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Management of root-knot nematode *Meloidogyne incognita* of eggplant using some growth-promoting rhizobacteria and chitosan under greenhouse conditions

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Abstract

Background: Eggplant (*Solanum melongena*) is one of the most popular vegetable crops in Egypt. It is affected by a wide range of pests; prominent among them is root-knot nematode (RKN) *Meloidogyne* spp. which constitutes a major group of plant-parasitic nematodes causing great economic losses worldwide especially in Egypt.

Main body: The present investigation was performed under greenhouse conditions to evaluate the potentials of 3 growth-promoting bacteria, *Azospirillum brasilense*, *Azotobacter chroococcum*, and *Bacillus subtilis*, as well as chitosan for the control of the nematode *Meloidogyne incognita* causes root-knot disease of eggplant cv. Baladi. All treatments reduced ($p \leq 0.05$) the nematode population in soil and roots as well as enhanced the plant growth parameters of eggplant remarkably than the control. The applied treatments varied in their efficacy against the plant nematode infection in correspondence to the time of application. The recorded results demonstrated that maximum reduction in J_2 in soil, egg mass/root, and eggs/egg masses were obtained by treating the soil with *Bacillus subtilis* followed by *Azospirillum brasilense* and *Azotobacter chroococcum*. Also, chitosan resulted in high reduction in root galls compared to control treatment.

Conclusion: The use of plant growth-promoting rhizobacteria, *Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus subtilis*, and chitosan achieved efficient control to *Meloidogyne incognita* and consequently increase eggplant growth parameters under greenhouse conditions. The present results suggested introducing such rhizobacteria in integrated nematode management program.

Keywords: Biocontrol, *Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus subtilis*, Chitosan, Eggplant, *Meloidogyne incognita*, Root-knot

Background

Eggplant (*Solanum melongena*) is one of the most popular vegetable crops of tropical and subtropical countries. Eggplant is affected by a wide range of pests; prominent among them is the root-knot nematode (RKN) *Meloidogyne* spp. which constitutes a major group of plant-parasitic nematodes causing economic losses. Infected plants showed stunting, chlorosis, reduction of water

uptake, nutrients transport lead to yield reduction (Oka et al. 2000). Using synthetic nematicides is the best method of managing this pest (Talavera et al. 2012), but the overuse of these agrochemicals is associated with being hazardous to human health, livestock, plants, and the beneficial soil fauna. This situation imposes a challenge to innovate effective, safe, and cheap alternative control strategies. In this concern, growth-promoting bacteria (rizobacteria) and chitosan were reported to be effective and safe for controlling plant-parasitic nematodes.

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It was reported that beneficial bacteria can compete with nematodes through different types of mechanisms: production of bioactive substances (toxins; metabolites such as protease, chitinase, glucanase, antibiotics, and antimicrobial peptides) which affect directly nematode's egg hatching and nematode mobility, interference with plant nematode recognition, synthesis of enzymes that can modulate plant hormone levels and indirectly by alteration of the root exudates, which make roots less attractive to nematodes and induced systemic resistance against nematode invasion. Furthermore, the bacteria can improve plant growth and productivity through production of growth hormones (IAA, gibberellins, and auxins) or converting unavailable minerals and organic compounds into available forms to plants (Khan et al. 2008). On the other hand, chitosan is a deacetylated and soluble form of chitin elicit plant defenses by increasing the biosynthesis of phytoalexins, callose, lignin, and other phenolic compounds resulted in inducing local and systemic resistance against root-knot nematode (RKN) *Meloidogyne* spp. also is biodegradable and environmentally safe (El-Hadrami et al. 2010). Broadly, because of the activities of such agents which are influenced by the soil biota and host plant factors, it is important to estimate them against the target nematode on the certain crop grown in native soil conditions in order to identify their effectivity.

The present work was carried out to evaluate the suppression effect of 3 rhizobacteria as well as chitosan against *M. incognita* infection and its population of eggplant cv. Baladi. under greenhouse conditions.

Main text

Nematicidal potential of 3 rhizobacteria, viz. *Azospirillum brasilense*, *Azotobacter chroococcum*, and *Bacillus subtilis* in addition to chitosan against the root-knot nematode (RKN); *Meloidogyne incognita* on eggplant cv. Baladi was evaluated. Bacterial source was the Plant Pathology Dept., National Research Centre, Giza, Egypt, where the experiment was conducted in its greenhouse. All the bacterial strains were used as cell suspension at concentration of 1×10^8 cell/ml after Abdel-Kader et al. (2012). Meanwhile, chitosan was used as water solution at concentration of 2% (El-Sayed and Mahdy 2014-15).

Rearing and identification of nematodes

Plastic pots (25-cm diameter) were filled with 2 kg loamy clay natural soil which was left for more than 6 months without cultivation. For nematode inoculum, pure culture of *M. incognita* was established from a single egg mass maintained on tomato plants under greenhouse conditions and the nematode identification was undertaken, using the perineal patterns technique (Taylor and Sasser 1978).

Nematode eggs were extracted by macerating infected tomato roots in 0.5% (v/v) NaClO as described by Hussey and Barker (1973).

Greenhouse experiment

Treatments were designated for each tested agent as follows: (1) 3 days before nematode inoculation, (2) simultaneously with nematode inoculation, and (3) simultaneously and 1 month after the first treatment. Each pot transplanted with 1-month-old eggplant seedling in the center and received 5 ml from each agent's preparation and 2000 *M. incognita* eggs around eggplant root zone. All treatments were replicated 5 times and the untreated plants were served as control. All pots were arranged in a complete randomized design on the bench of the greenhouse watered daily and fertilized with NPK as recommended.

Data recorded

Sixty days after nematode inoculum plants were uprooted and root galls were counted as mean for each treatment. The roots were stained for 15 min in an aqueous solution of Phloxine B stain (0.15 g/l water), then washed with running tap water to remove residual stain and the egg mass was determined (Holbrook et al. 1983). Soils at each treatment were thoroughly mixed to represent the whole sample and the nematodes were extracted using sieving and decanting technique from an aliquot of 250 g soil. Extracted second-stage juveniles (J_2 s) were counted in 1 ml suspension using a counting slide under light microscope and repeated 5 times then calculated as means. Eggs/egg mass was recorded by detaching 10 egg masses from infected roots of each treatment, using a needle and exposed to sodium hypochlorite (2%) for 3 min and released eggs were counted.

Eggplant growth parameters were recorded as root and shoot lengths, fresh and dry shoot weights, and numbers of leaves/plant.

$$\text{Nematode reduction \%} = [C1 - C2/C1] \times 100$$

where C1 = No. of live nematodes juveniles in control and C2 = No. of live nematodes juveniles in treatments

$$\text{Increase \%} = \frac{\text{Eggplant growth parameters in Treatment} - \text{Eggplant growth parameters in control}}{\text{Eggplant growth parameters in control}} \times 100$$

Statistical analysis

Collected data were subjected to the analysis of variance test (ANOVA) as complete randomized design for greenhouse experiment using (Assistant) program and mean values were compared using Duncan (1955) multiple range test.

Results and discussion

Effect of the different treatments on root-knot nematode population

Data presented in Tables 1 and 2 and illustrated in Figs. 1 and 2 revealed the significant ($p \leq 0.05$) suppression effects of the applied agents *A. brasilense*, *A. chroococcum*, *B. subtilis*, and chitosan on *M. incognita* population in terms of number of J_2 s in soil and roots, galls and egg mass per plant, and number of eggs/egg mass as well as improve of the different vegetative eggplant growth parameters. Among all tested agents *B. subtilis* performance was the best for enhancing plant growth characters and suppressing the RKN infection. Also, data in Table 1 demonstrated that soil treated with *B. subtilis* simultaneously and after 1 month from *M. incognita* egg inoculation resulted in announced low number in J_2 in soil as 91 J_2 /kg soil followed by chitosan, *A. chroococcum*, and *A. brasilense* which recorded 119, 140, and 210 J_2 /kg, respectively. Also, treated soil simultaneously with egg inoculation revealed moderate rate counts of J_2 in roots recorded as 29 J_2 in 5 g roots at *B. subtilis* application, followed by 48, 48, and 53 J_2 /in 5 g roots at applied treatments of *A. brasilense*, chitosan, and *A. chroococcum*, in respective order.

Illustrated data in Fig. 1 showed that at soil treated with chitosan 3 days before *M. incognita* eggs inoculation was recorded, a reduction in root galls by 81.4%, followed by 77.7, 62.9, and 22.2% at the treatments of *A. brasilense*, *B. subtilis*, and *A. chroococcum*, respectively. The most prominent effect in reducing egg mass was *B. subtilis* when applied simultaneously plus 1 month after the first treatment recording 95.4% over control, followed by 90.9 and 40.9 with chitosan and *A. chroococcum*, respectively. Meanwhile, the least effect was

calculated (18.2%) and was found at *A. brasilense* treatment over control treatment. Obtained results are in agreement with those of Tian et al. (2007) and Lugtenberg and Kamilova (2009) who reported that the living bacteria in plant rhizosphere (rhizobacteria) are mostly plentiful microorganisms that revealing different modes of action against nematodes, which include production of several antibiotics, enzymes, and toxins; in addition, they can also elevate systemic resistance of plants against nematodes. The present investigation showed that *B. subtilis* was the most prominent bacteria in reducing nematodes population. In a harmony of these results, Kavitha et al. (2012) and Engelbrecht et al. (2018) recorded substantial impact of *Bacillus* spp. as biocontrol agents on plant-parasitic nematodes. Under stressed conditions, these strains have multiple modes of action and a promising ability to sporulate. Furthermore, *B. subtilis* produce cyclic lipopeptides of surfactin and iturin which inhibit egg hatching of *M. incognita* and play an imperative role in increasing the percentage of juvenile mortality (El-Hadrami et al. 2010). In addition, a direct antagonism was also reported by *Bacillus* sp. against several plant-parasitic nematodes, i.e., *Meloidogyne*, *Heterodera*, and *Rotylenchulus* (Kokalis-Burelle et al. 2002, and Li et al. 2005). Also, the present study revealed more effectively of *A. brasilense* for reducing nematode population when applied 3 days before eggs inoculum that was referred to its role as resistance elicitors, which leads to the accumulation of phytoalexin suppress nematode viability (Bashan 1998). Also, plant growth-promoting rhizobacteria strains, e.g., *Pseudomonas fluorescens*, *Azotobacter chroococcum*, and *Azospirillum brasilense* inhibited egg hatching and killed

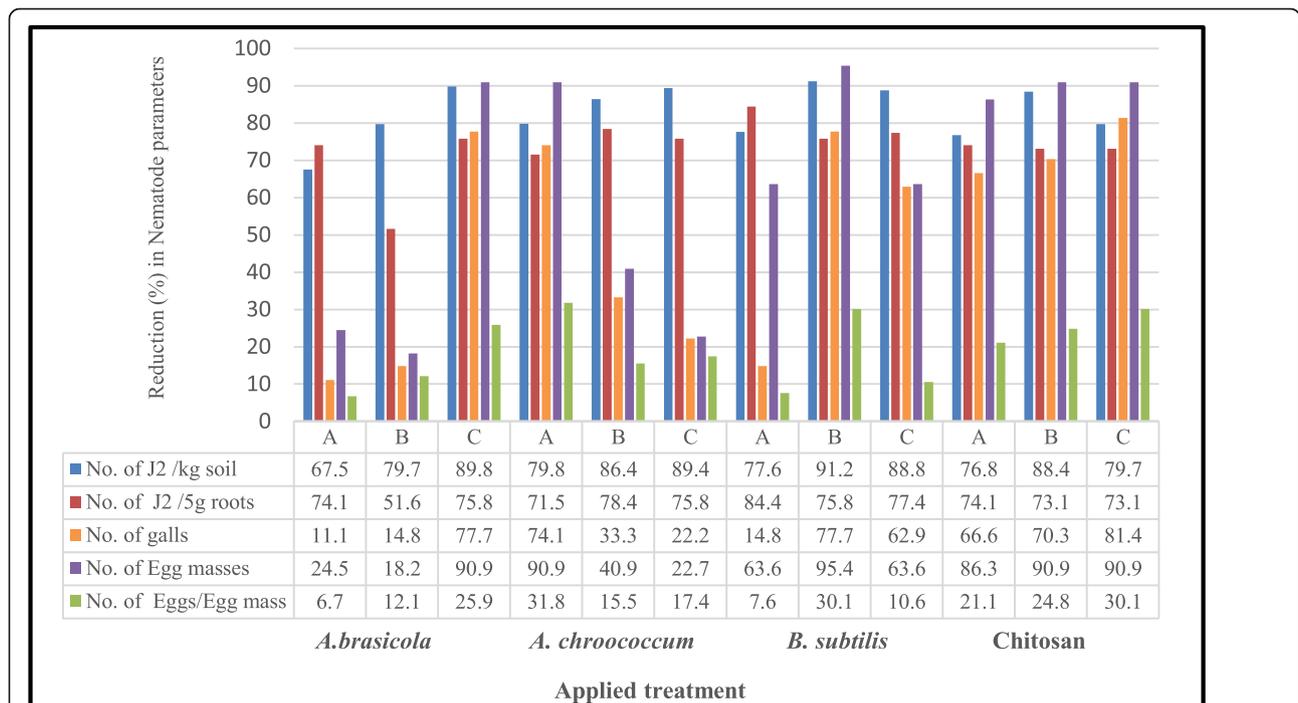
Table 1 Effect of growth-promoting bacteria and chitosan on development and multiplication of root-knot nematode *Meloidogyne incognita*

Treatment	Application time	Measured parameters				
		No. of J_2 /kg soil	No. of J_2 /5 g roots	No. of galls	No. of egg masses	No. of eggs/egg mass
<i>Azospirillum brasilense</i>	A	336 b	48 c	120 b	83 b	427 b
	B	210 d	90 b	115 b	90 b	403 d
	C	105 fg	45 c	30 gh	10 ef	339 g
<i>Azotobacter chroococcum</i>	A	214 d	53 c	35 fg	10 ef	312 h
	B	140 e	40 cd	90 d	65 c	387 e
	C	109 f	45 c	105 c	85 b	378 e
<i>Bacillus subtilis</i>	A	231 c	29 d	45 ef	15 e	423 bc
	B	91 g	45 c	40 ef	10 ef	320 h
	C	115 f	42 c	25 h	10 ef	409 cd
Chitosan	A	240 c	76.8 b	115 bc	40 d	361 f
	B	119 f	88.4 b	30 gh	5 f	344 g
	C	210 d	79.7 b	50 e	40 d	320 h
Untreated control		1035 a	186 a	135 a	110 a	458 a

Table 2 Effect of growth-promoting bacteria and chitosan on plant morphogenesis of eggplants infected with root-knot nematode *Meloidogyne incognita*

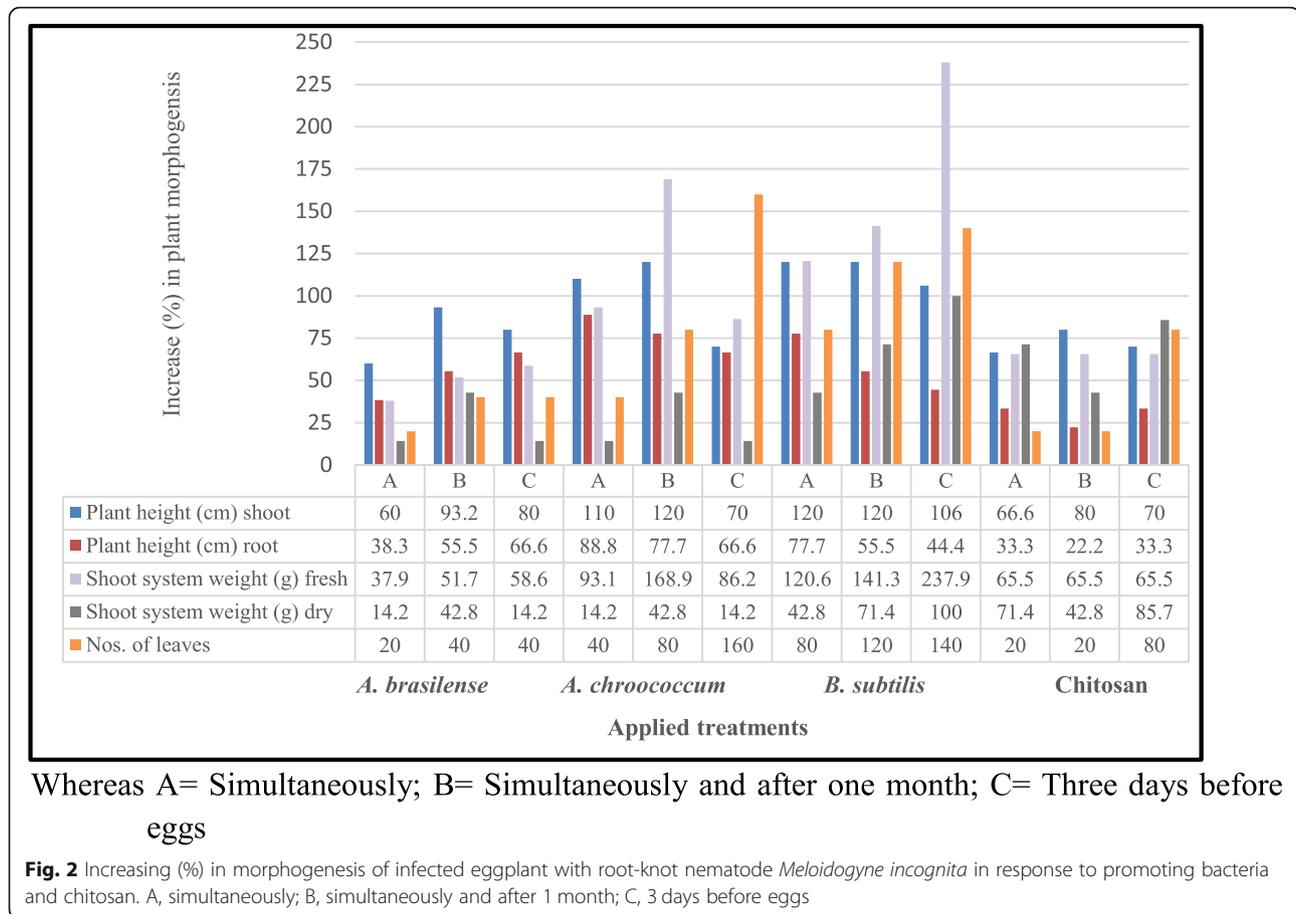
Treatments	Time of application	Plant morphogenesis				
		Plant height (cm)		Shoot system weight (g)		Nos. of leaves
		Shoot	Root	Fresh	Dry	
<i>Azospirillum brasilense</i>	A	24 de	12.5 cd	40 g	8 cd	6 d
	B	29 ab	14 bc	44 fg	10 bc	7 cd
	C	27 bc	15 ab	46 f	8 cd	7 cd
<i>Azotobacter chroococcum</i>	A	31.5 ab	17 a	56 e	8 cd	7 cd
	B	33 a	16 ab	78 b	10 bc	9 bc
	C	25.5 cd	15 ab	54 e	8 cd	13 a
<i>Bacillus subtilis</i>	A	33 a	16 ab	64 d	10 bc	9 bc
	B	33 a	14 bc	70 c	12 ab	11 ab
	C	31 ab	13 cd	98 a	14 a	12 a
Chitosan	A	25 de	12 cd	48 f	12 ab	6 d
	B	27 bc	11 de	48 f	10 bc	6 d
	C	25.5 cd	12 cd	48 f	13 a	9 bc
Control		15 e	9 e	29 h	7 d	5 d

A simultaneously, B simultaneously and after 1 month, C 3 days before eggs



Whereas A=Simultaneously; B=Simultaneously and after one month; C=Three days before eggs

Fig. 1 Reduction (%) in development and multiplication of root-knot nematode *Meloidogyne incognita* in response to growth-promoting bacteria and chitosan. A, simultaneously; B, simultaneously and after 1 month; C, 3 days before eggs



juveniles by producing a wide variety of antibiotics, siderophores, hydrolytic enzymes, organic compounds, HCN, phenol oxidation, and protease (Insunza et al. 2002, and Siddiqui et al. 2005). On the other hand, Chitosan is one of the auspicious agents utilized against plant pathogens as it displays a wide range of antimicrobial activities and shows a hypersensitive response to pathogens infection (Kulikov et al. 2006). Chitosan have revealed antiviral, antibacterial, and antifungal effects and have been suggested for many agricultural uses. Also, it has been utilized for controlling disease or reducing their development and enhancing plant inherent defenses (El-Hadrami et al. 2010). Obtained results of chitosan on *M. incognita* reproduction and eggplant growth in agreement with those of Khalil and Badawy (2012) who demonstrated that chitosan decrease the severity of root-knot disease by inducing physiological alterations in both nematodes and plants that prevent successful reproduction of the nematodes and supply local and systemic resistance (Aboud et al. 2002). Likewise, exhibition elicitor activity by chitosan has been reported through induction of local and systemic resistance mechanisms of tomato plants against *M. incognita* (Radwan et al. 2012).

Effect of different treatments on vegetative eggplant growth parameters

Results presented in Table 2 demonstrated different significant ($P \leq 0.05$) stimulatory effects of the evaluated agents on vegetative eggplant growth parameters in terms of shoot, root lengths, fresh, dry shoot weights, and numbers of leaves relative to different times of application. The highest percent increase in plant height 33.0 cm was achieved by the application of *B. subtilis* simultaneously and simultaneously plus 1 month later, while adding *B. subtilis* 3 days before inoculation resulted in 98 g and 14 g fresh and dry shoot weights and the numbers of leaves calculated as 12, in relevant respective order. Treatments of *A. chroococcum* ranked second for enhancing eggplant growth parameters than the control, followed by *A. brasiliense* and chitosan (Table 2).

Moreover, illustrated data in Fig. 2 revealed the highest calculated increase over control treatment in plant height shoot and root length as 120, 77.7 and 237.9, 100, 140.0% for fresh, weights of shoot system, and numbers of leaves at *B. subtilis* either applied as simultaneously and 3 days before inoculation treatments, in relevant respective order. The significant effects on eggplant

growth given by the rhizobacteria would mainly be due to the increase in root length which in turn increases the mineral uptake in plants, and the production and regulation of phyto-hormones lead to increase of biomass; moreover, these bacteria survive in soil around plants and can provide growth promotion materials and furthermore suppression of disease-causing organisms (Tian et al. 2007; Lugtenberg and Kamilova 2009). Also, Morsy et al. (2009) and Soliman et al. (2011) stated that *A. chroococcum* and *A. brasilense* improve plant growth parameters as well as chemical components by the production of growth hormones (IAA, gibberellins, and auxins) or by converting unavailable minerals and organic compounds into forms available to plants. Furthermore, Morgenstern and Okon (1987) found that *A. brasilense* increased root hair formation and produced more lateral roots which lead to increase the nutrient uptake and improve plant growth.

In the present investigation, the positive effect on eggplant growth parameters was recorded by chitosan. These results are in agreement with Khalil and Badawy (2012) and El-Sayed and Mahdy (2014-15) who revealed that the capability of chitosan to stimulate and manufacture defense compounds and modulate the soil's biological and physical properties which improve plant growth parameters, i.e., root and shoot length and weights compared to the plants treated with nematode alone. Likewise, Ashif et al. (2017) reported the affectivity of chitosan applied to maximize reduction in egg mass/root, eggs/egg mass, nematode population, and root-knot indices in cultivated eggplant compared to the control. They added that application of chitosan increased plant growth, i.e., plant length, fresh and dry weights, pollen fertility, and yield as well as some biochemical parameters such as chlorophyll, carotenoid content, and antioxidant enzymes. They conclude that the eliciting activity of chitosan and activating systemic resistance in the plant as well as during decomposition it release different toxic chemical compounds which have fatal effects against J₂ and multiplication of the RKN, *M. incognita*.

Conclusion

The results concluded that using the plant growth-promoting rhizobacteria, *A. brasilense*, *A. chroococcum*, *B. subtilis*, and chitosan achieved efficient control to the RKN, *M. incognita* with consequent increase in eggplant growth under greenhouse conditions. Such approach could be generally used successfully for controlling plant-parasitic nematode affecting economic crops.

Abbreviations

J₂: Second-stage juvenile; IAA: Indole acetic acid; RKN: Root-knot nematode; A: Simultaneously; B: Simultaneously and after one month; C: Three days before eggs

Acknowledgements

The authors are grateful to Dr. Hoda Hussein Ameen, Department of Plant Pathology, National Research Centre, Egypt, for her guidance throughout this work and manuscript revision.

Authors' contributions

MA suggested the aim of study, designed the experimental work, and wrote and revised the manuscript. NE shared in the experimental design, prepared the bioagents cultures and performed the statistical analysis of the collected data. UE shared in the experimental design, performed, followed the greenhouse work, carried out all the nematode investigations, and collected and tabulated data. All authors read and approved the final manuscript.

Funding

This work was supported in part with the In-House Project No. 11030132 of the National Research Center, Giza, Egypt. This support includes greenhouse, pots, eggplant transplants, and chemical used.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the authors on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that there are no competing interests.

Received: 15 June 2020 Accepted: 21 October 2020

Published online: 04 November 2020

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