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 × 10⁸*) 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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In the current years, nematotoxic or antagonistic compounds have greatly increased. This research aimed to evaluate two fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, commonly used as bio-insecticides in Egypt, for their nematicidal potential compared to *Paecilomyces lilacinus*. The tested fungi significantly reduced nematode numbers when used as filtrate or spore. In screen house conditions, *P. lilacinus* filtrate at standard dilution reduced nematode numbers by 84.5%. *B. bassiana* culture filtrate 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. All treatments significantly promoted plant growth and yield criteria.
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; Khorasani 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 bio-active 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 (J2s) 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 × 10^0, 1 × 10^1, and 1 × 10^2) 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/4] 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 containing 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 of 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. anisopliae, and P. lilacinus against root knot nematode, M. incognita eggs. Concentrations of 1 × 10⁶, 1 × 10⁷, and 1 × 10⁸ 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:

\[
\text{Egg inhibition} \% = \frac{(m-n)}{(100-n)} \times 100
\]

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 (I₂) + 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 J2 in soil and roots, egg masses, as well as number of galls per plant were counted in one half of roots. The number of J2 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 J2 from egg masses. All J2 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 $1 \times 10^8$, $1 \times 10^7$, and $1 \times 10^6$ 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. anisopliae* 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*, *Metarhizium anisopliae*, and *Paecilomyces lilacinus* after 24, 48, 72, and 96 h exposure

| Treatments       | Dilution | % egg inhibition | % recovery | % net inhibition |
|------------------|----------|-----------------|------------|-----------------|
| *Beauveria bassiana* | S        | 0.0             | 91.7       | 1.7             | 90.0            |
|                  | S/2      | 0.0             | 85.6       | 10.6            | 75.0            |
|                  | S/4      | 0.0             | 70.0       | 5.0             | 65.0            |
| *Metarhizium anisopliae* | S        | 0.0             | 92.5       | 5.0             | 90.0            |
|                  | S/2      | 0.0             | 87.5       | 10.0            | 77.5            |
|                  | S/4      | 0.0             | 80.0       | 3.0             | 77.0            |
| *Paecilomyces lilacinus* | S        | 0.0             | 85.0       | 10.0            | 75.0            |
|                  | S/2      | 0.0             | 72.5       | 2.5             | 70.0            |
|                  | S/4      | 0.0             | 65.0       | 0.0             | 65.0            |
| Distilled water (control) | –        | 0.0             | 0.0        | 0.0             | 0.0aday

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Table 2 Percentages of egg inhibition of root-knot nematode, *Meloidogyne incognita*, as influenced by three spore concentrations from *Beauveria bassiana*, *Metarhizium anisopliae*, 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*| $1 \times 10^8$ | 0      | 60.0    | 61.5    | 63.0    | 28.0    | 35.0    |            |
|                     | $1 \times 10^7$ | 0      | 47.0    | 49.0    | 50.0    | 24.5    | 25.5    |            |
|                     | $1 \times 10^6$ | 0      | 40.0    | 42.5    | 44.0    | 26.0    | 18.0    |            |
| *Metarhizium anisopliae* | $1 \times 10^8$ | 0      | 62.5    | 65.0    | 67.0    | 29.5    | 37.5    |            |
|                     | $1 \times 10^7$ | 0      | 57.5    | 60.5    | 62.5    | 30.5    | 22.0    |            |
|                     | $1 \times 10^6$ | 0      | 45.0    | 47.5    | 50.0    | 30.0    | 20.0    |            |
| *Paecilomyces lilacinus* | $1 \times 10^8$ | 0      | 65.0    | 72.5    | 80.0    | 37.5    | 42.5    |            |
|                     | $1 \times 10^7$ | 0      | 57.0    | 60.0    | 67.0    | 32.0    | 35.0    |            |
|                     | $1 \times 10^6$ | 0      | 47.5    | 50.0    | 72.0    | 44.5    | 27.5    |            |
| Distilled water (control) | – | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |            |

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. lilacinus* at S dilution which recorded the highest mean nematode reduction (84.5%) with the highest percentages 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. anisopliae* (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.
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. lilacinus* 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. lilacinus* (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 *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces lilacinus* provided significant control of the root-knot nematode, *Meloidogyne incognita*. The highest percentage reductions in the number of nematodes were achieved with *P. lilacinus* spores at the highest dilution (Table 5).
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 × 8⁸) which recorded the highest mean nematode reduction (85.3%) followed by 83.5% at medium concentration (1 × 8⁷) of spore concentration with the highest mean reduction of number of egg masses (84.2%) and J₂ 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 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*, *Metastrongylus anisopliae*, and *Paecilomyces lilacinus*

| Treatments             | Concentration | Mean No. of reproductive parameters of nematode | Mean No. of galls/root system | Mean no. of nodules/root system |
|------------------------|---------------|-----------------------------------------------|-----------------------------|---------------------------------|
|                        |               | J₂s in soil/pot | J₂s in roots/root system | Egg masses/root system          |                                 |
| *Beauveria bassiana*   | 1 × 10⁸       | 1000 g         | 98cde                      | 12bc                           | 22bc                            |
|                        | 1 × 10⁷       | 2500d          | 113bc                      | 15b                            | 24b                             |
|                        | 1 × 10⁶       | 4050b          | 120b                       | 16b                            | 27b                             |
| *Metastrongylus anisopliae* | 1 × 10⁸       | 800 h          | 55 g                       | 10 cd                          | 17 cd                            |
|                        | 1 × 10⁷       | 2200e          | 75f                        | 13bc                           | 18 cd                            |
|                        | 1 × 10⁶       | 3500c          | 75f                        | 15b                            | 25b                             |
| *Paecilomyces lilacinus* | 1 × 10⁸       | 500i           | 83ef                       | 6d                             | 11e                             |
|                        | 1 × 10⁷       | 600i           | 90def                      | 7d                             | 15de                            |
|                        | 1 × 10⁶       | 1750f          | 105bcd                     | 12bc                           | 22bc                            |
| Untreated (control)    | –             | 11000a         | 35oa                       | 38a                            | 66a                             |

Means followed by different letter(s) are significantly different at *P* ≤ 0.05

### 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*, *Metastrongylus anisopliae*, 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 |
|------------------------|---------------|---------------------------------|-----------------------------------------------|----------------------------|------------------------------|
|                        |               | J₂s in soil | J₂s in roots | Egg masses       |                                 |                              |
| *Beauveria bassiana*   | 1 × 10⁸       | 90.9      | 72.0         | 68.4            | 77.1                          | 66.7                          |
|                        | 1 × 10⁷       | 77.3      | 67.7         | 60.5            | 68.5                          | 63.6                          |
|                        | 1 × 10⁶       | 63.2      | 65.7         | 33.3            | 63.1                          | 59.1                          |
| *Metastrongylus anisopliae* | 1 × 10⁸       | 92.7      | 84.3         | 73.7            | 83.6                          | 74.2                          |
|                        | 1 × 10⁷       | 80.0      | 78.6         | 65.8            | 74.8                          | 72.7                          |
|                        | 1 × 10⁶       | 68.1      | 78.6         | 60.5            | 69.1                          | 62.1                          |
| *Paecilomyces lilacinus* | 1 × 10⁸       | 95.5      | 76.3         | 84.2            | 85.3                          | 83.3                          |
|                        | 1 × 10⁷       | 94.5      | 74.3         | 81.6            | 83.5                          | 77.3                          |
|                        | 1 × 10⁶       | 84.1      | 70.0         | 68.4            | 74.1                          | 66.7                          |
| Untreated (control)    | –             | 0.0       | 0.0          | 0.0             | 0.0                           | 0.0                           |
Also, the percentages of reduction of galls were significantly reduced by 83.3% caused by \textit{P. lilacinus} at the highest spore concentrations followed by 77.3% caused by the same fungus at the medium spore concentration. \textit{M. anisopliae} recorded reduction (74.2%) and \textit{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 \textit{M. anisopliae} at the highest spore concentration followed by 58.6% at medium concentration. \textit{P. lilacinus} recorded 51.7% increase in the number of bacterial nodules, while \textit{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 \textit{B. bassiana}, \textit{M. anisopliae}, and \textit{P. lilacinus} 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 8 Effect of Three Fungal Filtrates from \textit{Beauveria bassiana}, \textit{Metarhizium anisopliae}, and \textit{Paecilomyces lilacinus} on Vegetative Parameters and Yield of Cowpea Infected by Root-Knot Nematode, \textit{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) |
| \textit{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      |
| \textit{Metarhizium anisopliae} | 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    |
| \textit{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            | 39.2g          | 7.0e           | 5.0d           | 1.8d           | 3b  | 2.9f      |

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

### Table 9 Percentages of Increase of Vegetative Parameters and Yield of Cowpea Infected by Root-Knot Nematode, \textit{Meloidogyne incognita}, as Affected by Three Fungal Filtrates from \textit{Beauveria bassiana}, \textit{Metarhizium anisopliae}, and \textit{Paecilomyces lilacinus}

| Treatments          | Dilution | % shoot parameter increases | % root parameter increases | % pod parameter increases | % mean total percentages of plant growth and yield increases |
|---------------------|----------|-----------------------------|---------------------------|--------------------------|----------------------------------------------------------|
|                     |          | Length Fresh weight Dry Weight | Fresh Dry | No. Weight |                                     |
| \textit{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 | 0.0 | 3.0 | 22.1 |
| \textit{Metarhizium anisopliae} | 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 |
| \textit{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) | –        | 0.0 0.0 0.0 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
hatching inhibition and J2 mortality of the spore (Kershaw et al. 1999). In accordance, nematode egg which may play an important role in its pathogenicity cyclopeptides and destruxins were produced by fungus reported by Ghayedi and Abdollahi (2013). Also, some produce the infective hyphae inside the nematode body as cuticle, germinate, parasitize, directly penetrate, and pro-
conidial spores as they attach to nematode anisopliae effects on nematodes may refer to mode of action of M. which agree with (Zhao et al. 2013). These ef-
bassiana proportional with the concentration of culture filtrates of B. These 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
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 Ghayed 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 J2 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 (J2) 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.

| 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 × 10⁸       | 59.0b            | 63.8b           | 11.9c         | 8.9b            | 3.2a          | 5a  | 4.9a       |
|                          | 1 × 10⁷       | 48.0f            | 44.7e           | 9.7f          | 8.6b            | 2.7bc         | 4ab | 3.8c       |
|                          | 1 × 10⁶       | 47.7f            | 42.8f           | 7.9h          | 6.9ef           | 2.5cd         | 3b  | 3.1de      |
| Metarhiziam anisopliae   | 1 × 10⁸       | 55.3c            | 64.8b           | 13.1b         | 10.8a           | 2.9b          | 4ab | 4.5b       |
|                          | 1 × 10⁷       | 52.8d            | 56.9c           | 11.1d         | 7.9c            | 2.6c          | 3b  | 3.8c       |
|                          | 1 × 10⁶       | 50.1e            | 50.5d           | 10.1e         | 7.3d            | 2.5cd         | 3b  | 3.3d       |
| Paecilomyces lilacinus   | 1 × 10⁸       | 62.0a            | 79.6a           | 17.1a         | 7.2de           | 2.5cd         | 5a  | 4.5b       |
|                          | 1 × 10⁷       | 54.5c            | 50.9d           | 10.1e         | 6.8f            | 2.4cd         | 4ab | 3.9c       |
|                          | 1 × 10⁶       | 50.3e            | 50.0d           | 8.5g          | 5.8g            | 2.2d          | 3b  | 3.1de      |
| Untreated (control)      | –             | 46.3f            | 39.2g           | 7.0i          | 5.0h            | 1.8e          | 3b  | 2.9e       |

Means followed by different letter(s) are significantly different at P ≤ 0.05.
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; J2: 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 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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### Availability of data and materials

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

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Competing interests

The authors declare that they have no competing interests.

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### 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*, *Metarhizium anisopliae*, 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 | Length | Fresh weight | Dry weight | No. | Weight |
| *Beauveria bassiana*     | 1 × 10⁶       | 27.4   | 62.8        | 70.0       | 78.0   | 77.8        | 66.7      | 69.0 | 64.5    |
|                          | 1 × 10⁷       | 4.0    | 14.0        | 38.6       | 72.0   | 50.0        | 33.3      | 31.0 | 34.7    |
|                          | 1 × 10⁸       | 3.0    | 9.2         | 12.9       | 38.0   | 38.9        | 0.0       | 7.0  | 15.6    |
| *Metarhizium anisopliae* | 1 × 10⁶       | 19.4   | 65.3        | 87.1       | 116.0  | 61.1        | 33.3      | 55.2 | 62.5    |
|                          | 1 × 10⁷       | 14.0   | 45.2        | 58.6       | 58.0   | 44.4        | 0.0       | 31.0 | 35.9    |
|                          | 1 × 10⁸       | 8.2    | 28.8        | 44.3       | 46.0   | 38.9        | 0.0       | 13.8 | 25.7    |
| *Paecilomyces lilacinus* | 1 × 10⁶       | 33.9   | 103.1       | 104.4      | 44.0   | 38.9        | 66.7      | 55.2 | 63.7    |
|                          | 1 × 10⁷       | 17.7   | 29.8        | 44.3       | 36.0   | 33.3        | 33.3      | 34.5 | 32.7    |
|                          | 1 × 10⁸       | 8.6    | 27.6        | 21.4       | 16.0   | 22.2        | 0.0       | 7.0  | 14.7    |
| Untreated (control)      | –             | 0.0    | 0.0         | 0.0        | 0.0    | 0.0         | 0.0       | 0.0  | 0.0     |
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