11.50 | 6.4 | 7.3 | 6.85 | 584.31 | 543.20 | 563.76 |
| | TVM2 | 10.2 | 10.4 | 10.30 | 5.6 | 5.9 | 5.75 | 593.02 | 566.73 | 579.88 |
| | THM4 | 15.2 | 14.3 | 14.75 | 9.9 | 10.2 | 10.05 | 520.20 | 508.81 | 514.51 |
| | Rhizo-N | 17.4 | 16.3 | 16.85 | 10.5 | 10.9 | 10.70 | 513.09 | 500.00 | 506.55 |
| | Control | 25.2 | 18.7 | 21.95 | 14.5 | 17.5 | 16.00 | 445.21 | 426.80 | 436.01 |
| | Mean | 16.00 | 14.38 | 15.19 | 14.79 | 14.99 | 14.89 | 531.89 | 509.49 | 520.69 |
| 1st November | BSM1 | 8.6 | 9.1 | 8.85 | 4.5 | 5.3 | 4.90 | 664.05 | 653.22 | 658.64 |
| | BMM5 | 3.2 | 4.3 | 3.75 | 2.6 | 2.8 | 2.70 | 692.01 | 671.14 | 681.56 |
| | TVM2 | 5.9 | 6.2 | 6.20 | 2.5 | 2.6 | 2.55 | 714.33 | 702.00 | 708.17 |
| | THM4 | 7.4 | 7.2 | 7.30 | 6.4 | 7.3 | 6.85 | 660.56 | 653.22 | 656.89 |
| | Rhizo-N | 8.2 | 9.0 | 8.60 | 7.3 | 8.3 | 7.80 | 621.40 | 605.87 | 613.64 |
| | Control | 19.5 | 16.4 | 17.95 | 9.7 | 11.2 | 10.45 | 525.31 | 509.30 | 517.31 |
| | Mean | 9.38 | 8.7 | 8.78 | 5.5 | 6.25 | 5.86 | 646.28 | 632.46 | 639.37 |
| 1st December | BSM1 | 14.3 | 13.9 | 14.10 | 6.4 | 5.4 | 5.90 | 454.32 | 425.10 | 439.71 |
| | BMM5 | 14.3 | 11.2 | 12.75 | 5.4 | 6.3 | 5.85 | 481.23 | 448.39 | 464.81 |
| | TVM2 | 14.3 | 12.3 | 13.30 | 7.1 | 7.4 | 7.25 | 423.37 | 401.30 | 412.34 |
| | THM4 | 14.3 | 12.3 | 13.30 | 7.1 | 7.4 | 7.25 | 423.37 | 401.30 | 412.34 |
| | Rhizo-N | 14.3 | 12.4 | 13.35 | 6.2 | 7.1 | 6.65 | 410.31 | 394.85 | 402.58 |
| | Control | 17.3 | 16.4 | 15.85 | 9.2 | 9.6 | 9.40 | 375.40 | 359.53 | 367.49 |
| | Mean | 14.8 | 12.75 | 13.78 | 6.9 | 7.2 | 7.05 | 444.87 | 415.23 | 430.05 |

| LSD at 0.05 for: | | Damping-off | Dead plants | Total seed yield |
|---|---|---|---|---|
| Sowing date (A) | = | 1.272 | 0.493 | 6.50 |
| Tested Bioagents (B) | = | 0.799 | 0.483 | 23.87 |
| Seasons (C) | = | ns | * | * |
| Interaction (AxBxC) | = | 2.001 | 1.024 | 29.04 |

[a] Dead plants, % due to infection by root rot and/or stem rot.

[b] Values are means of 4 replicates.

BSM1= *B. subtilis*, BMM5= *B. megaterium*, TVM2= *T. viride* and THM4 = *T. harzianum*.

*T. viride* produced the highest seed yield in all sowing dates followed by seeds treated with *B. megaterium*. For sowing at 1st November, seeds treated with *T. viride* and/or *B. megaterium* resulted average seed yield, being 708.17 and 681.56 kg/fed. (average of both seasons), respectively compared to the control (517.31 kg/fed.). Chickpea seeds treated with Rhizo-N recorded the lowest seed yield compared to the other bioagents. Also, the obtained data showed that the sowing dates affected significantly the seed yield, in treated seeds or untreated seeds. Sowing at 1st December recorded the lowest seed yield (averaged 367.49 kg/fed.), while sowing at 1st November recorded the highest seed yield (averaged 517.31 kg/fed.). It was found that sowing at the late date, in case of control, recorded the lowest damping-off, root and stem rot diseases, but scored less the amount of seed yield.

## Discussion

During this investigation, eleven fungal isolates were isolated from chickpea roots collected from different locations in New Valley governorate. The isolated fungi were identified as *Rhizoctonia solani*, *Fusarium solani* and *Sclerotinia sclerotiorum*. In pathogenicity tests, all the isolated fungi were pathogenic to chickpea plants cv. Giza 3 with different degrees of disease severity. These results are in agreement with those reported by Kaur *et al.* (2007); Khalil (2007) and Siddiqui and Akhtar (2007).

The obtained data indicated that only Giza 1 was resistant to infection with any of the tested fungi and Giza 531 was moderately resistant, while the other cultivars were highly susceptible (Hassanein *et al.*, 1997; Rahhal *et al.*, 2000). Khalil (2007) reported that chickpea cv. Giza 1 proved to be the most resistant against damping-off and root and/or stem rot caused by *F. solani, S. sclerotiorum*. While, cvs. Giza 2, Giza 3 and Giza 4 were susceptible to all the tested fungi.

*In vitro* studies indicated that antagonistic fungi and bacteria inhibited the growth of the tested fungi with different degrees of inhibition. Many investigators reported that many *Trichoderma* spp. and *Bacillus* spp. are able to inhibit growth of the tested fungi (Zheng and Sinclair, 2000; Prasad *et al.*, 2002; Siddiqui and Akhtar; 2007 and Sallam *et al.*, 2008). Elad (1996) stated that mechanisms of the antagonism of *Trichoderma* spp. and *Bacillus* spp. against different pathogens may be due to mycoparasitism, competition and antibiosis.

Under greenhouse conditions, all the tested antagonistic isolates were able to cause significant reduction of damping-off, root and/or stem rot diseases in chickpea plants. *T. viride* (isolate TVM2) and *B. megaterium* (isolate BMM5) proved to be the most effective isolates for controlling the diseases.

These antagonistic isolates were selected to treat seeds of the resistant cultivar Giza 1 which were sown in three sowing dates to be an integrated control program to reduce infection by damping-off, root and/or stem rot diseases under field conditions. Data summarized that all tested antagonistic isolates caused significant reduction of damping-off and root and/or stem rot diseases under field conditions in all sowing dates

The reduction in disease severity under field conditions was reflected on seed yield. Plants grown from seeds treated with biocontrol agents produced seed yield greater than untreated ones. Seeds previously treated with *T. viride* produced the highest seed yield in all sowing dates followed by seeds treated with *B. megaterium*. Seed yield was significantly affected with sowing dates in treated seeds or untreated seeds, where sowing in first of November gave the highest seed yield compared with the other sowing dates. These results are relatively similar to those obtained by Prasad *et al.* (2002) who tested two antagonistic fungi, *T. harzianum* (PDBCTH-10) and *T. viride* (PDBCTV) against wilt (*Fusarium oxysporum* f.sp. *ciceris*) and wet root rot (*Rhizoctonia solani*) diseases of chickpea in the field. Zheng and Sinclair (2000) showed that there was a significant positive correlation ($r2= 0.78$) between root colonization by *B. megaterium* strain B153-2-2 or its mutants and suppression of Rhizoctonia root rot of soybean plants. Landa *et al.* (2004) studied the effect of sowing date, host resistance and biological control on spread of Fusarium wilt in chickpea plants. They found that the main effects of sowing date, partially resistant genotypes, and biocontrol agents caused a reduction in the rate of epidemic development over time, a reduction of disease intensity, and an increase in chickpea seedling emergence. Chickpea seed yield was influenced by the three tested factors in the study. The increase in chickpea seed yield was the most consistent effect of the biocontrol agents. However, the effect was primarily influenced by sowing date, which also determined disease development. Sallam *et al.* (2008) reported that the tested formulations of *Trichoderma* spp. proved to be effective for controlling *R. solani* and *F. oxysporum*, the causal agents of bean damping-off and wilt diseases, respectively under greenhouse and artificially infested field conditions and also enhanced green yield compared to infection control.

There are many mechanisms suggested to clarify the role of antagonistic organisms in suppression of growth pathogens and thus to control diseases. Their action could be through antibiosis (Walker *et al.*, 1998), mycoparasitism (Haran *et al.*, 1996). The competition for nutrients and/or space (Inbar *et al.*, 1994). Also, the other mechanisms involved are induction of resistance in plants through the increase of oxidative enzymes, *i.e.* polyphenoloxidase, peroxidase, enhanced lignifications (Jetiyanon *et al.*, 1997), induction of pathogeneses related protein (PR-1), chitinase and β, 1-3, gluconase in addition to increase salicylic acid (SA) level in plants (De Meyer *et al.*, 1998).

The increase of seed yield obtained in this study, could be attributed to the effect of biocontrol agents as plant growth promoters (Naseby *et al.*, 2000). Yuming *et al.* (2003) reported that three *Bacillus* strains, *B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 provided increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen and grain yield of soybean plants.

In summary, it could be concluded that management of damping-off, root and/or stem diseases of chickpea could be based on strategies that integrate several control measures in the form of selected chickpea resistant cv. Giza 1 and treated with *T. viride* (TVM2) or *B. megaterium* (BMM5) and sown in first of November lead to decrease of damping-off, root and/or stem rot diseases and increase of seed yield under field conditions. Also, the obtained bioagents isolated from chickpea rhizosphere proved to be a commercial biocide product, but this needs further studies on these isolates before using in the biological control programs.

## References

Anonymous (2005). Food and Agriculture Organization of the United Nations. FAO Production Yearbook, Rome, Italy.

Bailey, B.A; Bae, H.; Strem, M.D.; Crozier, J.; Thomas, S. E; Samuels, G.J.; Vinyard, B.T. and Holmes, K.A. 2008. Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biological Control*, **46**: 24-35

Barnett, H L. and Hunter, B.B. 1986. Illustrated Genera of Imperfect Fungi. 4th Ed., Macmillan Publishing Co., New York.

Booth, C. 1985. The genus *Fusarium*. Kew, Surrey Commonwealth Mycological Institute, 2nd Ed., 237 pp.

De Meyer, G.; Bigirimana , J.; Elad, Y. and Hofte, M. 1998. Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *Europ. J. Plant Pathol.*, **104**: 279-286.

Dhingra, O.D. and Sinclair, J.B. 1985. Basic Plant Pathology Methods. CRC, Boca Raton, Florida, USA.

Elad, Y. 1996. Mechanisms involved in the biological control of *Botrytis cinerea* incited diseases. *Europ. J. Plant Pathol.*, **102** (8): 719-732.

Fokemma, N.J. 1973. The role of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. *Physiol. Plant Pathol.*, **3**: 195-205.

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

Haran, S.; Schickler, H. and Chet, I. 1996. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. *Microbiology*, **142**: 2321-2331.

Hassanein, A.M.; El-Garhy, A.M. and Mekhemar, G.A.A. 2006. Symbiotic nitrogen fixation process in faba bean and chickpea as affected by biological and chemical control of root –rot. *J. Agric. Sci. Mansoura Univ.*, **31** (2): 963-980.

Hassanein, A.M.; El-Morsy, G.A.; Abou-Zied, N.M. and Mahmoud, Samia A. 1997. Integrated control of root rot/wilt diseases in faba bean, lentil and chickpea. *Egypt. J. Agric. Res.*, **75** (3): 543-550.

Inbar, J., Abramshy, D.; Cohen, DS. And Chet, I. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *Europ. J. Plant Pathol.*, **100**: 337-346.

Jetiyanon, K.; Tuzun, S. and Kloepper, J.W. 1997. Lignifications, peroxidase reaction and superoxidase dismutase as early plant defence associated with PGPR- mediated induced systemic resistance. Pp 265-268 in: Ogoshi, A., Kobayashi, K.; Homma, Y.; Kodama, F.; Kodo, N. and Akino, S. (eds.) "Plant Growth Promoting Rhizobacteria. Present Status and Future Prospects". Proc. 4th Internat. Sapporo, Japan.

Jiménez-Díaz, R.M. ; Porta-Puglia, A. and Tivoli, B. 1998. New approaches in the integrated management of legume diseases: Toward sustainable crop health. Pages 89-93 in: 3rd European Conference on Grain Legumes. Opportunities for High Quality, Healthy and Added-value Crops to Meet European Demands. European Association for Grain Legumes, Valladolid, Spain.

Kaur, R.; Singh, R.S. and Alabouvette, C. 2007. Antagonistic activity of selected isolates of fluorescent *Pseudomonas* against *Fusarium oxysporum* f.sp. *ciceris*. *Asian J. of Plant Sci.*, **6** (3): 446-454.

Khalil, Manal, S.M. 2007. Studies on Some Chickpea (*Cicer arietinum* L.) Fungal Diseases. M.Sc. Thesis, Fac. Agric., Minia Univ., Egypt

Landa, B.B.; Navas-Cortés, J.A. and Jiménez-Díaz, R.M. 2004. Integrated management of Fusarium wilt of chickpea with sowing date, host resistance, and biological control. *Phytopathology*, **94**: 946-960.

Landa, B.B.; Navas-Cortés, J.A., Hervás, A. and Jiménez-Díaz, R.M. 2001. Influence of temperature and inoculum density of *Fusarium oxysporum* f.sp. *ciceris* on suppression of Fusarium wilt of chickpea by rhizosphere bacteria. *Phytopathology*, **91**: 807-816.

Larkin, R.P. and Fravel, D.R. 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. *Plant Dis.*, **82**: 1022-1028.

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.

Naseby, D.C.; Pascual, J.A. and Lynch, J.M. 2000. Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. *J. App. Microbiol.*, **88**: 161-169.

Nene, Y. and Reddy, M.V. 1987. Chickpea diseases and their control. Pages 233-270. In: *The Chickpea*. M.C. Saxena and K.B. Singh (eds.). Commonwealth Agricultural Bureaux International, Oxon, UK, 320pp.

Prasad, R.D.; Rangeshwaran, R.; Anuroop, C.P.; Rashni, H.J. 2002. Biological control of wilt and root rot of chickpea under field conditions. *Ann. Pl. Prot. Sci.*, **10** (1): 72-75.

Rahhal, M.M. H.; Bastawisy, M.H.; Ismail, I.A.; El-Awadi, F.A. and Heweidy, M.A. 2000. Evaluation of some chickpea cultivars and entries to damping-off disease under greenhouse and field conditions. Proc. 9th Cong. of the Egypt. Phytopathol. Soc., pp. 385-392.

Sallam, Nashwa, M.A.; Abo-Elyousr, K.A.M. and Hassan, M.A.E. 2008. Evaluation of *Trichoderma* species as biocontrol agent for damping-off and wilt diseases of *Phaseolus vulgaris* L. and efficacy of suggested formula. *Egypt. J. Phytopathol.*, **36** (1-2): 81-93.

Siddiqui, Z.A. and Akhtar, M.S. 2007. Biocontrol of a chickpea root rot disease complex with phosphate- solubilizing microorganisms. *J. Plant Pathol.*, **9** (1): 67-77.

Sneath, P.H.A.; Mair, N.S.; Sharpe, M.E. and Holt, J.G. 1986. Bergey's Manual of Systemic Bacteriology. Williams & Wilkins Co., Baltimore, 1123pp.

Turner, D.; Kovacs, W.; Kuhis, K.; Lieckfeldt, E.W.; Peter, B; Arisan Atac, I.; Strauss, J.; Samuels, G.J.; Bomer, T. and Kubicek, C. 1998. Biogeography and phenotypic variation in *Trichoderma* sect. *longibraclatum* and associated *Hypocrea* species. *Mycol. Res.*, **101**: 449-459.

Walker, R.; Powell, A.A. and Seddon, B. 1998. *Bacillus* isolates from the spermosphere of peas and French beans with antifungal activity against *Botrytis cinerea* and *Pythium* species. *J. Appl. Microbiol.*, **84**: 791-801.

Yuming, B., Xiaomin, Z. and Smith, D.L. 2003. Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. *Crop Sci.*, **43**: 1774-1781.

Zheng, X.Y. and Sinclair, J.B. 2000. The effects of traits of *Bacillus megaterium* on seed and root colonization and their correlation with the suppression of Rhizoctonia root rot of soybean. *Bio. Control.*, **45**: 223-243.

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# المكافحة المتكاملة لأمراض سقوط البادرات وأعفان الجذور والسيقان فى الحمص بإستخدام الأصناف المقاومة وميعاد الزراعة والعوامل الحيوية

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أجرى هذا البحث بغرض معرفة أهم المسببات المرضية التى تسبب أمراض سقوط البادرات وأعفان الجذور والسيقان فى الحمص المنزرع فى الوادى الجديد وتحديد الأصناف المقاومة وميعاد الزراعة الأمثل وإستخدام الميكروبات المصاحبة للجذورفى مكافحة هذة الأمراض وزيادة محصول البذور وفى النهاية عمل برنامج مكافحة متكامل لخفض الإصابة بامراض الجذور وزيادة المحصول. ومن أهم النتائج المتحصل عليها:-

اتضح من الدراسة أن فطريات الريزوكتونيا سولانى والفيوزاريوم سولانى والاسكليروتنيا اسكليتيورم هى المسببات الرئيسية لأمراض سقوط البادرات وأعفان الجذور والسيقان فى الحمص. وجد أن صنف جيزة ١ من الاصناف المختبرة هو الصنف المقاوم للإصابة بإى من الفطريات المختبرة يلية صنف جيزة ٥٣١ بينما كانت باقى الأصناف (جيزة ٢ و٣ و٤ و ٨٨ و١٩٥) شديدة الحساسية للإصابة بأمراض أعفان الجذور والسيقان.

أمكن الحصول على ثمانى عزلات بكتيرية تتبع ثلاثة أنواع من جنس الباسيلس وهى *Bacillus subtilis, B. megaterium, B. cereus* وخمس عزلات فطرية تتبع نوعين من جنس التريكودرما وهى *Trichoderma viride, T. harzianum* وعزلتان تتبع الجنس *Aspergillus* sp. وعزلتان تتبع *Penicillium* sp. من منطقة الريزوسفير المحيطة بجذور الحمص- إستطاعت كل هذة العزلات تثبيط نمو الفطريات الممرضة وكانت العزلات *B. subtilis* (BSM1), *B. megaterium* (BSM5), *T. viride* (TVM2), *T. harzianum* (THM4) أكثر العزلات المختبرة تثبيطا لنمو الفطريات الممرضة فى الاطباق والتى تم إختيارها لمعاملة البذور وزراعتها تحت العدوى الصناعية فى الصوبة وتحت العدوى الطبيعية فى الحقل.

اتضح من معاملة البذور بهذة العزلات الميكروبية بالإضافة الى المبيد الحيوى التجارى ريزو- ان للمقارنة كلا" على حده مقدرتها على خفض نسبة سقوط البادرات واعفان الجذوز والسيقان التى تسببها الفطريات المختبرة تحت ظروف الصوبة وكانت عزلة الفطر *T. viride* (isolate TVM2) وعزلة البكتيريا *B. megaterium* (isolate BSM5) اكثرها فاعلية فى هذا الصدد.

تم عمل برنامج للمكافحة المتكاملة متمثلا فى معاملة بذور الصنف المقاوم (جيزة ١) بأقوى العزلات البكترية والفطرية تثبيطا للفطريات المرضة فى المعمل والتى اظهرت نتائج جيدة فى الصوبة بالإضافة الى المبيد الحيوى التجارى ريزو- ان للمقارنة وزراعتها فى ثلاثة مواعيد مختلفة ( الأول من كل" من أكتوبر ونوفمبر وديسمبر) تحت ظروف الحقل وتبين أن جميع المعاملات السابقة أدت الى خفض نسبة سقوط البادرات وشدة أمراض أعفان الجذور والسيقان مع زيادة محصول البذور للفدان فى كلا موسمى الزراعة تحت ظروف الحقل فى جميع مواعيد الزراعة المختلفة، ولكن تبين أن الزراعة فى أول نوفمبر هو أنسب ميعاد لزراعة البذور المعاملة بالعزلات الحيوية فى خفض الإصابة وزيادة المحصول بفروق معنوية عالية ، وتفوقت العزلات المعزولة من ريزوسفير نباتات الحمص على المبيد الحيوى التجارى ريزو- إن فى جميع الاحوال.

ونخلص من هذة الدراسة الى أن معاملة بذور الصنف المقاوم لأعفان الجذور والسيقان جيزة ١ بعزلة البكتيريا *B. megaterium* (isolate BSM5) أو عزلة الفطر *T. viride* (isolate TVM2) والزراعة فى أول نوفمبر يعتبر برنامجا ناجحا لحماية نباتات الحمص من الإصابة بأمراض أعفان الجذور والسيقان والتى تسببها فطريات الريزوكتونيا سولانى والفيوزاريوم سولانى والاسكليروتنيا اسكليتيورم، كما انها تزيد محصول البذرة للفدان.