Egyptian Journal of Phytopathology, Vol. 50, No. 2, pp 138-150 (2022)
DOI 10.21608/ejp.2022.179022.1077

ORIGINAL PAPER

Bioagents as Safe Control Agents against *Fusarium oxysporum, Rhizoctonia solani, Macrophomina phaseolina* of Cluster Bean (*Cyamopsis tetragonoloba* L.)

El-Rayes, M.M.[1*]; Ali, I.N.M.[2]; Abd El-Nabi, H.M.[2]; Morsy, K.M.M.[1] and Khalil, M.I.I.[2]

Received: 06 December 2022 / **Accepted**: 30 December 2022 / **Published online**:31 December 2022.

ABSTRACT

Seven different selected fungal and bacterial isolates *viz., Trichoderma viride, T. harzianum, Chaetomium globosum, Bacillus subtilis, Pseudomonas fluorescens, Debaryomyces* sp. and *Streptomyces lavendulae* and the mycorrhizal product of *Glomus* sp. were evaluated against the fungal pathogens, *Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani* the causal pathogens of damping-off and root-rot diseases of cluster bean *in vitro*, greenhouse and under field conditions. Data obtained indicated that all the tested bioagents resulted significant reduction in linear growth of the tested fungi. In this respect, *in vitro*. *B. subtilis*, *P. fluorescens*, *T. viride*, *T. harzianum* and *C. globosum* were the most effective in reducing the linear growth, whereas *S. lavendulae and Debaryomyces* sp. were the lowest effective. All the tested bioagents, mycorrhizae and the fungicide Rizolex-T50 significantly reduced the percentages of damping-off, root rot and wilt diseases of cluster bean when grown in artificially infested soil with *F. oxysporum, M. phaseolina* and *R. solani* under greenhouse conditions compared with control treatment. This reduction resulted in a significant increase in survived plants under soil infestation with the mixture of pathogenic fungi. Based on healthy survived plants both bacterial isolates followed by both fungal isolates were the most effective after fungicide. *In-vivo*, all the tested treatments, *i.e.*, bioagents, mycorrhizae, the fungicide Rizoles-T50 showed significant decrease in the percentages of damping-off (pre- and post-emergence) and dead plants due to infection by root rot and wilt with significant increase in survived plants under the natural infection at Dakahliya governorate during two successive seasons (2016 and 2017) compared with control treatment. In case of bioagents treatments the highest seed yield plot^{-1} occurred under the treatment of *T. harzianum* followed by *T. viride* then *P. fluorescens* and *B. subtilis* in both seasons. From the foregoing results, it may be concluded that using *T. harzianum* from fungal bioagents and *P. fluorescens* from bacteria bioagents as seed treatments could be applied for controlling damping-off, root rot and wilt diseases in cluster bean plants and improving crop parameters and seed yield.

Keywords: Guar, *Cyamopsis tetragonoloba*, biological control, bioagents, soil borne pathogens, root rot, damping-off,

***Correspondence: El-Rayes, M.M.**
E-mail: m.alrayes2020@yahoo.com
Mohamed M. El-Rayes
Kadry M.M. Morsy
1- Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

Ibrahem N.M. ALI
Heba M. Abd El-Nabi
Mohamed I.I. Khalil
2- Botany Department, Faculty of Agriculture, Suez Canal University, Egypt.

INTRODUCTION

Cluster bean or Guar (*Cyamopsis tetragonoloba* (L.) Taub.) belongs to the family Fabaceae (Leguminosae) is a coarse, upright, bushy, a drought tolerant summer annual legume and it is cultivated as a feed crop for human and livestock consumption. It is grown commercially for its seeds as a source of natural polysaccharide (galactomannan); commercially known as guar gum. Guar gum has several usages in food (Khalil, 2001) and other industries, such as paper, textiles, oil well drilling, and pharmaceuticals and a well-known traditional plant used in traditional 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 suffering from 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 host plant

(Pareek and Varma, 2014). Guar also suffers from *Macrophomina phaseolina, and Fusarium oxysporum,* which are the most common fungi causing root rot and wilt leading to considerable yield losses (Abd-El-Rahman *et al*., 2018).

Although application of fungicides is far from the most effective method to control guar damping-off and root rot and wilt diseases, it can be involved in many problems due to health risk concerns and environmental pollution. Also, using of chemical fungicides lead to reduction in population of beneficial microorganisms and death of Rhizobia (Al-Kahal *et al*., 2009). Thus, there is a growing need to develop alternative approaches for the management of these pathogens. An acceptable approach that is being actively investigated involves the use of plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* in controlling soil borne fungi (Rajkumar *et al*., 2008 and Abdel-Monaim, 2013). Using of biological control against several soil borne pathogens by various microbial antagonists including strains of *Trichoderma* species, and *Bacillus* spp. were widely used worldwide (Ziedan and Farrag, 2002; Howell, 2004, Jacobsen *et al* 2004 and Mousa *et al*., 2006). Some authors investigated the mode of action of antagonistic fungi and bacteria in the past two decades based on root and soil-borne fungal pathogens; *Bacillus* spp. exhibited high effect on *F. solani* and *R. solani* (Janisiewiez *et al*., 2000 and Gabr *et al*., 2003). *Bacillus* spp. caused the widest inhibition zone around to *R. solani*, *F. solani* and *M. phaseolina* (Mahmoud *et al*., 2006).

The present investigation was planned to investigate the antimicrobial activity of bio agents (*Trichoderma viride, Trichoderma harzianum, Chaetomium globosum, Bacillus subtilis, Pseudomonas fluorescens, Debaryomyces* sp., and *Streptomyces lavendulae* and the mycorrhizal product of *Glomus* sp) as safe fungicide alternatives against damping-off, root rot and wilt pathogens in cluster bean comparing with Rizolex-T50 fungicide.

MATERIALS AND METHODS

1-Isolation, purification, and identification of pathogenic fungi:

Samples of naturally infected cluster bean (guar) plants showing typical symptoms of root rot and wilt diseases were collected from five districts inside each of Dakahliya, Giza, Ismailia and Sharkia governorates. The collected plants were transferred to the laboratory for isolation process. The infected roots of guar samples collected from each district were thoroughly washed in running tap water to remove the adhering soil particles, cut into small pieces (5 mm) and then surface sterilized in 0.5 % sodium hypochlorite solution for two minutes. The sterilized pieces were rinsed several times in sterilized distilled water and placed between folds of sterilized filter paper until dryness then transferred onto potato-dextrose-agar medium (PDA) in Petri-dishes 9cm diameter. The Petri-plates were incubated at 25±1°C for 7 days. The emerged fungi were picked-up and transferred onto PDA plates, three replicates were used for each isolate. The isolated fungi were purified using single-spore technique and/or hyphal tip method. Then the isolated fungi were identified according to their morphological features using the descriptions of Gilman (1957); Booth (1971) and Nelson *et al.* (1983). The identified fungi were maintained on PDA for further studies.

2-Pathogenicity tests:

The pathogenicity of the isolated fungi, *i.e., A. alternata* (one isolate), *F. oxysporum* (6 isolates), *F. solani* (3 isolates), *M. phaseolina* (4 isolates), *R. solani* (5 isolates) and *Sclerotium rolfsii* (2 isolates), was carried out at Hosinia Agric. Res. Stat., Sharkia Governorate, Egypt. Preparation of fungal inoculum for soil infestation was carried out as follows:

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

Clay silt soil was disinfested with 5% formalin solution and kept under plastic sheet for two weeks until the disappearance of formalin odor.

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

$$\textbf{Pre-emergence damping-off (\%)} = \frac{\text{Total number of sown seeds} - \text{emerged seedlings after 15 d}}{\text{Total number of sown seeds}} \times 100$$

Post-emergence damping-off (%) =

$$\frac{\text{Total number of sown seeds – post-emerged seedling after 30 d}}{\text{Total number of sown seeds}} \times 100$$

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

Dead plants (%) =

$$\frac{\text{Total number of sown seeds – Survived plants}}{\text{Total number of sown seeds}} \times 100$$

Then the survived plants were calculated, also, 90 days after sowing.

The survived plants (infected and healthy) (%) =

$$\frac{\text{Total sown seeds – total dead seedlings and plants}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Fusarium wilt severity (\%)} = \frac{\sum n \times v}{5\ N} \times 100$$

Where:

n = Number of discolored roots in each category,

v = Numerical value of each category,

N = Total number of examined roots in the samples.

The most aggressive isolates were selected for further studies based on the pathogenicity test. While other fungal isolates were considered negligible towards pathogenicity of guar plants. The most aggressive isolates in this study were *F. oxysporum* No.1, *R. solani* No.2 *and M. phaseolina* No.2.

4-*In vitro* experiments:

4.1. Evaluation the antagonistic activity of the tested antagonists:

After preparation of bioagents culture filtrate as mentioned below were added individually to conical flasks containing PDA medium before solidification to obtain a concentration of 25%. The medium poured into the Petri-dishes (10 ml/plate). After solidification the Petri plates were carefully inoculated with 5 mm. discs in the center of any plate of the tested three pathogens (*F. oxysporum* No.1, *R. solani* No.2 and *M. phaseolina* No.2) obtained from the periphery of five days old culture. PDA plates inoculated with the tested pathogens, but not amended with culture filtrate (medium free culture filtrate), were maintained as control. The plates were incubated in an incubator at 28±1°C. Four replications were prepared for each treatment. Periodic observations on the linear growth of the mycelium were recorded and the average diameter of the fungal growth was measured when any treatment of the tested pathogen showed full growth by reaching the plates edge.

Inhibition percentages of the linear growth of the tested pathogens were calculated using the following formula:

$$\text{\% Inhibition} = (C - T)/C \times 100$$

Where:

C = Linear growth of the pathogen (mm) in control,

T = Linear growth of the pathogen (mm) in treatment.

5-Pot experiments:

5-1. Effect of bioagents, mycorrhiza, and fungicide Rizolex-T50 on disease incidence of cluster bean plants artificially inoculated with desired pathogen under greenhouse conditions:

Based on the results of *in vitro* experiments, the bioagent treatments which showed to be more efficient were selected and applied in further investigations in addition to *Glomus* sp.

1. Biocontrol agents:

In this study, 7 isolates of bio agents *i.e., Trichoderma viride*, *Trichoderma harzianum, Chaetomium globosum*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Debaryomyces* sp., and *Streptomyces lavendulae* in addition to the mycorrhizal product of *Glomus* sp. were kindly obtained from Biofertilizers Production Unit, Soils, Water & Environment Research Institute, Agricultural Research Center, Giza, Egypt.

5-2. The tested treatments:

The tested bioagents *T. viride*, *T. harzianum, Ch. globosum* and *B. subtilis*, *P. fluorescens, Debaryomyces* sp., *S. lavendulae* and the mycorrhizal product of *Glomus* sp. and the fungicide Rizolex-T50 (20% Tolclophos-methyl and 30% Thiram) were used to evaluate their efficiency on management of cluster bean damping off and root-rot and/or wilt diseases caused by the three tested fungi *viz. F. oxysporum, M. phaseolina* and *R. solani* in pot experiment under greenhouse conditions.

5-3. Preparation of the bioagents as powder:

Flasks containing the inoculum of each the bioagents, *Ch. globosum, Debaryomyces* sp., *T. harzianum* and *T. viride* (grown in potato-dextrose both medium), were filtered with sheet cloth to obtain the spores. The filtrates of these fungi and the inoculum growth of *B. subtilis*, *P. fluorescens* and *S. lavendulae* (grown in nutrient medium) were centrifuged (10.000 rpm for 10 minutes), and the supernatant of each bioagent was mixed with sterilized talc powder at the rate of 1:2 (v/v), then dried at room temperature for 24 h. and kept in a refrigerator at 5-7ºC until usage.

5-4. Seed treatments with bioagents:

Cluster bean seeds were dressed with the above-mentioned prepared inoculum of each microbial powder product and the mycorrhiza (*Glomus* sp.) at the rate of 4g/kg. seeds.

The seeds of cluster bean were soaked at 1.5g/l of the fungicide Rizolex-T50 just before sowing.

The treated seeds were sown in pots (25 cm. in diameter) contained infested soil with any of the three tested pathogenic fungi *viz. F. oxysporum*, *M. phaseolina* and *R. solani* at the rate of 2% inoculum level w/w. Ten untreated seeds were sown in each pot for comparison, and the seeds were sown in soil infested with the tested pathogenic fungi, each alone. A set of five replicates were used for each treatment.

The percentages of pre- and post-emergence damping off and were recorded 15 and 30 days, respectively after sowing, root rot and wilt as well as the survived plants were recorded 90 days after sowing, same pots of the survived plants were irrigated, pulled-up and examined for the apparent infection by the tested fungi.

5-5. Effect of bioagents, mycorrhiza, and fungicide Rizolex-T50 on the incidence of cluster bean plants infection with the mixture of the tested pathogens under greenhouse conditions:

The treated seeds (as in the experiments) were sown in pots (25 cm. in diameter) contained the inoculum mixture of the tested pathogenic three fungi at the rate of 2% inoculum level composed an equal amount of each fungal inoculum. Ten seeds were sown in each pot. Un-treated seeds were sown in soil infested with inocula mixture of the tested pathogenic fungi. A set of five replicates was used for each treatment.

The percentages of pre- and post-emergence damping off were recorded 15 and 30 days, respectively after sowing and root rot, wilt as well as the survived plants were counted 90 days after sowing. The pots of the survived plants were irrigated, pulled-up and examined for the apparent infection by the tested fungi.

Field experiments:

5-6. Effect of bioagents, mycorrhiza and fungicide Rizolex-T50 on disease incidence of cluster bean plants under natural infection:

These experiments were conducted at El - Mataria, Dakahliya governorate, Egypt during 2016 and 2017 growing seasons for controlling damping-off, and dead plants resulted from root-rot and/or wilt diseases of cluster bean, both locations have a back history of high infection with the causal pathogens of damping-off, root-rot and wilt diseases. The land of each location was prepared for sowing cluster bean and divided into plots of 10.5 m^2 (3×3.5m) with 5 rows in each plot. The treated seeds with the tested bioagents, the mycorrhizal product of *Glomus* sp., and the fungicide Rizolex-T50 as mentioned before under pot experiment were sown in hills at 20 cm. distance (two seeds in each hill) during June 1st, 2016, and 2017 growing seasons. The field of each experiment received the recommend agricultural practices as recommended by Min. of Agric. and Land Reclamation.

The percentages of pre- and post-emergence damping-off were recorded at 15 and 30 days after sowing. Also, dead plants and the survived plants in each plot were counted 90 days after sowing.

5-7. Effect of bioagents, mycorrhiza and fungicide Rizolex-T50 on growth and yield characters of cluster bean plants under field conditions:

Samples were taken after harvesting to estimate growth parameters *i.e*., plant height "cm" and number of branches plant-1, and yield components *i.e*., No. of pods/ plant, seed weight (g)/plant, 100 seed weight (g) and seed yield (kg)/plot.

Statistical analysis:

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

RESULTS

1. Isolation, purification, and identification of the associated fungi with root-rot and wilt diseases as well as their frequency:

Isolation trials from the diseased roots of cluster bean plants showing typical symptoms of root-rot and wilt diseases, collected from Sharkia, Ismailia, Dakahliya and Giza governorates during 2015 growing season yielded 138 fungal isolates belonging to more than six genera. The isolated fungi were purified and identified as: *Alternaria alternata* **(**Fr.) Keissler., *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* Schlecht., *Rhizoctonia solani* Kühn, *Macrophomina phaseolina* (Tassi) Goid., *Sclerotium rolfsii* Sacc. in addition to un-known fungi. *F. oxysporum* recorded the highest occurrence and frequency (39 isolates and 28.3% frequency) followed by *M. phaseolina* (34 isolates and 24.6% frequency), *R. solani* (31 isolates and 22.4% frequency) and *F. solani* (25 isolates and 18.1% frequency). Meanwhile, *S. rolfsii* recorded the lowest occurrence and

frequency (2 isolates and 1.5% frequency) after *A. alternata* (3 isolates and 2.2% frequency). Four isolates from un-known fungi were isolated with 2.9% frequency.

The isolated fungi from Dakahliya governorate recorded the highest occurrence, being 47 isolates of 34.0% frequency followed by Sharkia governorate (36 isolates of 2.1% frequency) then Ismailia governorate (34 isolates of 24.6% frequency) and Giza governorate (21 isolates of 15.2% frequency).

Table (1): Occurrence and frequency percentage (%) of the isolated fungi from diseased cluster bean collected from four governorates during 2015 growing season.

Governorate	*F. solani*		*F. oxysporum*		*A. alternata*		*R. solani*		*S. rolfsii*		*M. phaseolina*		Un-known fungi		Total	
	*Oc.	**Fr.%	Occ.	Fr%	Occ.	Fr%	Occ.	Fr%	Occ.	Fr%	Occ.	Fr%	Occ.	Fr%	Occ.	Fr%
Sharkia	5	20.0	11	28.2	0.0	0.0	9	29.0	1	50.0	10	29.4	0.0	0.0	36	26.1
Ismailia	7	28.0	9	23.1	1	33.3	7	22.5	0.0	0.0	8	23.5	2	50.0	34	24.6
Dakahliya	9	36.0	12	30.8	2	66.7	10	32.2	1	50.0	11	32.4	2	50.0	47	34.0
Giza	4	16.0	7	18.0	0.0	0.0	5	16.1	0.0	0.0	5	14.7	0.0	0.0	21	15.2
Total	25	18.1	39	28.3	3	2.2	31	22.4	2	1.5	34	24.6	4	2.9	138	---

*Oc. = Occurrence, **Fr% = Frequency (%).

2. Pathogenicity test of the isolated fungi:

Data shown in Table (2) show that all the isolated fungi were pathogenic to cluster bean plants. However, the isolated fungi varied in their pathogenicity. In this respect, the isolates of *F. oxysporum* failed to cause pre-emergence damping-off. In addition, isolate-2 of *R. solani* caused the highest figure of pre-emergence damping-off (36.7%) followed by isolate-2 of *M. phaseolina* (23.3%). Isolate-2 of *R. solani* caused the highest percentage of post-emergence damping-off (30.0%) followed by isolates 1, 4 and 6 of *F. oxysporum*, isolate-2 of *R. solani* and isolate-2 of *M. phaseolina* (26.7% for the isolates of the three fungi) then isolates-2 of *F. oxysporum* and isolates 1, 3 and 4 of *M. phaseolina*, recorded similar value (23.3%).

Meanwhile, the lowest percentage of survived plants was occurred by isolate-1 of *F. oxysporum* and isolate-2 of *R. solani* (26.7% for both fungi). In this respect, *M. phaseolina* isolate No.2 came the third order. No infection by damping-off and no dead plants were found in the control treatment. The most aggressive isolates were selected for further studies (*Fusarium oxysporum* No.1, *Rhizoctonia solani* No.2 and *Macrophomina phaseolina* No.2).

Table (2): Pathogenicity test of the isolated fungi from the roots of cluster bean showing root-rot and wilt symptoms, greenhouse experiment.

Fungal isolates	% Damping-off		% Dead plants [a]	% Survived plants
	Pre-emergence	Post-emergence		
F. oxysporum-1	0.0 g	26.7 b	46.7 a	26.7 k
F. oxysporum-2	0.0 g	23.3 d	33.3 c	43.3 i
F. oxysporum-3	0.0 g	20.0 d	36.7 b	43.3 i
F. oxysporum-4	0.0 g	26.7 b	30.0 d	43.3 i
F. oxysporum-5	0.0 g	20.0 d	30.0 d	50.0 g
F. oxysporum-6	0.0 g	26.7 b	36.7 b	36.7 m
R. solani-1	13.3 d	30.0 a	16.7 f	40.0 j
R. solani-2	36.7 a	26.7 b	10.0 h	26.7 q
R. solani-3	16.7 c	20.0 d	20.0 e	43.3 i
R. solani-4	10.0 e	20.0 d	16.7 f	53.3 f
R. solani-5	13.3 d	16.7 e	13.3 g	56.7 e
M. phaseolina-1	16.7 c	23.3 c	13.3 g	46.7 h
M. phaseolina-2	23.3 b	26.7 b	13.3 g	36.7 k
M. phaseolina-3	13.3 d	23.3 c	16.7 f	46.7 h
M. phaseolina-4	16.7 c	23.3 c	16.7 f	43.3 i
Control	0.0 h	0.0 k	0.0l h	100 a

[a] Dead plant: Resulted from the infection by root-rot and/or wilt and assessed 90 days after sowing.
Figures in the same column followed by the same letters are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

3-*In vitro* experiments:

3.1. Antagonistic effect of the two tested bacterial and actinomycetes isolates *in-vitro*:

Results shown in Fig. (1) show the antagonistic effect of two tested bacterial isolate *Bacillus subtilis*, and *Pseudomonas fluorescens* and actinomycetes isolate *Streptomyces lavendulae* against the three selected fungal pathogenic isolates *F. oxysporum*, *M. phaseolina* and *R. solani in-vitro*. the obtained results indicate that the three tested isolates significantly inhibited the linear growth of the tested fungi, *B. subtilis* was the most effective isolate in reducing the linear growth of *F. oxysporum*, caused 62.5% inhibition followed by *Ps. fluorescens* and *S. lavendulae*, being 60.4 and 40.3% reduction in the linear growth respectively, as well as the *Ps. fluorescens* was the most effective isolates in case of both *M. phaseolina* and *Rhizoctonia solani* as recorded 62.5 and 70.7% reduction in the linear growth, followed by *B. subtilis*, recorded 60.4 and 62.5% reduction, respectively. Meanwhile *S. lavendulae* resulted in the lowest values in reducing the linear growth, being 40.3, 48.5 and 50.6% against the three tested fungal isolates, respectively.

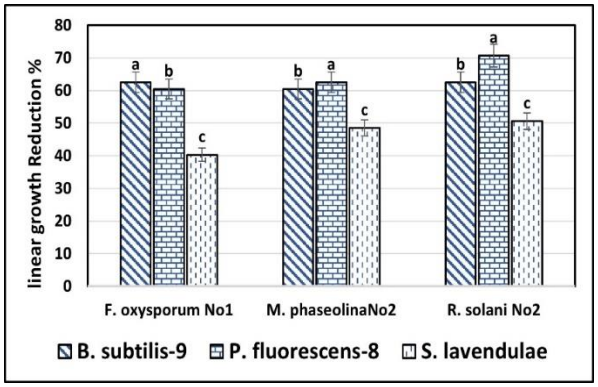

Fig. (1): Antagonistic effect of the tested *B. subtilis*, *Ps. fluorescens* and *S. lavendulae* against *F. oxysporum*, *M. phaseolina* and *Rhizoctonia solani in-vitro*.

3.2. Efficacy of *Debaryomyces* sp., *Trichoderma viride*, *Trichoderma harzianum* and *Chaetomium globosum* isolates on the linear growth reduction of the pathogenic fungi:

Results shown in Fig. (2) show the antagonistic effect of *Debaryomyces* sp., *T. viride*, *T. harzianum* and *Ch. globosum* isolates against the three selected fungal pathogenic isolates *F. oxysporum*, *M. phaseolina* and *R. solani in-vitro*. The obtained results indicate that the three tested isolates significantly inhibited the linear growth of the tested fungi, *Ch. globosum* was the most effective isolate for reducing the linear growth of the three tested pathogens *F. oxysporum*, *M. phaseolina* and *R. solani*, recorded 76.4, 70.9 and 72.3% reduction, respectively followed by *T. harzianum* and *T. viride* which recorded (74.6, 68.5 and 60.5% reduction) and (70.5, 60.4 and 61.3% reduction) of the linear growth of the three tested pathogens, respectively. Meanwhile, *Debaryomyces* sp. resulted in the lowest values in reducing the linear growth, being 57.5, 56.6 and 55.4% reduction against the three tested fungal pathogenic isolates, respectively.

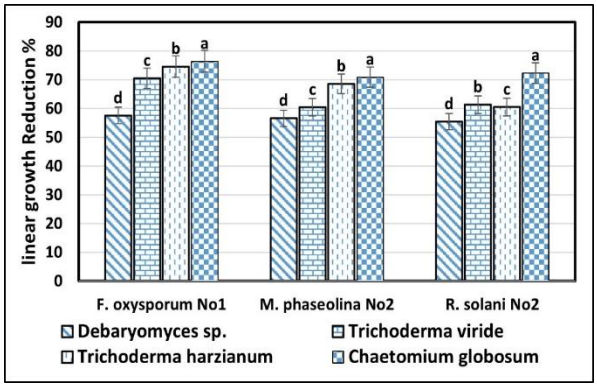

Fig. (2): Antagonistic effect of the tested *Debaryomyces* sp., *T. viride*, *T. harzianum* and *Ch. globosum* against *F. oxysporum*, *M. phaseolina* and *R. solani in-vitro*.

3-3. Greenhouse experiment:

3-4. Effect of seed treatment with the tested bioagents, mycorrhizae and fungicide Rizolex-T50 on the incidence of damping-off, root rot, wilt, and survived plants of cluster bean plants artificially inoculated with *F. oxysporum*, *M. phaseolina* and *R. solani* under greenhouse conditions:

Results in Table (3) indicate that all the tested bioagents, mycorrhizae, and the fungicide Rizolex-T50 significantly reduced the percentages of damping-off, root rot and wilt of cluster bean when grown in artificially infested soil with *F. oxysporum*, *M. phaseolina* and *R. solani* under greenhouse conditions compared with control treatment. This reduction led to increasing the survived plants significantly. Moreover, the treatment with Rizolex-T50 was the superior treatment for lowering the infection by damping-off, root rot, and wilt and increasing the survived plants (infected and healthy) caused by the three tested pathogenic fungi.

In case of *F. oxysporum*, all the tested treatments resulted in the absence of cluster bean infection by pre-emergence damping-off. In addition, treatment with *T. viride* was the

most efficient treatment in reducing the infection by post-emergence damping-off (6.7%) followed by treatment with *T. harzianum*, *B. subtilis*, *P. fluorescens*, (10.0%). Meanwhile, *Debaryomyces* sp. and *S. lavendulae* were the lowest efficient in reducing the infection by post-emergence damping-off (16.7%) after by *C. globosum* and *Mycorrhizae* (13.3%). The reduction in the infection by damping-off and wilt was reflected on increasing the healthy survived plants. The lowest percentage of wilt was resulted from the treatment with Rizolex-T50 (3.3%), while other treatments resulted in wilt incidence ranged from 6.7 to 10.0%.

Control treatment recorded 23.3, 20.0, 20.0, and 36.7% post-emergence damping-off, wilt, infected survived plants and healthy survived plants, respectively.

In case of *M. phaseolina*, percentages of pre-emergence damping-off ranged from 6.7 to 13.3%, post-emergence damping-off and root rot ranged from 6.7-10.0%, respectively due to the tested treatments rather than Rizolex-T50, which resulted in 3.3 to 6.7% infected survived plants and the healthy survived plants ranged from 63.3 to 73.3%. Meanwhile, control treatment recorded 26.7, 20.0, 20.0, 13.3 and 30.0% pre-emergence damping-off, post-emergence damping-off**,** root rot, and survived plants, respectively.

In case of *R. solani* the percentages of pre-emergence and post-emergence damping-off ranged from 6.7 to 10.0%, root rot, ranged from 3.3 to 10.0% due to the tested treatments rather than Rizolex-T50 treatment which resulted 3.3% survived plants ranged from 60.0 to 76.7%.

Control treatment recorded 23.3, 23.3, 13.3, 13.3 and 26.7% pre- emergence damping-off, pot-emergence damping-off, root rot**,** infected survived plants, and healthy survived plants, respectively.

Concerning the effects of bio agents, both bacterial isolates followed by both fungal isolates were the most effective based on healthy survived plants.

Table (3): Effect of seed treatment with the tested biotic agents, on damping-off, root rot, wilt diseases of cluster bean under greenhouse conditions.

Treatments	*F. oxysporum*					*M. phaseolina*					*R. solani*				
	% Damping-off		wilt (%)	% Survived plants		% Damping-off		Root rot (%)	% Survived plants		% Damping-off		Root rot (%)	% Survived plants	
	Pre-	Post-		*In.	**He.	Pre-	Post-		In.	He.	Pre-	Post-		In.	He.
T. viride	0.0	6.7 e	6.7 c	13.3 b	73.3 c	6.7 d	10.0 c	6.7 c	3.3 b	73.3 b	10.0 b	6.7 d	6.7 c	3.3 c	73.3 c
T. harzianum	0.0	10.0 d	6.7 c	10.0 c	73.3 c	6.7 d	10.0 c	6.7 c	3.3 b	73.3 b	6.7 c	10.0 c	6.7 c	3.3 c	73.3 c
C. globosum	0.0	13.3 c	10.0 b	13.3 b	63.3 h	13.3 b	10.0 c	6.7 c	3.3 b	66.0 d	10.0 b	10.0 c	10.0 b	6.7 b	63.3 e
B. subtilis	0.0	10.0 d	6.7 c	6.7 d	77.0 b	10.0 c	6.7 d	6.7 c	3.3 b	73.3 b	6.7 c	10.0 c	3.3 d	3.3 c	76.7 b
P. fluorescens	0.0	10.0 d	6.7 c	6.7 d	77.0 b	10.0 c	6.7 d	6.7 c	3.3 b	73.3 b	6.7 c	10.0 c	3.3 d	3.3 c	76.7 b
Debaryomyce sp.	0.0	16.7 b	6.7 c	10.0 c	67.0 e	13.3 b	10.0 c	10.0 b	3.3 b	63.3 e	10.0 b	13.3 b	6.7 c	6.7 b	63. e
S. lavendulae	0.0	16.7 b	6.7 c	6.7 d	70.0 d	10.0 f	10.0 c	6.7 c	3.3 b	70.0 c	10.0 b	10.0 c	10.0 b	6.7 b	63.3 e
Mycorrhizae	0.0	13.3 c	10.0 b	13.3 b	63.3 e	13.3 b	10.0 c	10.0 b	3.3 b	63.3 e	16.7 b	10.0 c	6.7 c	6.7 b	60.0 h
Rizolex-T 50	0.0	6.7 e	3.3 d	6.7 d	83.3 a	3.3 e	3.3 e	3.3 d	0.0 c	90.0 a	6.7 c	6.7 d	3.3 d	3.3 c	80.0 a
Control	0.0	23.3 a	20.0 a	20.0 a	36.7 f	26.7 a	20.0 a	20.0 a	13.3 a	30.0 f	23.3 a	23.3 a	13.3 a	13.3 a	26.7 f

* In. = infected; ** He. = healthy; Values in the same column followed by the same letters are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

3-5. Effect of seed treatment with the tested bioagents, mycorrhizae and the fungicide Rizolex-T50 on the incidence of damping-off, root rot, wilt, and survived plants under soil infestation with the mixture of the three tested pathogenic fungi in the greenhouse:

Obtained data in Table (4) reveal that the tested bioagents, mycorrhizae, and the fungicide Rizolex-T50 resulted significant reduction to the incidence of damping-off, root rot, and wilt. In turn, increased survived plants under soil infestation with the inocula of mixture of the three tested pathogenic fungi. In addition, the fungicide was the superior treatment in this regard, which resulted in 10.0% pre- emergence damping-off, 6.7% post-emergence and 3.3% wilt. This reduction in the infection by damping-off and the lower percentage of root rot led to increasing the survived plants to 80.0%. Meanwhile, the lowest efficient treatment was the mycorrhiza in case of pre- and post-

emergence damping-off as well as the survived plants, being 23.3, 16.7 and 50.0%, respectively and *B. subtilis* in case of root rot (16.7%). Control treatment recorded 33.3% pre-emergence damping-off, 26.7% post-emergence damping-off and 16.7%root rot, 3.3% wilt plants and 20.0 % survived plants.

Table (4): Effect of seed treatment with the tested bioagents, mycorrhizae, and the fungicide Rizolex-T50 on the incidence of damping-off, root rot, wilt and survived plants of cluster bean plants growth in soil artificially infested with the mixture of *F. oxysporum, M. phaseolina* and *R. solani* inocula under greenhouse conditions.

Treatments	Damping-off %		Root rot %	Wilt %	Survived plants %
	Pre-emergence	Post-emergence			
T. viride	13.3 e	10.0 d	6.7 d	10.0 a	60.0 b
T. harzianum	16.7 d	10.0 d	6.7 d	10.0 a	57.0 c
C. globosum	20.0 c	13.3 c	13.3 b	3.3 c	50.0 e
B. subtilis	16.7 e	6.7 e	16.7 a	6.7 b	53.3 d
P. fluorescens	16.7 g	6.7 e	13.3 b	6.7 b	57.0 c
Debaryomyces sp.	20.0 c	13.3 c	10.0 c	6.7 b	50.0 e
S. lavendulae	20.0 c	13.3 c	6.7 d	6.7 b	53.3 d
Mycorrhizae	23.3 b	16.7 b	6.7 e	3.3 c	50.0 e
Rizolex-T 50	10.0 f	6.7 e	3.3 e	0.0 d	80.0 a
Control	33.3 a	26.7 a	16.7 a	3.3 c	20.0 f

Values in the same column followed by the same letters are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

3-6. Field trials:

3-7. Effect of seed treatment with the tested bioagents, mycorrhizae, and the fungicide Rizolex-T50 on cluster bean plants damping-off and dead plants at Dakahliya governorate under natural infection during 2016 and 2017 growing seasons:

Data in Table (5) show that all the tested treatments clearly reduced the percentages of damping-off (pre- and post-emergence) and dead plants with significant increase to the survived plants under the natural infection by the causals of root-rot and wilt diseases at Dakahliya governorate during two successive seasons (2016 and 2017). In this regard, the fungicide Rizolex-T 50 was, also, the superior treatment in this regard, being 3.3, 4.7, 3.3 and 88.7% for 2016 growing season and 4.7, 4.3, 3.5 and 87.5% for 2017 growing season, respectively.

In case of the first season, the highest percentage of pre-emergence damping-off was occurred due to using the bioagents *B. subtilis*, *S. lavendulae*, mycorrhizae and *C. globosum*, being 9.7% (for the five treatments), post-emergence damping-off from the treatment with the mycorrhizae, being 9.3%. The lowest percentage of pre-emergence damping-off after using the fungicide was occurred due to treatment with the bioagent *T. harzianum*, being 7.3%, post-emergence damping-off was due to treatment with the bioagents *B. subtilis* and *T. harzianum* being 5.3%.

In case of the second season, the highest percentage of pre-emergence damping-off was occurred after treatment with the bioagents *T. viride* and *C. globosum*, being 10.3 % (for both treatments), post-emergence damping-off due to treatment with the mycorrhizae, being 10.7% overall fungicide the lowest percentage of pre-emergence damping-off was occurred due to treatment with the bioagent *T. harzianum*, being 4.7%.

The highest percentage of dead plants was resulted due to treatment with the bioagent *S. lavendulae* (7.0%) during the first season and from the mycorrhizae (8.0 %) during the second season. The lowest percentage of survived plants was occurred with mycorrhizae treatment, being 75.0 and 71.3% during 2016 and 2017 growing seasons, respectively.

Control treatment recorded 16.7, 12.3, 7.5, and 63.5% pre- post-emergence damping-off, dead plants and survived plants, during 2016 growing season and 16.3, 7.5, 10.7 and 65.5% during 2017 growing season, respectively.

Table (5): Effect of seed treatment with the tested bioagents, mycorrhizae, fungicide Rizolex-T50 on cluster bean plants damping-off and dead plants, at Dakahliya governorate under the natural infection during 2016 and 2017 growing seasons.

Treatment	Season 2016				Season 2017			
	% Damping- off		[a] Dead plant (%)	Survived plants %	% Damping- off		Dead plants (%)	Survived plants %
	Pre-	Post-			Pre-	Post-		
T. viride	9.0 b	9.0 b	5.5 bc	76.5 g	10.3 b	8.3 c	5.7 e	75.7 e
T. harzianum	7.3 d	5.3 g	4.3 e	83.0 b	7.3 f	4.7 f	6.3 d	81.7 b
C. globosum	9.7 a	7.5 c	5.3 c	77.5 efg	10.3 b	8.0 cd	4.7 f	77.0 d
B. subtilis	9.7 a	5.3 f	3.5 f	81.5 c	8.7 d	8.3 bc	4.5 f	78.5 c
P. fluorescens	8.0 c	7.3 c	3.5 f	81.2 c	8.3 de	8.0 cd	6.3 d	77.4 cd
S. lavendulae	9.7 a	6.3 ef	7.0 a	77.0 fg	9.3 d	7.0 e	6.3 d	77.4 cd
Debaryomyces sp.	9.3 ab	7.0 cd	5.7 b	78.0 ef	8.7 d	8.3 c	7.0 c	76.0 de
Mycorrhizae	9.7 a	9.3 b	6.0 b	75.0 h	10.0 b	10.7 a	8.0 b	71.3 f
Rizolex-T 50	3.3 e	4.7 g	3.3 f	88.7 a	4.7 g	4.3 f	3.5 h	87.5 a
Control	16.7 a	12.3 a	7.5 a	63.5 i	16.3 a	7.5 de	10.7 a	65.5 g

Values in the same column followed by the same letters are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

[a] Dead plant: due to the infection by the causals of root-rot and wilt disease.

3.8. Effect of seed treatment with the tested bioagents, fungicide Rizolex-T50 on cluster bean growth and crop parameters at Dakahliya governorate under natural field conditions during 2016 and 2017 growing seasons:

Results presented in Table (6) show the effect of seed treatment with the tested bioagents and fungicide Rizolex-T50 on some cluster bean growth and crop parameters at Dakahliya governorate under the natural infection by the causals of root-rot and wilt diseases during 2016 and 2017 growing seasons.

The obtained data indicate that all the tested bioagents, fungicide Rizolex-T50 resulted in considerable increase in the assessed growth and crop parameters during 2016 and 2017 growing seasons *viz.* plant height, No. of branches/plant, No. of pods/ plant, seed weight (g)/plant, 100 seed weight (g) and seed yield (Kg)/plot compared with control treatment.

Table (6): Effect of seed treatment with the tested bioagents, fungicide Rizolex-T50 on cluster bean growth and crop parameters, at Dakahliya governorate under natural infection during 2016 and 2017 growing seasons.

Treatment	Season 2016						Season 2017					
	Plant height (cm)	No. of branches /plant	No. of pods/ Plant	Seed weight (g)/ plant	100seed weight (g)	Seed yield (kg) /plot	Plant height (cm)	No. of branches /plant	No. of pods/ plant	Seed weight (g)/ plant	100seed weight (g)	Seed yield (kg) /plot
T. viride	135.3 f	13.5 d	145.2 d	23.5 bc	3.3 ab	3.83 c	132.3 d	12.9 c	142.5 c	22.3 c	3.0 a	3.58 cd
T. harzianum	140.7 c	14.3 a	147.5 c	24.7 ab	3.4 a	4.15 b	135.6ab	13.0 b	145.2 b	23.9 a	3.1 a	3.76 ab
C. globosum	133.5 g	12.2 g	132.3 g	20.5 e	2.9 ab	3.18 de	129.4 f	11.8 f	128.3 h	20.0 f	2.7 a	3.03 f
B. subtilis	136.5 ef	13.2 e	143.2 e	22.4 cd	3.0 ab	3.73 c	133.3cd	12.8 d	135.3 g	22.2 c	2.8 a	3.27 e
P. fluorescens	138.5 d	13.7 c	147.3 c	24.5 ab	3.1 ab	3.81 c	135.2 b	13.3 a	140.5de	23.3 b	3.0 a	3.42 de
S. lavendulae	135.5 f	13.0 f	140.1 f	22.0 d	3.0 ab	3.67 c	130.8 e	12.5 e	139.7 ef	20.7 e	2.8 a	2.88 f
Debaryomyces sp.	132.7 g	13.3 e	143.5 e	22.0 d	3.1 ab	3.75 c	130.3 ef	13.0 b	141.3 d	21.5 d	3.1 a	3.35 e
Mycorrhizae	130.3 h	12.0 h	130.7 h	20.1 e	2.7 ab	3.11 e	127.3 g	11.5 g	127.5 h	19.5 g	2.7 a	2.96 f
Rizolex-T 50	142.3 b	13.9 b	150.3 b	24.9 a	3.3 ab	4.22 b	135.3ab	13.3 a	145.2 b	23.3 b	3.1 a	3.70 bc
Control	111.6 i	9.5 i	113.5 i	18.5 f	2.6 b	2.86 f	115.3 h	12.9 c	108.7 j	16.3 h	2.6 a	2.63 g

Figures in the same column followed by the same letters are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

The obtained data during 2017 growing season were, in most cases, in the same trend with those obtained data during 2016 growing season. Meanwhile, the treatment with mycorrhizae exerted the lowest figures, among the tested treatments, being130.3 cm, 12.0 branch, 130.7 pod 20.1g, 2.7g and 3.11 Kg during 2016 growing season, 127.3 cm, 11.5 branch, 127.5 pod 19.5 g, 2.7g and 2.96 Kg during 2017 growing season. Other treatments recorded intermediate figures through both seasons.

Control treatment recorded poor figures, being 111.6 cm, 9.5 branch, 113.5 pod 18.5 g, 2.6 g and 2.86 Kg during 2016 growing season, 115.3 cm, 9.2 branch, 108.7 pod 16.3 g, 2.6g and 2.63 Kg. during 2017 growing season. In case of bioagents treatment the highest seed yield plot^{-1} occurred under the treatment of *T. harzianum* followed by *T. viride* then *P. fluorescens* and *B. subtilis* in both seasons.

DISCUSSION

The constant growth of the world's population requires substantial resources to produce food. One of the greatest challenges of the world is to produce enough food for the growing population as well as animal feeding. Production as well as protection of food commodities is necessary to nourish the ever-growing population. The situation is particularly critical in developing countries, where the rate of net food production is slowing down in relation to population growth. The world food situation is aggravated by the fact that in spite of the use of all available means of plant protection, a major proportion of the yearly production of food commodities of the world is destroyed by various pests, including bacteria, fungi, viruses, insects, rodents, nematodes, etc. Losses at times are so severe as to lead to famine in large areas of the world that are densely populated. So, increasing the productivity is considered as a national goal.

Cluster bean or guar (*Cyamopsis tetragonoloba* (L.) Taub.) belongs to the family Fabaceae (Leguminosae) which emerging as a potential source of vegetable protein for human beings. It is liable to infection by many soil-borne pathogens causing great loss to the plant stand and both quality and quantity (Mohamed *et al*., 2006; Yadva *et al.*, 2007; Pareek and Varma, 2014; Choudhary and Sindhu, 2015 and Abdel-Monaim, 2016).

In this investigation, isolation trials from the roots of cluster bean plants showing typical symptoms of root-rot and wilt diseases, collected from Sharkia, Ismailia, Dakahliya and Giza governorates during 2015 growing season yielded 138 fungal isolates belonging to more than six genera. The isolated fungi were purified and identified as: *Alternaria alternata, Fusarium solani, Fusarium oxysporum* and *Macrophomina phaseolina, Rhizoctonia solani* and *Sclerotium rolfsii* in addition to un-known fungi. The fungus *F. oxysporum* recorded the highest occurrence and frequency followed by *M. phaseolina* then *R. solani* and *F. solani*. Meanwhile, *S. rolfsii* recorded the lowest occurrence and frequency followed by *A. alternata.* The isolated fungi from Dakahliya governorate recorded the highest occurrence and frequency followed by Sharkia governorate then Ismailia governorate and Giza governorate, the results may be due to the environmental and soil differences among governorates. The isolated fungi were previously isolated from the roots of cluster bean plants causing root-rot and wilt symptoms these results are in agreement with those of Lodha, (1998); Lodha *et al.* (2002); Sharma *et al.* (2005); Bajoria *et al*. (2008); Jaiman and Jain, (2011); Pareek and Varma, (2014); Choudhary and Sindhu, (2015) and Abdel-Monaim, (2016), who reported that these complex fungi infect cluster bean plants and cause root rot and wilt diseases. Variation in the number, type, and frequency of pathogens with the location may be due to the difference of soil texture, moisture and composition of the soil, in addition to the ability of fungal genera to live and adapt to different conditions.

The obtained data revealed that all the isolated fungi were pathogenic to cluster bean plants. However, the isolated fungi varied in their pathogenicity. In this respect, the isolates of *F. oxysporum* failed to cause pre-emergence damping-off. In addition, isolate-2 of *R. solani* caused the highest figure of pre-emergence damping-off followed by isolate-2 of *M. phaseolina*.

Generally, *Fusarium oxysporum* No1, *Rhizoctonia solani* No.2 and *Macrophomina phaseolina* No.2 were the most aggressive isolates based on pathogenicity test. The pathogenicity of the isolated fungi was previously proved by many investigators (Mohamed *et al.*, 2006; Muthomi *et al.*, 2007; Pareek and Varma, 2014; Choudhary and Sindhu, 2015 and Abdel-Monaim, 2016). The injurious effects of fungal pathogens also might be due to enzyme production which causes rotten lesions on seed cotyledons followed by seed rot and plumule soft rot in turn occurs

damping off (Mahmoud *et al*., 2013). The different of pathogenic ability among fungal isolates may be due to genetic variance among them.

To control fungal diseases, synthetic fungicides are usually applied as effective, dependable, and economical control measures. However, the indiscriminate use of chemical fungicides has resulted in several problems, such as toxic residues in food, water and soil and disruption of the ecosystem, leading to the fear that their regular use may harm the environment further.so, there is an urgent to apply alternative safe efficient methods against these diseases rather than fungicides. An acceptable approach that is being actively investigated involves the use of plant growth promoting such *Bacillus subtilis* and *Pseudomonas fluorescens* in managing soil borne fungi (Abdel-Monaim, 2013 and Sarhan and Shehata, 2014).

In our study, the twelve fungal, bacterial and *an* Actinomycetes isolates of cluster bean resulted in significant reduction in the linear growth of the pathogenic fungi, *in vitro*, 5 days after incubation at 28±1°C. *B. subtilis*, *P. fluorescens*, *T. viride*, *T. harzianum* and *C. globosum* were the most effective in reducing the linear growth against the three tested fungal pathogens. The role of antagonistic bacteria in reducing pathogens linear growth may be due to competition, antibiosis and siderophores production (Sarhan and Shehata, 2014). They added that *B. subtilis* inhibited growth of *F. oxysporum* in vitro by production of several antibiotics such as bacilysin, iturin and mycobacillin. As well as *B. subtilis*, *B. cereus*, *B. pumilus* and *B. amyloliquefaciens* were found to be antagonistic against *F. solani* in vivo (Ajilogba *et al*., 2013).

Trichoderma genus is the most common mycoparasitic and saprophytic fungi. Trichoderma can inhibit plant pathogens by producing secondary metabolites such as antibiotics (Howell 2004) and cell wall-degrading enzymes (Elad *et al*., 2000). Qi and Zhao (2013) found that *Trichoderma asperellum* exhibited some plant growth-promoting attributes of phosphate solubilization, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, auxin and siderophore production. Culture filtrate of the selected isolate (*T. harzianum*) was analyzed using GC-MS system to determine their chemical constituents. *T. harzianum* produced the phytohormone indole-3-acetic acid (IAA) on tryptophan free medium, a marked dependency on tryptophan for the production of IAA was noticed by Saber *et al*. (2009).

The obtained results indicated that, mycorrhizae, and the fungicide Rizolex-T50 resulted significant reduction to the incidence of damping-off, root rot and wilt, in turn increased survived plants under inoculation with the inoculums mixture of the three tested pathogenic fungi. Shaul-Keinan *et al*. (2002) explained the positive role of Mycorrhizal colonization application on enhancement plants growth parameters might be due to the hormonal change throughout the entire plant by the influence of the symbioses mycorrhiza such as increase of gibberellins.

The application of Rizolex-T50 WP (Tolclofos-methyl) partially improved and induced of faba bean growth and yield by increasing the resistance against fungal pathogens. This effect may be due to the seeds of plant requires treatment with any fungicide for protection it within germination, seedling stages to assure an adequate plant stand in the field. Similar findings were obtained by Abd El-Hai *et al*. (2010) and Abd El-Hai and Ali (2017).

It could be concluded that the application of *Trichoderma harzianum* from fungal bioagents and *P. fluorescens* from bacteria bioagents as seed treatments could be applied for safe controlling damping-off, root rot and wilt diseases in cluster bean plants and improving growth parameters and seed yield.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest

REFRENCES

Abd El-Hai, K.M. and Ali, A.A. 2017. Down-Regulation of damping-off and root rot diseases in lentil using kinetin and *Trichoderma*. J. Plant Protection and Pathology, Mansoura Univ., 8(2): 45-54.

Abd El-Hai, K.M.; El-Metwally, M.A. and El-Baz, S.M. 2010. Reduction of soybean root and stalk rots by growth substances under salt stress conditions. Plant Pathol. J., 9(4): 149-161.

Abdel-Monaim, M.F. 2013. Improvement biocontrol of damping-off and root rot/wilt of faba bean by salicylic acid and hydrogen peroxide. Mycobiology, 41(1):47-55.

Abdel-Monaim, M.F. 2016. Salicylic acid and *Pseudomonas fluorescens* as safe control means against *Rhizoctonia solani* in guar

(*Cyamopsis tetragonoloba* (L.) Taub.), Egypt. J. Phytopathol., 44(1): 25-47.

Abd-El-Rahman, S.S.; Khalil, A.A. and Balabel, N.M. 2018. Influence of rice compost fortified with bioagents on guar root-rot disease. Egypt. J. Phytopathol., 46(2): 195-214.

Ajilogba, C.F.; Babalola, O.O. and Ahmad, F. 2013. Antagonistic effects of *Bacillus* species in biocontrol of tomato Fusarium wilt. Studies on Ethno-Medicine, 7: 205-216.

Al-Kahal, A.A.; Mansoor, H.M. and Ashmawy, E.A. 2009. Effect of Copper oxychloride, elemental sulphur, and rhizobium inoculation on root rot disease, nodulation, and growth of faba bean plants. Journal of Soil Sciences and Agricultural Engineering, 34(5): 5773-5783.

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

Booth, C., 1971. The Genus Fusarium. CMI, Kew, Surray, England, 237 pp.

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.

Elad, Y.; Freeman, S. and Monte, E. 2000. Biocontrol agents: (eds) Mode of action and interaction with other means of control. IOBC wprs Bulletin, vol 24, Sevilla, Spain

Gabr, M. R; M. N. Shaat, M. H. Hassanein and M. F. Abd El-Monaim, 2003. Lentil (*Lens esculenta*) damping-off and root rot diseases and their chemical and biological control in El-Minia governorate. J. Agric. Sci. Mansoura Univ. 28: 7289-7306

Gilman, J.C. 1957. A Manual of Soil Fungi. The Iowa State College Press, USA. 450 pp.

Howell, C.R., 2004. Understanding the mechanisms employed by *Trichoderma virens* to affect biological control. Phytopathology 94: S138 (Supplement).

Jacobsen, B.J.; Zidack, N.K. and Larson, B.J. 2004. The role of *Bacillus* based biological control agents in integrated pest management systems: plant diseases. Phytopathology 94:1272-1275.

Jaiman, R.K. and Jain, S.C. 2011. Bio-control of root rot of cluster bean caused by *Macrophomina phaseolina*. Environmental Ecology, 28(24): 1135-37.

Janisiewiez, W.J.; Tworkoski, T.J. and Sharer, C. 2000. Characterizing the mechanism of biological control of post-harvest diseases on fruits with a simple method to study competition for nutrients. Phytopathol., 90: 1196-2000.

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

Lodha, S. 1998. Effect of sources of inoculum on population dynamics of *Macrophomina phaseolina* and disease intensity in cluster bean. Indian Phytopathol., 51(2) 175-179.

Lodha, S.; Sharma, S.K. and Agrawal, R.K. 2002. Inactivation of *Macrophomina phaseolina* propagules during compositing and effect of composts on dry root rot severity and on seed yield of cluster bean. Eur. J. Plant Pathol.,108(3): 253-261.

Mahmoud, E.Y.; Shokry, S.Y.M. and Hussin, Z.N. 2006. Efficiency of some antagonistic bacteria to reduce incidence of damping-off, wilt and peanut root rot. J. Agric. Sci. Mansoura Univ., 31(6): 3525-3536.

Mahmoud, S.Y.M.; Hosseny, M.H.; Shaikh, K.A.A.; Obiaalla, A.H.A. and Mohamed, Y.A. 2013. Seed borne fungal pathogens associated with common bean (*Phaseols vulgaris* L.) seeds and their impact on germination. J. Enviro. Studies, 11: 19-26.

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.

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.

Mousa, M.S.; Ali, M.K.; Mosa, A.A. and Elewa, I.S. 2006. Root rot disease of olive transplants and its biological control. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 14(1), 395-409.

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. Biol. Sci., 7(7): 1163-1170.

Nelson, P.E.; Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium* species: An

illustrated manual for identification. Pennsylvania State University Press, University Park, 193 pp.

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.

Qi, W. and Zhao, L. 2013. Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress. J. Basic Microbiol., 53(4): 355-64.

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. S. Afr. J. Bot., 74: 268-273.

Saber, W.I.A.; Abd El-Hai, K.M. and Ghoneem, K.M. 2009. Synergistic effect of *Trichoderma* and *Rhizobium* on both biocontrol of chocolate spot disease and induction of nodulation, physiological activities and productivity of *Vicia faba*. Res. J. Microbiol., 4(8): 286-300.

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

Sharma, S.; Pathak, D.V.; Kumar, S. and Kumar, N. 2005. Efficacy of plant formulations and bioagents in controlling root rot of cluster bean. Forage Res., 31: 103-105.

Shaul-Keinan, O.; Gadkar, V.; Ginzberg, I.; Grunzweig, J. and Chet, I.; Elad, Y.; Wininger, S.; Belausov, E.; Eshed, Y.; Atzmon, N.; Ben-Tal, Y. and Kapulnik, Y. 2002. Hormone concentration in tobacco roots change during arbuscular colonization with *Glomus intraradices*. New Phytol., 154(2): 501-507.

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

Yadav, E.; Pathak, D.V.; Sharma, S.K.; Kumar, M. and Sharma, P.K. 2007. Isolation and characterization of mutants of *Pseudomonas maltophila* PM-4 altered in chitinolytic activity and antagonistic activity against root rot pathogens of cluster bean (*Cyamopsis tetragonoloba*). India. J. Microbiol., 64: 64-71.

Ziedan, E.H.E. and Farrag, E.S.H. 2002. Biological control of root-rot disease on mandarin by antagonistic strain of *Bacillus megatherium*. Annals Agric. Sci. Ain Shams Univ. Cairo. 47: 1021- 1031.