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Evaluation of the fungal activity of *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces lilacinus* as biocontrol agents against root-knot nematode, *Meloidogyne incognita* on cowpea

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Abstract

Background: In the current years, nematotoxic or antagonistic compounds for example, toxins, enzymes, or compounds derived from the metabolites of fungal culture filtrates have greatly increased.

Objective: This research was designed to evaluate two fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, commonly used only as bio-insecticides in Egypt for their nematicidal potential compared to *Paecilomyces lilacinus*, one of the most important fungi parasitizing on eggs of root-knot nematode, *Meloidogyne incognita*.

Results: The tested fungi either as filtrate or spore affected egg hatching and survival of second stage juveniles at different degrees according to fungal filtrate dilution and spore concentration and exposure period under in vitro study. Under screen house conditions, the tested fungi as filtrates or spores were used to control root-knot nematode on cowpea. The overtopped significant results were gained with *P. lilacinus* filtrate at standard dilution and recorded the highest mean overall percentages nematode reduction (84.5%). The second rank was obtained by *B. bassiana* culture filtrate, where it significantly reduced all nematode numbers with a mean of 81.1% at standard dilution. *M. anisopliae* caused 78.5% as a mean percentages nematode reduction followed by other dilutions and untreated check. When using spore concentrations, the overtopped significant results were gained with *P. lilacinus* at the highest spore concentration (1×10^8) and recorded the highest mean percentages nematode reduction (85.3%). The second rank was obtained by *M. anisopliae*, where it reduced all nematode numbers as an average of 83.6%. *B. bassiana* caused 77.1% as a mean percentages nematode reduction at the highest spore concentration. At all cases, all treatments significantly promoted plant growth and yield criteria and these increases were positively proportional to the filtrate dilution or spore concentration higher than the untreated plants.

Conclusions: It can be concluded that *B. bassiana*, *M. Anisopliae*, and *P. lilacinus* as antagonistic fungi proved to be efficient against root-knot nematode, *incognita* infecting cowpea as they reduced nematode criteria which subsequently improved plant growth and yield of cowpea.

Keywords: *Beauveria bassiana*, Cowpea, Fungal bioagents, In vitro, In vivo, Meloidogyne incognita, Metarhizium anisopliae, Paecilomyces lilacinus

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Background

Biological control of nematodes is one of the most important approaches in nematode management directed towards a sustainable agriculture (Mokhtari et al. 2009). Some soil inhabiting fungi have ability to controlling the nematodes (Tian et al. 2007). Endophytic entomopathogens are known to colonize several horticultural and agronomic crops, providing protection from herbivore damage and also regulating insect populations (Vianna et al. 2018). As fungi cohabit together with plant-parasitic nematodes in the rhizosphere, their toxic metabolites may keep a low level of nematode populations (Kerry 2000). The search for nematotoxic or antagonistic compounds in fungal culture filtrates has greatly increased in the last years, due to the toxins, enzymes, or compounds derived from their metabolites (Ciancio 1995; Liu et al. 2008). Among these fungi, green muscardine, *Metarhizium anisopliae*, is considered a soil dwelling fungus with entomopathogenic characteristics. The effect of this fungus against reniform nematode, *Rotylenchulus reniformis*, was studied (Tribhuvaneshwar Sharma and Bhargava 2008). Biocontrol potential of *M. anisopliae* against some species of root-knot nematodes was studied by some investigators (Jahanbazian et al. 2014; Khosrawi et al. 2014; Jahanbazian et al. 2015). Ghayedi and Abdollahi (2013) purified the isolated fungus, *Beauveria bassiana*, and showed the biocontrol potential of the isolate on *Heterodera avenae*, with 47.1% of larval mortality and has a suppressive action on nematodes of the genus *Meloidogyne* spp. (Bekanayake and Jayasundar 1994; Caroppo et al. 1990). *B. bassiana* may have more than a single bioactive metabolite with nematicidal activity, and each metabolite may act on a different site. It was shown that *B. bassiana* produces beauvericin and oosporin, and beauvericin proved to have nematicidal activity against *M. incognita* (Hamil et al. 1969; Suzuki et al. 1977; Anke et al. 1995). Little parasitism of nematode eggs by *B. bassiana* was shown by Chen et al. (1996), but it inhibited hatching of *Heterodera glycines*. As reported by Cayrol et al. (1992), that egg-parasitic fungi can infect nematodes, destroying their eggs. Most of these fungi act as saprophytes, and can secondarily invade already-dead eggs. Among these fungi, *P. lilacinus* which is considered probably the most effective egg parasites and has been shown to successfully control root knot nematodes, *M. javanica* and *M. incognita*, on tomato, eggplant, potato, and other vegetable crops (Cayrol et al. 1989; Aboul-Eid and Youssef 1998; Goswami and Mittal 2004; Goswami et al. 2006; Haseeb and Kumar 2006). Nearly, no work was done on the effect of two fungi, *B. bassiana* and *M. anisopliae*, on root knot nematode or other plant-parasitic nematodes in Egypt.

Therefore, this research was designed to evaluate two fungi, *B. bassiana* and *M. anisopliae*, commonly used only as bio-insecticides for their nematicidal potentials against root-knot nematode, *M. incognita*, on cowpea compared to *P. lilacinus* fungus, under screen house conditions.

Methods

Pure culture of root-knot nematode inoculum

M. incognita was the tested species of root knot nematode, identified from nematode adult female on the basis of the morphological characteristics of the female perineal pattern (Taylor and Sasser 1978). Pure culture of *M. incognita* was reared on eggplant cv. Ice in a screen house of Nematology Lab., Plant Pathology Department, National Research Centre at 30 ± 5°C by using a single egg mass of this nematode. Newly hatched second stage juveniles (J_2s) and eggs were used as inocula.

Fungus culture

Isolates of *B. bassiana*, *M. anisopliae*, and *P. lilacinus* were obtained from Assiut University, Mycological Center, Faculty of Science. The isolates were cultured on Sabouraud dextrose yeast agar (SDYA) medium (Sabouraud 1892) which contained 40 g glucose, 20 g peptone, 20 g agar, and 2 g yeast extract in 1000 ml of distilled water in flasks which were autoclaved at 21 °C for 15–20 min.

Preparation of spore suspension

Fungal cultures grown on Sabouraud dextrose yeast agar (SDYA) medium were incubated at 25 ± 2 °C in darkness for 14 days. Conidial medium suspensions were prepared by scraping cultures with a sterile objective glass and transferred to 10 ml of sterile water containing 0.05% Tween 80 in a laminar flow chamber. The conidia were harvested by scraping the surface of the culture with inoculation needle. The mixture (spores+ hyphae) was stirred for 10 min and the hyphae were removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined (1×10^8 viable conidia) by direct count using hemocytometer. Serial dilutions were prepared in distilled water containing 0.1% Tween 80 and preserved at 5 °C until used. In vitro nematode tests were applied to evaluate efficacy of fungal spores against root knot nematode, *M. incognita* eggs. A volume of the adjustable concentrations (1×10^6, 1×10^7, and 1×10^8) viable conidia were directly applied to the eggs.

Preparation of supernatant

The filtrates of *B. bassiana*, *M. anisopliae*, and *P. lilacinus* were produced on broth semi-synthetic Sabouraud dextrose yeast. The medium was prepared and adjusted

at PH (5.5–6.6). After sterilization, flasks were inoculated with the fungal species and incubated for 2 weeks at 25 °C and 50–60% Rh. At the end of the incubation period, the supernatant was separated from the mats by filtration through Whatman filter paper No.1 under aseptic conditions and the supernatant at different dilutions [S (Standard), S/2 and S/] were used for bioassay against nematodes (Barker 1985).

Laboratory tests

In vitro test was carried out to determine the effect of culture filtrates of the studied fungi, *B. bassiana*, *M. anisopliae*, and *P. lilacinus*, at dilutions, S, S/2, and S/4 *M. incognita* egg hatching from infected tomato roots. Eggs were extracted by Clorox (NaOCl 1.0%.), then the suspension was poured onto a 500 mesh sieve and washed by excess tap water to remove NaOCl (Hussey and Barker 1973). Then, extracted eggs were transferred to into clean beaker with sterilized water. One milliliter of distilled water containing 300 nematode eggs was put in plastic capsule with 9 ml of each fungal filtrate dilution. Control treatment was made by adding 1 ml of distilled water containing 300 nematode eggs to 9-ml distilled water as comparison. There were 5 replicates for each treatment.

Also, in vitro test was applied to evaluate efficacy of three conidial spore concentrations from *B. bassiana*, *M. aniisopliae*, and *P. lilacinus* against root knot nematode, *M. incognita* eggs. Concentrations of 1×10^6, 1×10^7, and 1×10^8 viable conidia were directly applied to eggs by adding 1 ml distilled water containing 300 eggs in plastic capsule with 9 ml of each fungal spore's suspension concentration. Equal number of eggs was also transferred to separate plastic capsule containing 9-ml distilled water to serve as control.

Observations on the number of non-hatched eggs by light microscope were made 24, 48, 72, and 96 h after treatment. Data on non-hatched eggs were converted to the percentages of egg inhibition at each period and dilution according to Abbott's formula (Abbott 1925) as follows:

$$\text{Egg inhibition } (\%) = (m\text{–}n)/(100\text{–}n) \times 100$$

where *m* and *n* stand for the percentages of non-hatched eggs in the treatment and control, respectively. Net percentage egg inhibition was calculated by subtracting percentage of recovery (hatched eggs in distilled water) from the percentage inhibition after 96 h.

Mortality of second stage juveniles (J_2)

For determining the effect of fungal filtrates of *B. bassiana*, *M. anisopliae*, and *P. lilacinus* on second stage juvenile mortality (J_2) of *M. incognita*, the number of J_2 in the soil per pot was extracted using a sieving and decanting technique (Barker 1985) and counted. For extraction of second stage juveniles (J_2) of *M. incognita* from roots, galled eggplant roots with egg masses per plant were washed thoroughly with tap water to avoid debris and cut into small pieces. Then, they were placed in plastic capsule containing sufficient water, covered to avoid loss of water by evaporation, and collected every 24 h (Young 1954). The same procedures were carried when 1 ml of distilled water containing 200 J_2 was added to 9 ml of filtrate of each fungus. Control treatment was made by adding 9 ml of distilled water to 1 ml of nematode suspension containing the same number of nematodes.

Number of dead and alive juveniles per each treatment was determined under light microscope 24, 48, and 72 h after treatment. The J_2 were considered dead when they did not move when probing with a fine needle. Data on nematode mortality were converted to the percentages of nematode mortality according to Abbott's formula (Abbott 1925) as follows:

$$\text{Juvenile mortality } (\%) = (m\text{–}n)/(100\text{–}n) \times 100$$

where *m* and *n* are for the percentages of dead juveniles in the treatment and control, respectively. Net percentage of mortality was calculated by subtracting percentage of nematode recovery in distilled water from the percentage of mortality after 72 h.

Screen house experiments

Pot experiment design

The experiment was carried out in pots in screen house of Plant Pathology Department, National Research Centre (NRC). Seeds of cowpea (*Vigna unguiculata* (L.) Walp.) cv. Baladi were sown in each pot in April 5, 2018 in pots (20-cm diameter) containing 2 kg of solarized sandy loamy soil. Each pot was inoculated with 2000 newly hatched juveniles (J_2) + 1000 eggs of *M. incognita* in April 19, 2018. This inoculum was made in four holes made around the plant. At the same time of nematode inoculation, cowpea plants were treated with the tested three cultural filtrates of *B. bassiana*, *M. anisopliae*, and *P. lilacinus*. These fungi were tested at dilutions, S, S/2, and S/4 at the rate of 10 ml per pot from each dilution in four holes around the plant and nematode only with liquid medium (control) used as untreated check. Pots were arranged in a completely randomized design with 5 replicates for each treatment on a bench under screen house conditions maintained at 30 ± 5 °C. Then, the plants were irrigated as needed.

After 3 months of nematode inoculation (harvest stage of cowpea plant) in July 2018, plants of cowpea were carefully uprooted and roots were washed thoroughly

with running tap water to avoid debris. Then, roots were cut into two halves. Numbers of J_2 in soil and roots, egg masses, as well as number of galls per plant were counted in one half of roots. The number of J_2 in the soil per pot was extracted using a sieving and decanting technique (Barker 1985) and counted. Then, the second half of roots was incubated in tap water by incubation method (Young 1954) to help hatching J_2 from egg masses. All J_2 numbers of nematodes were counted under a light microscope.

At the same time, plant growth criteria of cowpea including shoot length (cm), fresh and dry shoot weights (g), and fresh and dry weights of roots (g) were recorded. Also, number and weight of pods (g) were recorded.

Also, in the second experiment, the same procedures were applied except that three concentrations of 1×10^8, 1×10^7, and 1×10^6 spores of the tested fungi at the rate of 10 ml per each concentration were tested. Mean percentages of nematode reduction, plant growth, and yield increases were calculated by dividing sum percentages of all parameters of each treatment/number of these parameters. This measurement was used to compare among treatments within all groups.

Statistical analysis

This experiment has been carried out according to analysis of variance (ANOVA) procedures. Duncan's multiple range test as reported by Snedecor and Cochran (1989) was used for comparing among treatments at 5% level of probability. This was done by Computer Statistical Package (COSTAT) User Manual Version 3.03, Barkley Co.

Results

Laboratory studies

Effect of fungal culture filtrates on egg hatching

Data in Table 1 illustrated that culture filtrates of *B. bassiana*, *M. anisopliae*, and *P. lilacinus* inhibited *M. incognita* egg hatching at each exposure period (24, 48, 72, and 96 h) compared to those of control. Generally, the percentages of hatching gradually increased with time and concentration of filtrate. In other words, the percentages of hatching were maximum at 96 h, but after 24 h, no egg hatching occurred. It was noticed that the highest percentage of net egg inhibition (90.0%) was achieved at S dilution of fungi, *B. bassiana* and *M. anisopliae*, followed by 77.5% mortality occurred by *M. aniisopliae* at S/2 dilution and 77.0% at S/4, 75.0% mortality occurred by *P. lilacinus* at S dilution. The rest dilutions of each fungus recorded less percentages of egg inhibition, whereas the least percentage of egg inhibition was recorded by S/4 dilution.

Effect of fungal spores on egg hatching

Data in Table 2 illustrated that spore concentrations of *B. bassiana*, *M. anisopliae*, and *P. lilacinus* inhibited *M. incognita* egg hatching at each exposure period (24, 48, 72, and 96 h) compared to those of control. Generally, the percentages of hatching gradually increased with time and concentration of spores. In other words, the percentages of hatching were maximum at 96 h, but after 24 h, no egg hatching occurred. It was noticed that the highest percentage of net egg inhibition (42.5%) was achieved at S dilution of spores of fungus, *P. lilacinus*, followed by 37.5% occurred by *M. anisopliae* at S dilution, and 35.0% by *B. bassiana* at S dilution. The rest of dilutions of each fungus recorded less percentages of egg inhibition, whereas the least percentage of egg inhibition was recorded by S/4 dilution.

Effect of fungal culture filtrates on mortality of nematode juveniles

Data in Table 3 illustrated that culture filtrates of *B. bassiana*, *M. anisopliae*, and *P. lilacinus* inhibited *M. incognita* juveniles at each exposure period (24, 48, and 72 h)

Table 1 Percentages of egg inhibition of root knot nematode, *Meloidogyne incognita*, as influenced by three culture filtrate dilutions from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus* after 24, 48, 72, and 96 h exposure

Treatments	Dilution	% egg inhibition				% recovery	% net inhibition
		24 h	48 h	72 h	96 h		
Beauveria bassiana	S	00.0	77.5	90.0	91.7	1.7	90.0
	S/2	00.0	75.0	80.0	85.6	10.6	75.0
	S/4	00.0	62.5	67.5	70.0	5.0	65.0
Metarhiziam aniisopliae	S	00.0	82.5	92.5	95.0	5.0	90.0
	S/2	00.0	80.0	85.0	87.5	10.0	77.5
	S/4	00.0	75.0	77.5	80.0	3.0	77.0
Paecilomyces lilacinus	S	00.0	70.0	80.0	85.0	10.0	75.0
	S/2	00.0	65.0	67.0	72.5	2.5	70.0
	S/4	00.0	55.0	62.5	65.0	00.0	65.0
Distilled water (control)	–	00.0	00.0	00.0	00.0	00.0	00.0

Table 2 Percentages of egg inhibition of root-knot nematode, *Meloidogyne incognita*, as influenced by three spore concentrations from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus* after 24, 48, 72, and 96 h exposure

Treatments	Concentration	% egg inhibition				% recovery	% net inhibition
		24 h	48 h	72 h	96 h		
Beauveria bassiana	1×10^8	0	60.0	61.5	63.0	28.0	35.0
	1×10^7	0	47.0	49.0	50.0	24.5	25.5
	1×10^6	0	40.0	42.5	44.0	26.0	18.0
Metarhiziam aniisopliae	1×10^8	0	62.5	65.0	67.0	29.5	37.5
	1×10^7	0	57.5	60.5	62.5	30.5	22.0
	1×10^6	0	45.0	47.5	50.0	30.0	20.0
Paecilomyces lilacinus	1×10^8	0	65.0	72.5	80.0	37.5	42.5
	1×10^7	0	57.0	60.0	67.0	32.0	35.0
	1×10^6	0	47.5	50.0	72.0	44.5	27.5
Distilled water (control)	–	0	00.0	00.0	00.0	00.0	00.0

compared to those of control. Generally, the percentages of mortality gradually increased with time and dilution of filtrate. In other words, the percentages of mortality were maximum at 96 h. It was noticed that the highest percentage net juvenile mortality (100.0%) was achieved at S dilution of fungus, *M. aniisopliae*, followed by 97 and 95% occurred by the same fungus at S/2 and S/4, respectively. This followed by fungus *P. lilacinus* caused 76% mortality at S dilution. The rest of dilutions of each fungus tested recorded less percentages of egg inhibition, whereas the least percentage of egg inhibition was recorded by S/4 dilution.

Screen house experiment

Effect of the tested fungal culture filtrates on root-knot nematode

Tables 4 and 5 show that three culture filtrates from *B. bassiana*, *M. anisopliae*, and *P. lilacinus* were selected for their efficacy to control *M. incognita* infecting cowpea. Number of nematode juveniles in soil and roots, egg masses, as well as number of galls and number of bacterial nodules were significantly increased compared to untreated check (Table 4). In general, on the basis of mean total percentages nematode reduction, data in Table 5 indicated that all chosen fungal culture filtrates had suppressed the previous criteria according to fungus and filtrate dilution compared to untreated check. The overtopped significant results were gained with *P. lilicanus* at S dilution which recorded the highest mean nematode reduction (84.5%) with the highest reduction of number of egg masses (84.2%) and higher percentage reduction in soil (86.4%) and roots (82.9%). The second rank was obtained by *B. bassiana* culture filtrate, where it significantly reduced all nematode numbers as a mean of 81.1% at S dilution with the highest percentages reduction of number of juveniles in roots (85.7%.) and number of second stage juveniles in soil (86.4%) at the same dilution. *M. anisopliae* caused 78.5% as a mean percentage of nematode reduction followed by other dilutions and untreated check.

Also, the percentages of reductions of galls were significantly reduced by 77.3% caused by *P. lilacinus* at S dilution followed by *B. bassiana* (75.8%) and *M. aniisopliae* (69.7%) at the same dilution compared to other treatments. On the other hand, control treatment (untreated infected plants) registered the highest numbers of reproductive parameters of nematode and galls of root knot nematode.

Number of bacterial nodules significantly increased by 72.4% and 65.5% caused by *B. bassiana* at S and S/2 dilutions, respectively. These were followed by 62.1 and 51.7% occurred by *P. lilacinus* at S and S/2, respectively. Percentages of increases 44.8 and 34.5% were achieved by *M. anisopliae* at S and S/2 dilutions, respectively. S/4 recorded the least ones.

Table 3 Effects of three culture filtrate dilutions from *Beauveria bassiana*, *Metarhiziam aniisopliae* and *Paecilomyces lilacinus* on the mortality of second-stage juveniles of *Meloidogyne incognita* under in vitro test

Treatments	Dilution	% mortality			% recovery	% net mortality
		24 h	48 h	72 h		
Beauveria bassiana	S	89	94	95	25	70
	S/2	85	91	95	20	75
	S/4	78	80	82	17	65
Metarhiziam aniisopliae	S	94	98	100	00	100
	S/2	91	95	97	00	97
	S/4	82	90	95	00	95
Paecilomyces lilacinus	S	91	93	96	20	76
	S/2	87	90	92	38	54
	S/4	64	68	88	62	26
Distilled water (control)	–	00	00	00	00	00

Table 4 Number of the root-knot nematode, *Meloidogyne incognita* infecting cowpea, number of gall and number of bacterial nodules as affected by three culture filtrates from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus*

Treatments	Dilution	Mean no. of reproductive parameters of nematode			Mean no. of galls/ root system	Mean no. of bacterial nodules/ root system
		J_2s in soil/pot	J_2s in roots/root system	Egg masses/root system		
Beauveria bassiana	S	1500ef	50e	11ef	16de	50a
	S/2	2200cd	60e	13def	18de	48ab
	S/4	4000b	110c	18bc	26c	36e
Metarhiziam aniisopliae	S	1300f	83d	11ef	20de	42cd
	S/2	2000de	128c	14de	21d	39de
	S/4	2700c	150b	20b	32b	35e
Paecilomyces lilacinus	S	1500ef	60e	6g	15e	47abc
	S/2	2500cd	80d	10f	17de	44bcd
	S/4	4200b	80d	15cd	20de	38de
Untreated (control)	–	11,000a	350a	38a	66a	29f

Means followed by different letter(s) are significantly different at $P \leq 0.05$

Effect of the tested fungal culture filtrates on cowpea plant growth and yield

Concerning cowpea plant growth, a significant augmentation of shoot length and fresh and dry weights and root fresh and dry weights and number and weight of pods as influenced by the tested filtrates of *B. bassiana*, *M. anisopliae*, and *P. lilicanus* were illustrated in Table 6. The treatments significantly promoted plant growth and yield criteria and these increases were positively proportional to the filtrate dilution higher than the untreated plants. These treatments can be ranked in descending order as follows: *M. anisopliae* > *P. lilacinus* > *B. bassiana* > as they achieved the highest mean percentages of increases of plant growth and yield by 68.5, 66.0, and 48.0%, respectively at the highest dilution compared to other treatments and untreated check. As for weight of pods, its highest increase was achieved by *P. lilicanus* (86.2%) followed by *M. anisopliae* (55.2%). Other treatments differed in their responses according to fungus and dilution tested. The least plant growth and yield increases were recorded by the least dilution (Table 7).

Effect of the tested fungal spores on root-knot nematode

Tables 8 and 9 show that three conidial spore concentrations from *B. bassiana*, *M. anisopliae*, and *P. lilacinus*

Table 5 Percentages of reduction of number of the root-knot nematode, *Meloidogyne incognita* infecting cowpea, number of galls and percentages of increase in number of bacterial nodules as affected by three culture filtrates from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus*

Treatments	Dilution	% reductions in no. of nematodes			% mean total percentages nematode reduction	% reduction in no. of galls	% increases in no. of bacterial nodules
		J_2s in soil	J_2s in roots	Egg masses			
Beauveria bassiana	S	86.4	85.7	71.1	81.1	75.8	72.4
	S/2	80.0	82.9	65.8	76.2	72.7	65.5
	S/4	63.6	68.8	52.6	61.7	60.6	24.1
Metarhiziam aniisopliae	S	88.2	76.3	71.1	78.5	69.7	44.8
	S/2	81.8	63.4	63.2	69.5	68.2	34.5
	S/4	75.5	57.1	47.4	60.0	51.5	17.1
Paecilomyces lilacinus	S	86.4	82.9	84.2	84.5	77.3	62.1
	S/2	77.3	77.1	73.7	76.0	74.2	51.7
	S/4	61.8	77.1	60.5	66.5	69.7	31.0
Untreated (control)	–	00.0	00.0	00.0	00.0	00.0	00.0

Table 6 Number of root-knot nematode, *Meloidogyne incognita* infecting cowpea, number of galls and number of nodules as affected by three spore concentrations from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus*

Treatments	Dilution	Mean no. of reproductive parameters of nematode			Mean No. of galls/ root system	Mean no. of nodules/ root system
		J_2s in soil/pot	J_2s in roots/root system	Egg masses/root system		
Beauveria bassiana	1×10^8	1000 g	98cde	12bc	22bc	35de
	1×10^7	2500d	113bc	15b	24b	33de
	1×10^6	4050b	120b	16b	27b	32de
Metarhiziam aniisopliae	1×10^8	800 h	55 g	10 cd	17 cd	50a
	1×10^7	2200e	75f	13bc	18 cd	46ab
	1×10^6	3500c	75f	15b	25b	34de
Paecilomyces lilacinus	1×10^8	500i	83ef	6d	11e	44abc
	1×10^7	600i	90def	7d	15de	39bcd
	1×10^6	1750f	105bcd	12bc	22bc	35de
Untreated (control)	–	11000a	350a	38a	66a	29e

Means followed by different letter(s) are significantly different at $P \leq 0.05$

were selected for their efficacy to control *M. incognita* infecting cowpea plant. Numbers of nematode juveniles in soil and roots, egg masses, as well as number of galls in roots and number of bacterial nodules were used as indicators for the efficacy of the tested fungi compared to untreated check. Table 6 illustrates mean numbers of treatments and untreated check. In general, on the basis of mean total percentages nematode reduction, data in Table 7 indicate that all chosen fungal spore concentrations had significantly suppressed the previous criteria according to fungal hypha and spore concentrations compared to untreated check. The overtopped significant results were gained with *P. lilicanus* at the highest spore concentration (1×8^8) which recorded the highest mean nematode reduction(85.3%) followed by 83.5% at medium concentration(1×8^7) of spore concentration with the highest mean reduction of number of egg masses (84.2%) and J_2 in soil (96.5%). The second rank was obtained by *M. anisopliae* where it reduced all nematode numbers as a mean of 83.6% at the highest spore concentration and with the highest percentage reduction of number of juveniles in roots (84.3%.) at the same spore concentration. *B. bassiana* caused 77.1% as a mean percentage of nematode reduction at the highest spore concentration followed by other concentrations and untreated check.

Table 7 Percentages of reduction of number of the root-knot nematode, *Meloidogyne incognita* infecting cowpea, number of galls and percentages of increase of number of nodules as affected by three spore concentrations from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus*

Treatments	Concentration	% reduction in no. of nematodes			% mean total percentages of nematode reduction	% reduction in no. of galls	% increase in no. of nodules
		J_2s in soil	J_2s in roots	Egg masses			
Beauveria bassiana	1×10^8	90.9	72.0	68.4	77.1	66.7	20.7
	1×10^7	77.3	67.7	60.5	68.5	63.6	13.8
	1×10^6	63.2	65.7	33.3	63.1	59.1	10.3
Metarhiziam aniisopliae	1×10^8	92.7	84.3	73.7	83.6	74.2	72.4
	1×10^7	80.0	78.6	65.8	74.8	72.7	58.6
	1×10^6	68.1	78.6	60.5	69.1	62.1	17.2
Paecilomyces lilacinus	1×10^8	95.5	76.3	84.2	85.3	83.3	51.7
	1×10^7	94.5	74.3	81.6	83.5	77.3	34.5
	1×10^6	84.1	70.0	68.4	74.1	66.7	20.7
Untreated (control)	–	00.0	00.0	00.0	00.0	00.0	00.0

Table 8 Effect of three fungal filtrates from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus* on vegetative parameters and yield of cowpea infected by root-knot nematode, *Meloidogyne incognita*

Treatments	Dilution	Shoot parameters			Root parameters		Pod parameters	
		Length (cm)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	No.	Weight (g)
Beauveria bassiana	S	54.3c	58.4b	11.6c	9.5ab	2.7ab	4ab	3.8de
	S/2	54.2c	55.6c	11.4c	7.1c	2.6abc	4ab	3.1f
	S/4	50.3c	50.9e	10.0d	6.8c	2.4bc	3b	3.0f
Metarhiziam aniisopliae	S	57.5ab	62.1a	13.6b	10.7a	3.0a	5a	4.5b
	S/2	57.0b	58.9b	13.3b	8.9b	2.8ab	5a	4.4bc
	S/4	52.0d	54.1d	9.2d	6.6c	2.6abc	4ab	3.9cde
Paecilomyces lilacinus	S	58.8a	61.4a	16.3a	7.8bc	3.0a	5a	5.4a
	S/2	52.7d	55.7c	14.2b	7.0c	2.4bc	4ab	4.3bcd
	S/4	52.2d	48.2f	10.2d	6.8c	2.1cd	4ab	3.6e
Untreated (control)	–	46.3f	39.2 g	7.0e	5.0d	1.8d	3b	2.9f

Means followed by different letter(s) are significantly different at $P \leq 0.05$

Also, the percentages of reduction of galls were significantly reduced by 83.3% caused by *P. lilacinus* at the highest spore concentrations followed by 77.3% caused by the same fungus at the medium spore concentration. *M. anisopliae* recorded reduction (74.2%) and *B. bassiana* (66.7%) at the highest concentration of spores compared to other treatments. On the other hand, control treatment (untreated infected plants) registered the highest numbers of reproductive parameters and galls of root knot nematode.

Number of bacterial nodules significantly increased by 72.4% caused by *M. aniisopliae* at the highest spore concentration followed by 58.6% at medium concentration. *P. lilacinus* recorded 51.7% increase in the number of bacterial nodules, while *B. bassiana* and other concentrations registered the least ones.

Effect of the tested fungal spores on cowpea plant growth and yield

Concerning cowpea plant growth, a significant augmentation of shoot length, fresh and dry weights, root fresh and dry weights, and number and weight of pods as influenced by the tested fungal spore concentrations of *B. bassiana*, *M. anisopliae*, and *P. lilicanus* was illustrated in Table 10. The treatments significantly promoted plant growth and yield criteria than the untreated plants and these increases were positively proportional to the concentration of spores. These treatments can be ranked in

Table 9 Percentages of increase of vegetative parameters and yield of cowpea infected by root-knot nematode, *Meloidogyne incognita*, as affected by three fungal filtrates from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus*

Treatments	Dilution	% shoot parameter increases			% root weight parameter increases		% pod parameter increases		% mean total percentages of plant growth and yield increases
		Length	Fresh weight	Dry Weight	Fresh	Dry	No.	Weight	
Beauveria bassiana	S	17.3	49.0	65.7	90.0	50.0	33.3	31.0	48.0
	S/2	17.1	41.8	62.9	42.0	44.4	33.3	7.0	34.5
	S/4	9.6	29.8	42.9	36.0	33.3	00.0	3.0	22.1
Metarhiziam aniisopliae	S	24.1	58.4	94.3	114.0	66.7	66.7	55.2	68.5
	S/2	23.1	50.3	90.0	78.0	55.6	66.7	51.7	59.3
	S/4	12.3	38.0	31.4	32.0	44.4	33.3	33.3	36.4
Paecilomyces lilacinus	S	27.0	56.6	103.0	56.0	66.7	66.7	86.2	66.0
	S/2	13.8	42.1	102.8	40.0	33.3	33.3	48.3	44.8
	S/4	12.7	23.0	45.7	36.0	16.7	33.3	24.1	27.4
Untreated (control)	–	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0

Table 10 Effect of three spore concentrations from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus* on vegetative parameters and yield of cowpea infected by root-knot nematode, *Meloidogyne incognita*

Treatments	Concentration	Shoot parameters			Root parameters		Pod parameters	
		Length (cm)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	No.	Weight (g)
Beauveria bassiana	1×10^8	59.0b	63.8b	11.9c	8.9b	3.2a	5a	4.9a
	1×10^7	48.0f	44.7e	9.7f	8.6b	2.7bc	4ab	3.8c
	1×10^6	47.7f	42.8f	7.9h	6.9ef	2.5cd	3b	3.1de
Metarhiziam aniisopliae	1×10^8	55.3c	64.8b	13.1b	10.8a	2.9b	4ab	4.5b
	1×10^7	52.8d	56.9c	11.1d	7.9c	2.6c	3b	3.8c
	1×10^6	50.1e	50.5d	10.1e	7.3d	2.5cd	3b	3.3d
Paecilomyces lilacinus	1×10^8	62.0a	79.6a	17.1a	7.2de	2.5cd	5a	4.5b
	1×10^7	54.5c	50.9d	10.1e	6.8f	2.4 cd	4ab	3.9c
	1×10^6	50.3e	50.0d	8.5g	5.8g	2.2d	3b	3.1de
Untreated (control)	–	46.3f	39.2 g	7.0i	5.0h	1.8e	3b	2.9e

Means followed by different letter(s) are significantly different at $P \leq 0.05$

descending order as follows: *B. bassiana* > *P. lilacinus* > *M. anisopliae* as they achieved the highest mean increases of plant growth and yield by 64.5, 63.7%, and 62.5%, respectively at the highest spore concentration compared to other treatments and untreated check. As for weight of pods, its highest increase was achieved by *B. bassiana* (69.0%) > each of *P. lilacinus* and *M. anisopliae* (55.2%) at the highest spore concentration. Other treatments differed in their responses according to fungus and dilution tested. The least percentage of plant growth and yield increase was recorded by the least spore concentration (Table 11).

Discussion

Bioassay tests proved that the tested fungi either as filtrate or spore affected egg hatching and survival of second stage juveniles at different degrees according to fungal filtrate dilution, spore concentration, and exposure period. The percentages of juvenile mortality and egg inhibition of root-knot nematode were directly proportional with the concentration of culture filtrates of *B. bassiana* which agree with (Zhao et al. 2013). These effects on nematodes may refer to mode of action of *M. anisopliae* conidial spores as they attach to nematode cuticle, germinate, parasitize, directly penetrate, and produce the infective hyphae inside the nematode body as reported by Ghayedi and Abdollahi (2013). Also, some cyclopeptides and destruxins were produced by fungus which may play an important role in its pathogenicity (Kershaw et al. 1999). In accordance, nematode egg hatching inhibition and J_2 mortality of the spore's suspension of *P. lilacinus* produced variable effects on root-knot nematode. The fungus caused 94% reduction in *M. javanica* egg hatching, especially at high concentration of *P. lilacinus* spore's suspension (3000 spores/ml) after 48 h and also killed 57% of *M. javanica* juveniles (J_2) after 72 h as shown by Al Ajrami (2016).

The present results showed that fungal culture filtrates and spores of *B. bassiana*, *M. anisopliae*, and *P. lilacinus* under screen house conditions can significantly reduce nematode reproductive parameters and improve the growth and yield of cowpea plants as well. The effect of Beauveria may due to that it can produce beauvericin and oosporin as beauvericin has an activity against *M. incognita* (Hamil et al. 1969; Suzuki et al. 1977; Anke et al. 1995). The mode of action of *P. lilacinus* against plant parasitic nematodes was explained by many investigations as follows: directed penetration of fungal hyphae to the female cuticle of *M. javanica* as reported by Khan et al. (2006). Whereas, Park et al. (2004) reported that *P. lilacinus* could produce leucino toxin and other nematicidal compounds, destroying the egg embryos of *M. incognita* within 5 days because of simple penetration of the egg cuticle by individual hypha. This may be due to mechanical and/or enzymatic activities resulting in killing juveniles and females of *M. incognita* and *Globodera pallida* (Jatala 1986), deformed eggs in *M. incognita* never matured or hatched (Jatala 1985) and penetration of the fungus through the egg shell of the nematode by serine protease produced by *P. lilacinus* (Bonants et al. 1995; Khan et al. 2004).

The significant results in most cases by using the tested fungi in the present study indicate their higher efficacy as promising bioagents on root-knot nematode and consequently on plant growth and yield of cowpea plants, one of the most important leguminous crops in Egypt.

Our results on using the tested fungi for nematode control can be generalized and carried out on a field scale for controlling root-knot nematode in Egypt. This

Table 11 Percentages of increase of vegetative parameters and yield of cowpea infected by root-knot nematode, *Meloidogyne incognita*, as affected by three spore concentrations from *Beauveria bassiana*, *Metarhiziam aniisopliae*, and *Paecilomyces lilacinus*

Treatments	Concentration	% increases in shoot parameters			% increases in root parameters		% increases in pod parameters		% mean total percentages of plant growth and yield increases
		Length	Fresh weight	Dry weight	Fresh weight	Dry weight	No.	Weight	
Beauveria bassiana	1×10^8	27.4	62.8	70.0	78.0	77.8	66.7	69.0	64.5
	1×10^7	4.0	14.0	38.6	72.0	50.0	33.3	31.0	34.7
	1×10^6	3.0	9.2	12.9	38.0	38.9	00.0	7.0	15.6
Metarhiziam aniisopliae	1×10^8	19.4	65.3	87.1	116.0	61.1	33.3	55.2	62.5
	1×10^7	14.0	45.2	58.6	58.0	44.4	00.0	31.o	35.9
	1×10^6	8.2	28.8	44.3	46.0	38.9	00.0	13.8	25.7
Paecilomyces lilacinus	1×10^8	33.9	103.1	104.4	44.0	38.9	66.7	55.2	63.7
	1×10^7	17.7	29.8	44.3	36.0	33.3	33.3	34.5	32.7
	1×10^6	8.6	27.6	21.4	16.0	22.2	00.0	7.0	14.7
Untreated (control)	–	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0

can be done by producing higher quantities of biomasses from these biogents by rearing the tested fungi in pure cultures in the laboratory (Tawfiq 1997) and applied them in experiments in the field to explore and increase their effects on root-knot and the other most economically important nematodes. Khudhair et al. (2016) proved that *B. Bassiana* isolate (MARD 92) was identified to have endophytic property which enables it to be established within plant tissues and increases its field efficacy in controlling some pests.

Conclusions

It can be concluded that *B. bassiana*, *M. Anisopliae*, and *P. lilacinus* as antagonistic fungi proved to be efficient against root-knot nematode. These fungi reduced *M. incognita* infectivity which subsequently improved plant growth and yield. This effect may be due to either some toxic compounds secreted by the tested fungi or nematode egg deformation by *P. lilacinus*. These results are considered the first report in Egypt because of *B. bassiana* and *M. Anisopliae* were commonly used previously as bio-insecticides only against some insects, but were not used to control nematodes. Further studies are needed to explore the most efficient method by these two bioagents for controlling root-knot nematode on a field scale in different crops.

Abbreviations

ANOVA: Analysis of variance; COSTAT: Computer Statistical Package; S: Standard; J_2: Second stage juveniles; *M*: The percentages non-hatched eggs or dead juveniles in the treatment; *n*: The percentages non-hatched eggs or dead juveniles in the control

Authors' contributions

MMAY suggested the idea and problem, participated in the design of the study, wrote the manuscript, and helped in conducting the experimental work. WMAE carried out the most experimental work, performed statistical analysis, and drafted the manuscript. DEML provided with some literature papers related to the tested fungi, identified, and prepared the fungal extracts tested. All authors read and approved the final manuscript.

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The tested fungi and nematodes are available in Egyptian environment and identified in the laboratory.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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