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ISOLATION, IDENTIFICATION AND BIOMANAGEMENT OF ROOT ROT OF BLACK CUMIN (*NIGELLA SATIVA*) USING SELECTED BACTERIAL ANTAGONISTS

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A B S T R A C T

The study deal with potentiality of some bioagents for controlling the root rot of black cumin under greenhouse conditions, caused by *Fus*arium spp. Eight fungal isolates were obtained from diseased of back cumin plants collected from Assiut Governorate. These isolates were belonged to the genus *Fusarium* spp. They were identified as, four isolates of *F. comptoceras*, three isolates of *F. solani* and one isolate *Fusarium lateritium.* Pathogenicity tests indicated that all tested fungal isolates were able to infect black cumin plants causing symptoms of root rot resulted in dwarfism and death before the capsules mature. They varied in their pathogenicity, *Fusarium comptoceras* No.1 gave the highest percentage of disease severity and percentage of infection on black cumin plants (53 and 50% respectively), while isolates *F. comptoceras* Nos. 3 and *F. solani* No. 6 gave the lowest percentage of infection (15 and 17% respectively) the rest of isolates showed moderate of percentage of infection. Antagonistic capability of 15 isolates (PGPR) was tested *in vitro* against growth of three isolates of *Fusarium* spp. the causal pathogen of root rot of black cumin. Seeds black cumin plant treated with all bioagents as a suspension significantly increased the root dry weigh and foliar dry weigh compared to infected control. In conclusion, our study confirmed that used of bioagents may be applied as future ecofriendly alternatives to synthetic fungicides for controlling the disease of black cumin.

Keywords: *Fusarium*, Root rot, Black cumin, Rhizobacteria, Number of colony fungi.

INTRODUCTION

Medicinal and aromatic plants are considered to be important crops in Egypt as well as many countries in the world. Black cumin is one of medicinal plants with potential uses, which can be explored for safe and effective herbal medicine for human benefit (Hilal, 1985; Pastirava *et al*., 2004; Eldegwy, 2004). It have great economic importance as they occupy an export priority in the first rank, they have especial importance among the other traditional crops in the Middle and upper Egypt, especially in Giza, Fayoum, BeniSuef, El-Minya and Assiut governorates Mohmed *et al*. (2012).Black cumin root rot is one of the most important diseases in Egypt, this disease caused by *Fusarium* spp. this fungus is considered to be soil and seed borne pathogen Sharma and Meena (2012).

Chemical control caused the development of resistant pathogenic populations, environmental contamination and affecting human health. Therefore, many trials for using biocontrol to overcome the plant diseases were carried out Sallam Nashwa *et al*. (2004). Biological control was found to be an attractive alternate strategy for control of soil - borne diseases as well as it was useful in reducing harmful side effect of pesticides on environment Cook and Baker (1983). In recent years, plant growth promoting rhizobacteria (PGPR) has been suggested as a potentially attractive alternative disease management approach since PGPR are known for growth promotion and disease reduction in crops Jetiyanon and Kloepper (2002). The use of PGPR has become a common practice in many regions of the world. Although significant control of plant pathogens has been demonstrated by PGPR in laboratory and greenhouse studies, results in the field have been inconsistent Siddiqui (2006). The present work was planned to study the pathogenicity of isolated fungi from diseased black cumin plants, and improvement of biological control by using this bacterium to control the disease under greenhouse.

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MATERIALS AND METHODS

Isolation and identification of the causal pathogens: The diseased plants of black cumin were collected from different localities in Assiut Governorate, infected roots were removed and washed in running tap water to remove any soil remains. Diseased roots were isolated on Potato Dextrose Agar medium (PDA). Single spores or hyphal tip were taken from the developing fungal colonies and transferred onto PDA medium. The isolated fungi were microscopically examined and preliminary microscopic examination the isolated fungi that may be related to *Fusarium* spp. were purified by single spore Booth (1971). While the reminder of fungi were purified by hyphal tip technique Brown, 1924). Identification of the pathogenic fungi isolates were performed according to (Gilman, 1957; Gams, 1971; and Nelson *et al.,* 1983).

Pathogenicity tests: Conical flasks each contained about 100 g sorghum grains and about 120 ml of tap water were autoclaved at 1 kg/cm²pressure for 25 minutes. Flasks were subsequently inoculated with each of the fungi isolates using1cm fungal disc (grown on PDA medium for 4 days) then incubation at 30°C for 2 weeks. Soil were sterilized by wetting them with formalin 5%, Clay pots (30 cm diameter) were sterilized similarly as soil, by dipping them in 5% formalin for 2 minutes and left in the open air for three weeks. About 5 kg soil were placed in each pot infestation was performed by mixing about 150 g of the inoculums with soil in each pot (rate of 3%) and pots were then irrigated. Sterilized soil un-inoculated were used in the control treatment Gabr *et al.* (1998). Ten disinfestations seeds of Black cumin were seeded in each pot one week after soil infestation with each pathogen, 4 replicates were used for each fungal isolate Hilal (1985). Pots were irrigated directly after sowing and subsequently as needed tell 8 weeks, respectively. The experiment was repeated twice.

The root rot was scored on 0 - 3 scale, where

0 = healthy, creamy white on discoloration of sub crown internode and crown roots, 1 = light brown discoloration of sub crown internode and crown roots, 2 = brown discoloration of sub crown internode and crown roots, 3 = dark brown to black discoloration of sub crown internode and crown roots and/or roots mostly decayed Tinline *et al.* (1975).

The estimation of root rot index (disease index) was carried out according as:

$$Root\ rot\ index = \frac{(nx1) + (nx2) + ..}{tn} \times 100$$

n = number of plants in each group of disease plants (1, 2, 3 ..)

Biocontrol of root rot disease of black cumin using bioagents

Isolation of bioagents from rhizosphere: This experiment was performed to isolate the native microflora from the rhizosphere that may antagonize the pathogenic fungi causing root rot disease of black cumin. Isolation of bacterial isolates from rhizosphere of black cumin plants was carried out according to the method described by Dhingra and Sinclair (1995). Plants were carefully uprooted then the excess soil gently shake off discarded and only that soil which was adhering closely to the root system were leave. Roots were cut to species and placed in flasks (500 mL) containing 200 mL sterile water. Flasks were gently shaked until most of the closely adhering rhizosphere soil was removed, then roots removed, placed into another flasks containing 200 mL sterile water and flasks shaked again. For isolation of the antagonistic bacteria, one ml from the suspension was added to Petri plates containing Nutrient Sucrose Agar (NSA) and plates were incubated for 48 hours at 25± 2°C.

Identification of the bioagent bacteria: The isolated bacteria used in bioagents were identified according to their morphological, cultural Dye (1968) and Schaad, (1988). Bergey's Manual of Determinative Bacteriology 9th edition Holt *et al*. (1994).

Antagonistic effect of isolated bacteria on the pathogenic fungus *In vitro:* Agar disks carrying mycelium from each pathogen, *Fusarium solani. F. coptoceras* and *F. latentium,* each was placed at the center of PDA plate between two parallel streaks of the tested bacteria, 15 bacteria isolates that isolated from rhizosphere of black cumin. Plates inoculated with the fungi alone served as control. When the fungal growth of the control approached the edge of the plates, the antagonistic effect was assessed by relating mycelial diameters on plates inoculated with bacteria to mycelial diameter on control plates and computing percentage inhibition. Eight plates were used for each treatment Dubey *et al*. (2007).

Percentage of reduction in linear growth of the tested fungi was determined using the following formula:

$$R = \frac{C - T}{C} \times 100$$

Where R= Percentage of growth reduction, C = Diameter of the control hyphal growth, T= Diameter of the treated hyphal growth

Evaluation of antagonistic microorganisms under greenhouse

Preparation of suspension antagonistic bacteria: The highly antagonistic isolates of bacterial isolates (*Pseudomonas fluorescens, Bacillus simplex, B. simplex, P. aeruginosa* and *P. fluorescens*) against growth of the pathogens were selected for this study. Inoculum of each bacteria isolates (*P. fluorescens, B. simplex, B. simplex, P. aeruginosa* and *P. fluorescens*) was prepared by growing the bacterial isolates in nutrient yeast extract broth, incubated at 25 °C on an orbital shaker at 200 rpm for 24 h. Bacteria were subsequently pelleted by centrifugation at 15000 rpm for 5 min and washed in 0.1% saline solution.

Effect of bioagents as suspension on disease severity and certain parameters of black cumin under greenhouse conditions: Inocula of the tested pathogenic isolate *Fusarium* spp. were prepared as previously mentioned in pathogenicity test the inoculated flasks were incubated at 27–30 °C for 15 days. Soil infestation with pathogenic fungi *Fusarium* spp. was carried out as mentioned before by mixing 3 % of fungal inocula in each pot. Pots irrigated immediately and later as needed.

Each pot was planted with 6 seeds sterilized black cumin after soaked it in the antagonistic bacterial suspension for 20 min. Untreated seeds were seeded as two controls, one with soil infested by the pathogenic fungi and the other is healthy one (without infested by the pathogen). The tested treatments were arranged in a randomized completes block design with 4 replicates. Results were recorded and calculated as previously mentioned before uprooted plants black cumin from pot's washed in running tap water the root rot was scored on 0-3 scalene

Where;

0 – healthy. Creamy white, no discoloration of sub crown interanode and crown roots, 1- light brown discoloration of discoloration of sub crown internode and crown roots, 2-brown discoloration sub crown internode and crown roots, 3-dark brown to black of sub crown internode and crown roots mostly decayed.

The score was transformed to root rot index using the formula Tinline *et al.* (1975).

The estimation of root rot index (disease index) was carried out according as:

$$Root\ rot\ index = \frac{(nx1) + (nx2) + ..}{tn}$$

tn = the total number of plants, n = number of plants in each group of disease plants (1, 2, 3 ..)

At the end of the experiment, plants from different treatments were removed, washed thoroughly with running water, blotted with tissue paper, weighed to determine fresh weights, and then oven dried at 65°C for 72 h for dry weights.

Effect of bioagents on number colony of fungal pathogen under greenhouse: To study the effect of bioaganets on the number of propagules of the pathogen in soil core soil samples were taken from experimental greenhouse pots after 85 days from planting, they were taken from the top 2 inches of soil. The size of each sample was 20 and 30 gm soils represent pot treatment, the collected soil samples of each treatment were mixed together to form one composite sample, each treatment were process through serial dilution technique for the fungal 10^{-3}-10^{-4}. The dilution was platted on the peptone dextrose, rosbengal agar medium plate the poured plates were incubated at 72 h in 25+ 2°C. The numbers of fungal colony were count according to the method of CFU was calculated using the formula given by Aneja (2003).

Fungal pathogen per ml/gm of the soil =
Number of colonies/amoun plated× dilution factor

Statistical analysis: Data were subjected to statistical analysis using analysis of variance and means were compared using L.S.D. test as described by Gomez and Gomez (1984).

RESULTS

Isolation and identification of the causal pathogens: Eight fungal isolates were isolated from infected roots of black cumin plants collected from different localities in Assiut, Egypt. Fungal isolates were identified by using the morphological features of mycelia and spores as described by Domsch *et al.* (1980) and confirmed by Assiut University Mycological Center (AUMC), Assiut, Egypt. All isolates belong to Genus *Fusarium* and four of them belong to *F. comptoceras* species, 3 belong to *F. solani* species and one of then belong to *F. lateritium.*

Pathogenicity tests: Data in Figure 1 illustrate that all tested fungi isolate were able to infect black cumin plants caused root rot disease. Regarding percentage of infection, the isolates *Fusarium comptoceras* No. 1 gave the highest

percentage of infection on black cumin plants (50%), while isolates *F. comptoceras* No. 3 and *F. solani* 6 gave the lowest percentage of infection (15 and 17%) the rest of isolates showed moderate of percentage of infection. About disease severity (DS%) isolates Nos. (*F. solani No 7. F. comptoceras No 2 and F. comptoceras No. 1)* were the highest isolates in DS%. Isolates *F. lateritium* No. 8 was the lowest in DS %, while the other isolates gave the moderate effect of disease infection % on black cumin plant.

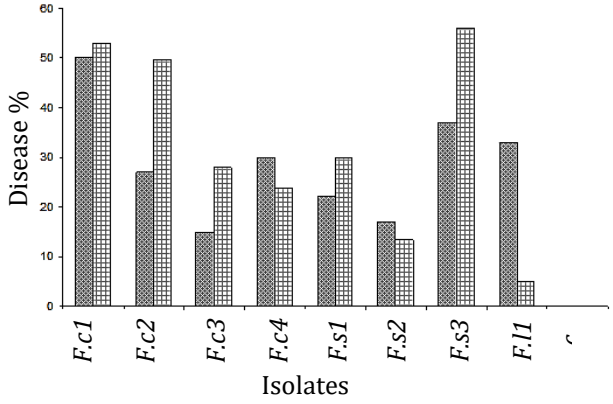


Figure 1. Pathogenic capabilities of *Fusarium* spp. isolates on black cumin under greenhouse.

Effect of certain plant growth promoting rhizobacteria (PGPR) on the growth of *fusarium* spp. black cumin *in vitro*: Fifteen isolated of PGPR were isolated from rhizosphere of black cumin plant. Antagonistic capability of 15 isolates was tested *in vitro* against growth of three isolates of *Fusarium* spp. the causal pathogen of root rot of black cumin.

Table 1 show that the tested bacterial isolates exhibit different inhibitory effect against growth of the tested pathogen. Isolates Nos. (2, 8 and 10) displayed the greatest percentage of inhibition to growth of *Fusarium* spp., followed by isolates Nos. 1, 14 and 15. Isolates Nos. (9 and 13) show the lowest inhibitory effect on the growth of the pathogens. In case of *Fusarium comptoceras* the isolates Nos. (10, 8 and 1) gave the highest effect (4.90, 4.46 and 4.26) followed by isolates Nos. (5, 7, 2 and 3). While the isolates Nos. (5, 9, 12 and 13) gave the lowest effect against *F. comptoceras*, the other tested bacterial isolates showed the moderate effect against the tested pathogen. Also, in case of *F. solani* the tested bacterial isolate Nos. (2 and 11) show the highest effect on the pathogen, followed by isolates Nos. (5, 14, 13.7.10.8 and 1) while the isolates No. (4) gave the lowest effect against *F. solani*.

Table 1. Effect of treatment with different plant growth promoting rhizobacteria (PGPR) on the growth of *Fusarium* spp. invitro.

Isolates No.	*F. comptoceras*	*F. solani*	*F. lateritium*	Mean
01	4.26 bcdefg	4.00 cdefghi	3.96 defghi	4.07 bcd
02	3.46 ghijklm	6.33 A	4.66 bcd	4.82 a
03	3.23 Ijklm	3.00 Jklmn	3.66 efghijkl	3.30 ef
04	2.96 klmn	2.00 opq	4.64 bcd	3.20 ef
05	4.16 bcdefgh	3.50 ghjkl	4.00 cdefghi	3.88 cd
06	2.26 bcdefgh	2.83 lmno	4.00 cdefghi	3.70 cde
07	3.86 defghijk	4.66 bcd	3.30 hijklmn	3.94 cd
08	4.46 bcdef	4.20 bcdefgh	6.36 a	5.01 a
09	1.66 pq	3.46 ghijklm	2.53 nop	2.55 g
10	4.90 q	4.33 bcdefgh	4.36 bcdefg	4.53 ab
11	2.56Mnop	5.00 b	2.86 lmno	3.12 F
12	1.23mnop	3.90 defghij	4.20 bcdefgh	3.55 def
13	1.23 q	4.73 bcd	1.36 q	2.44 g
14	4.03cdefghi	4.56 bcde	3.60 fghijkl	4.06 bcd
15	4.26 bcdefghi	3.96 defghi	4.16 bcdefgh	4.13 bcd
Control	0.00 r	0.00 r	0.00 r	0.00
Mean	3.179 b	3.781 a	3.606 ab	-

Identification of bacterial isolates: Five pure cultures of non-pathogenic bacteria isolated from black cumin Rhizospheres were identified according to their morphological and biochemical characteristics.

Results represented in Tables 2 and 3 showed that the morphological and biochemical characterization of the isolates revealed that isolates RB1 and RB2 were identified as *P. fluorescens*. All these were rod shaped and they showed negative reaction to gram stain, spore formation and gas from glucose cellebios, manhtal, ramnose, and urease tests. Also, they showed positive reaction to motility, casin hydrolysis. esculin hydrolysis positive, levan production positive, negative in urease, voges proskauer test and gelatine liquefaction, starch hydrolysis, H_2S production, mythyle red test, esculin hydrolysis, and casin hydrolysis. Isolated BR5 had the same morphological and biochemical characterization of the previous strains except that they showed negative reaction to glucose and they were identified as *P. aeruginosa*. Isolate no. Isolates RB3 and RB4 were identified as *B. subtilis* 1 and 2 were rode-shape, motile, sporing, Gram postive, urease postive, and postive in gelatine liquefaction, starch hydrolysis, H_2S production, mythyle red test, vogesproskauer, esculin hydrolysis, levan production and casin hydrolysis.

Table 2. Morphological and physiological characteristics of bacterial isolates.

Isolate No.	RB1	RB2	RB3	RB4	RR5
Shape of cell	Rod	Rod	Rod	Rod	Rod
Motility	-	-	+	+	-
Sporulation	-	-	+	+	-
Gram staining	-	-	+	+	-
Starch hydrolysis	-	-	+	+	-
Urease	-	-	+	+	-
Esculin hydrolysis	-	-	+	+	-
H_2S production	-	-	-	-	-
Levan production	+	+	+	+	-
Methyl-red test (MR)	-	-	-	-	
Voges-proskauer test (VP)	+	+	+	+	+
Casein hydrolysis	+	+	+	+	-

Table 3. Fermentation of carbon compounds by the bacterial isolates.

Isolates		RB1	RB2	RB3	RB4	RR5
Glucose	Acid	+	+	+	+	+
	Gas	-	-	-	-	-
Sucrose	Acid	+	-	+	-	-
	Gas	-	-	-	-	-
Lactose	Acid	+	-	+	+	-
	Gas	-	-	-	-	-
Maltose	Acid	+	+	+	+	+
	Gas	-	-	+	+	-
Manitol	Acid	+	+	+	-	+
	Gas	-	-	-	-	-
Xylose	Acid	+	+	+	+	+
	Gas	-	-	-	-	-
Cellobiose	Acid	+	+	-	+	+
	Gas	-	-	-	-	-
Dextrin	Acid	+	+	+	+	+
	Gas	-	-	-	-	-
Rahminose	Acid	+	+	+	+	+
	Gas	-	-	-	-	-

Effect of treatments with bioagents on % infection and disease severity of *Fusarium* spp. under greenhouse conditions in 2015: All bacterial isolates tested in a suspension form gave a positive decrease on percentage of infection against the three pathogenic fungi of black cumin root rot.

Data Figs. 2A, B indicate that application of isolates (PGPR) *P. fluorescens* No. 2, *B. simplex* No. 2, *P. aeruginosa* No. 1, *P. fluorescens* No. 1, and *P. simplex* No. 1 gave the less rate of percentage of infection (31.67, 33.33, 34.58, 36.67 and 38.58) respectively, but without significant difference between them. All the treatments were gave the medium effect except treatments *P. fluorescens* No.2/ *F. solani* and *B. simplex* No.2/*F. comptoceras* it exhibited highest rate percentage of inhibition compared with infected control (47.50).

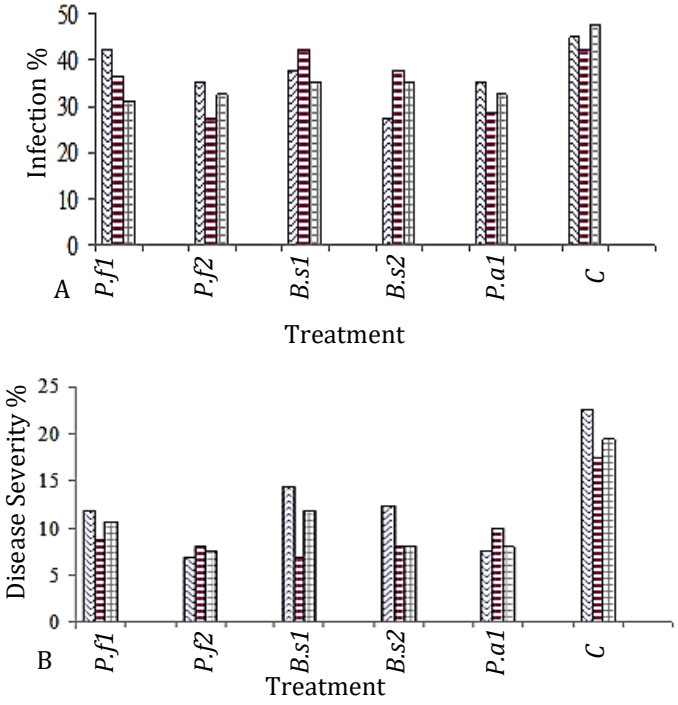


Figure 2 A, B. Effect of treatments with different bioagents as suspension on % infection and disease severity of *Fusarium* spp. under greenhouse conditions in 2015 season.

Application of the isolates PGPR *B. simplex* No. 1, *P. fluorescens* No. 1, *B. simplex* No. 2, *P. fluorescens* No. 2 and *P. aeruginosa* No. 1 reduced the severity of disease (11.04, 10.42, 9.54, 8.54 and 7.5%) respectively compared with infected control (19.79%). Results also, show that all treatments reduced the disease severity of all tested pathogenic fungi compared to infected control without significant between them except in case *B. simplex* No.1/ *F. comptoceras*. Treatment with *B. simplex* No.1 with *F. solani*, *P. fluorescens* No.2 with *F. comptoceras* exhibited the best reduction of the disease followed by *P. fluorescens* No.2 with *F. lateritium* and *P. aeruginosa* No.1 with *F. comptoceras*.

Effect of treatments with different bioagents on number of colony fungi of *Fusarium* spp. under greenhouse conditions in 2015 season: Data Figure 3 show that seeds black cumin treated by bioagent as a suspension gave positive effect in reducing the number of colony fungi compared to infected control. All bioagents *P. fluorescens* No. 1, *B. simplex* No. 1, *B. simplex* No. 2, *P.* aeruginosaNo.1 and *P. fluorescens* No. 2 were reduced the number of *Fusarium* spp. (9.22, 8.55, 8.00, 7.11 and 6.88) respectively compared to infected control (17.56). The treatment *B. simplex* No. 1/*F. lateritium* gave the less effect in the reducing the number of colony fungi (15.33).

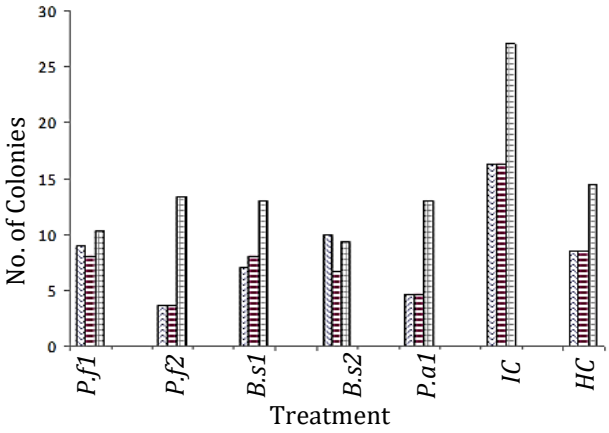


Figure 3. Effect of treatments with different bioagents on number of colony fungi of *Fusarium* spp. under greenhouse conditions in 2015 season.

Effect of treatments with different bioagents as suspension of root and foliar fresh weigh of black cumin after inoculated with *Fusarium* spp. under greenhouse conditions 2015 season: The black seeds treated with the tested bacterial isolates gave the highest vegetable weight (root and foliar) of the plant under greenhouse better than control plant.

Root Fresh Weight: Data in Figure 4A show that seeds black cumin plant treated with isolate *P. fluorescens*No.1 gave the highest root fresh weight in the rate of (4.07) while other isolates have no significant difference.

Treatments by *B. simplex* No.1/*F. solani, P. fluorescens* No.1/ *F. lateritium* and *P. fluorescens*No.1/ *F. solani* and gave the highest fresh weight of the root (4.60, 4.52 and 4.35) and and the treatment by *B. simplex* No.2/ *F. comptoceras* gave the lowest root fresh weight (1.90).

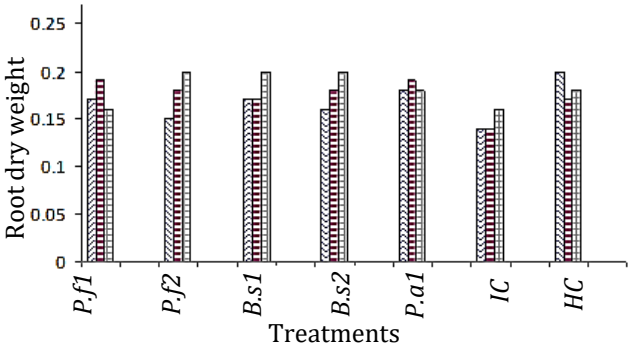


Figure 4A. Effect of treatments with different bioagents suspension on root dry weight of black cumin after inoculated by *Fusarium* spp. under greenhouse conditions 2015 growing season**.**

Foliar Fresh Weight: Data in Figure 4B show that seeds black cumin plant treated by isolate *P. aeruginosa*No.1 as a suspension gave the lowest foliarfresh weight (21.69). While the other isolates significant increased the foliar fresh weight compared to infected control (15.00). Results show that treatments by *B. simplex* No. 2/ *F. solani, B. simplex* No.1/*F. solani, P. fluorescens* No.2/ *F. solani* and *P. fluorescens* No.1/ *F. solani* No.1 gave the highest fresh weight of the foliar (35.70, 34.35, 31.35 and 29.77).

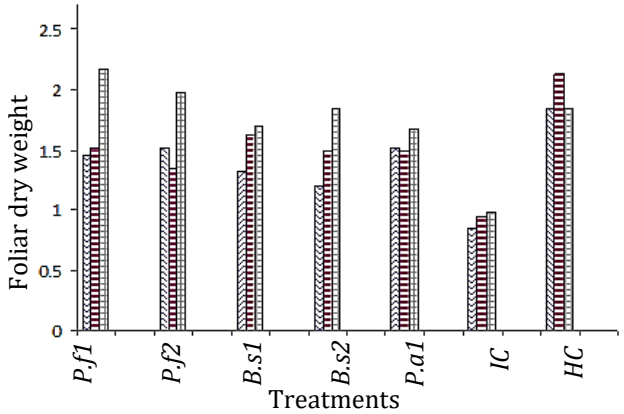
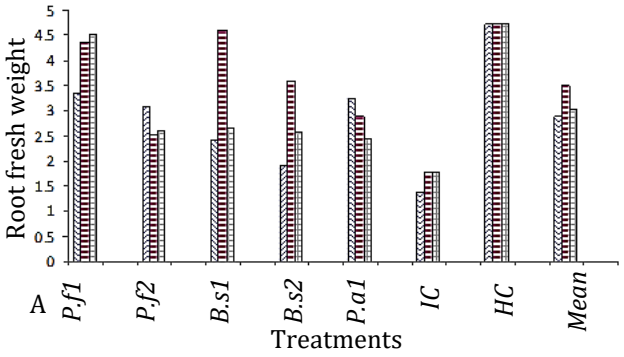


Figure 4B. Effect of treatments with different bioagents suspension on foliar dry weight of black cumin after inoculated by *Fusarium* spp. under greenhouse conditions 2015 growing season**.**

Effect of treatments with different bioagents on suspension on root and foliar dry weight growth of black cumin after inoculated by *fusarium* spp. under greenhouse conditions in 2015 season

Root dry weight: Data in Figure 5A showed that seeds black cumin plant treated with all bioagents as a suspension significantly increased the root dry weigh and foliar dry weigh compared to infected control. Application of all bacterial isolates gave positive effect on root dry weight and no significant differences between isolates *P aeruginosa* No. 1, *P. aeruginosa* No. 2, *B. simplex* No. 1, *B. simplex* No. 2 and *P. aeruginosa* No. 1 (0.18) compared to infected control in the rate of (0.15) and health control (0.18).

Foliar dry weight: Data in Figure 5B show that seeds black cumin plant treated by bioagent isolate as a suspension *P. fluorescens* No. 1and *P. fluorescens* No. 2 gave highest foliar dry weight (1.71 and 1.66) respectively followed by isolates *P. aeruginosa* No. 1, *B. simplex* No. 1 and *B. simplex* No. 2 (1.56, 1.55 and 1.52) compared to infected control (0.93).



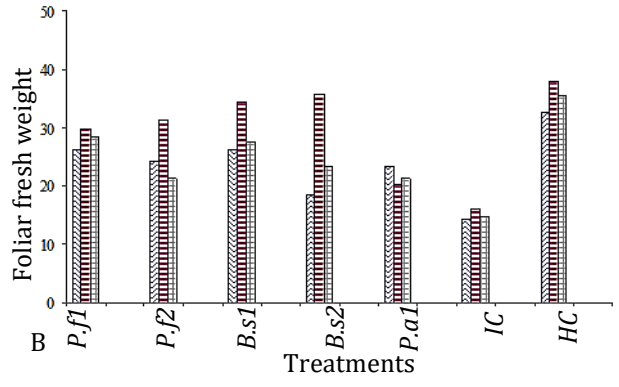


Figure 5 A,B. Effect of treatments with different bioagents suspension on root and foliar fresh weight of black cumin after inoculated by *Fusarium* spp. under greenhouse conditions 2015 growing season.

Results also, show that treatments by *P. fluorescens* No. 1/ *F. lateritium* and *B. simplex* No. 2/ *F. lateritium* gave the highest total root dry weight without any significant difference between them. The treatments *B. simplex* No. 2/ *F. comptoceras* and *B. simplex* No.1/ *F. comptoceras* gave the lowest effect on dry weight to the root (1.20 and 1.32).

DISCUSSION

Fusarium root rot of black cumin caused by *F. comptoceras, F. solani* and *F. lateritium* was detected widely in black cumin fields located in Assiut governorate provinces. The pathogen was observed to cause typical root rot symptoms including a drooping and yellowing of the leaves, browning of underground plant parts and eventual death of the plant in infected black cumin fields. Root rot of black cumin, among the major diseases that caused by a soil and seed borne vascular wilt pathogen *Fusarium* sp. Koike, (2005).

Eight fungal isolates were isolated from infected root of black cumin plants fungal isolates were identified by using the morphological features of mycelia and spores as described by Domsch *et al*. (1980). Pathogenic potentialities of the tested isolates revealed that all tested fungal isolates were able to infect black cumin plants cultivar caused root disease with different degrees of severity. Such results are in agreement with those obtained by Goksel and Harun (2015).

The isolates identified *as F. comptoceras, F. solani*and *F. lateritium* were highly pathogenic and caused disease root rot black cumin symptoms including stunted growth, root rot and death of the plant. These results are consistent with in previous studies, *F. solani* and *F. equiseti* were reported to be pathogenic to cumin plant (Reuveni 1982; Mohammadi and Mofrad, 2009; Ramchandra and Bhatt, 2012).

Biological control was found to be an attractive alternate strategy for control of soil-borne disease as well as it was useful in reducing harmful side effect of pesticides on environment (Cook and Baker, 1983; Seleim *et al.,* 2011; Hoda El-Hendawy and Abo-Elyousr 2016). *In vitro* study, the test of the 15 isolates of bacteria against the growth of high pathogenic isolates of *F. comptosiras, F. solani* and *F. lertritum*showed that all test isolates (PGPR) inhibit the growth of disease causing varying degrees of inhibition. *Pseudomonas fluorescens* No.1 and *P. fluorescens* No.2, *Bacillus simplex* No.1, *B. simplex* No.2 and *P. aeruginosa* No.1 (5.01). The least was growth inhibition No.13. (2.44) these results agree with those reported by Zdravković *et al*. (2015).

Antagonistic effect might be due to direct influence of (PGPR) against pathogens on the interaction between *P. fluorescens* on *F. comptoceras* revealed that the Pf caused complete mycoparasitism on the fungal growth. The bacterial growth was seen adhering and colonizing the hyphae, thus leading to maceration of hyphal tissues. The isolates *Bacillus simplex* No.1 and *Bacillus simplex* No.2 gave the highest inhibitory rate on *Fusarium* sp. these results correspond to Tawfik, Azza and Allam (2004) they mentioned that *Pseudomonas fluorescens* is one such proven biological control agent. Many success reports by several scientists around the world have described different Pseudomonas strains able to significantly control a number of fungal, bacterial and nematode diseases in cereals, horticultural crops, oil seeds and others. The efficacy of bacterial antagonism in controlling diseases was often better than with fungicide fungicides *Pseudomonas* spp. is a well-known plant growth promoting bacteria. Our results suggest that all *Pseudomonas* strains produced normal PGPR activity of IAA, siderophore and P-solubilization, some plant growth promoting rhizobacteria effectively colonize in rhizosphere and improves the plant growth. Recently, Deshwal and Kumar (2013) mentioned that *Pseudomonas* strains effectively produced PGPR activity under stress condition, also able to produce antibiotics, bacilysin, iturin and mycosubtilin and siderophores which are suppressing fungal spores germination Shoda, (2000).

The black seeds treated with the tested bacterial PGPR isolates stimulated plant growth promotion under greenhouse better than control plant without any treatment. Growth enhancement by biological control methods has been reported in other crop species. Bacteria may play a role in the nitrogen fixation. These results are agreement with those reported by several researches (Chang *et al*., 1986; Linderman, 1994; Zhang *et al*., 1996; Dashti *et al*., 1998 and Kumar *et al*., 2001). Or may be due to the output Phytohormone Idris *et al*. (2004).

Results showed seeds black cumin treated by bioagent as a suspension gave positive effect in reducing the number fungi compared with infected control. *P. fluorescens* No.1, *B. simplex* No.1, *B. simplex* No.2 and *P. aeruginosa* No.1 as a suspension reduced the number of pathogens fungi compared with infected control, whereas *P. fluorescens* No.2 had the lowest effect in reducing the number of fungi.

In conclusion, our study confirmed that used of bioagents

may be applied as future ecofriendly alternatives to synthetic fungicides for controlling the disease of black cumin.

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