Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Integrated management of *Meloidogyne incognita* on tomato using combinations of abamectin, *Purpureocillium lilacinum*, rhizobacteria, and botanicals compared with nematicide

R. M. El-Ashry[1], Mohamed A. S. Ali[2], Ahmed E. A. Elsobki[1] and Ahmed A. A. Aioub[1*]

Abstract

Background: Acceptable alternative eco-friendly tools in the present study were tested to control the root-knot nematode, *Meloidogyne incognita*, on greenhouse-cultivated vegetables. The nematicidal effect of rhizobacteria (*Pseudomonas* and *Serratia*), egg parasitic fungus (*Purpureocillium lilacinum*), abamectin (*Streptomyces avermitilis*), and 3 botanicals (colocynth, *Citrullus colocynthis*; moringa, *Moringa oleifera*; marigold, *Tagetes erecta* L.) singly or in combination was tested against *M. incognita*, in comparison with emamectin benzoate.

Results: In vitro treatments revealed that egg hatching and juvenile mortality were influenced by the type of bioagents, plant species of botanicals, and exposure time. All the tested bioagents and botanicals displayed nematicidal potential via their ovicidal and larvicidal action on egg hatching and J2 mortality of *M. incognita*. Three and 5 days post-treatment, abamectin and emamectin benzoate were more effective than *P. lilacinum*, *Serratia* and *Pseudomonas*, and *C. colocynthis* in inhibiting egg hatching: 96.31 and 94.88%; 95.79 and 94.05%; 94.11 and 94.46%; 85.54 and 87.28%; 88.87 and 84.30%, respectively. On the other hand, after 10 days, *P. lilacinum* gave the highest inhibition percentage (99.00%), followed by abamectin (89.25%). However, the difference was insignificant compared with the inhibition percentage of rhizobacteria, *Serratia* and *Pseudomonas* (88.69%; $p \leq 0.05$). Moreover, juvenile mortality was 100.0, 96.80, and 91.60% after 10 days of treatment, respectively. However, botanicals showed a lower effect on egg hatching and juvenile mortality. Under greenhouse conditions, potential antagonism towards *M. incognita* by application the mixture of biocontrol agents and botanicals was more effective in controlling *M. incognita* than single treatments.

Conclusions: The combination of abamectin and/or emamectin benzoate with *P. lilacinum* and rhizobacteria was the most effective against *M. incognita*, followed by rhizobacteria and *P. lilacinum*, not only in decreasing galls and reproduction of *M. incognita* but also in increasing plant growth of tomato parameters than the control. The application of various bioagents including abamectin might be a potential antagonism strategy against phytonematodes in protected agricultural areas.

Keywords: Abamectin, *Meloidogyne incognita*, Botanicals, Rhizobacteria, *Purpureocillium lilacinum*, Emamectin benzoate, Egg hatching, Juvenile mortality, Tomato

* Correspondence: ahmedaioub1991@gmail.com
[1]Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
Full list of author information is available at the end of the article

Background

Economic damage and yield losses in fields of vegetables are globally caused by root-knot nematodes (RKNs), *Meloidogyne* spp. (Jones et al. 2013). Sedentary endoparasitic nematode, *Meloidogyne incognita*, has a broad host range and infects plants that belong to important plant families such as tomatoes, cucumbers, eggplants, okra, and potatoes. Galling and subsequent reproduction of RKN in roots of infected plants are the primary symptoms affecting the plant's ability to uptake water and nutrients. There is an urgent need to choose an appropriate control method to secure yield and profitable production.

Generally, phytonematodes are managed by chemical nematicides or fumigants. Nowadays, alternative strategies or tools and bioagents are used to decrease populations of *Meloidogyne* spp., reduce pesticide usage, and avoid problems caused by chemical pesticides such as resistant cultivars, rhizobacteria, fungi, nematodes, and botanicals. Several eco-friendly methods are effective against eggs and infective juveniles of *M. incognita* in vitro and under greenhouse conditions (El-Ashry et al. 2020).

Rhizobacteria is one of these new strategies used in managing plant-parasitic nematodes as an alternative to chemical nematicides (Fatima et al. 2021). Moreover, fungi and botanical extracts solely or combined might be a more robust strategy to suppress nematode populations or downregulate the nematode density at-plant, keeping the nematode population below an economic threshold (Kayani et al. 2017).

To optimize the potential benefits of the various agents, researchers have recommended applying a combination of two or more beneficial microbes in biocontrol preparation for the management of plant-parasitic nematodes (Meyer and Roberts 2002). The combination of rhizospheric microorganisms with antagonistic fungi and plant growth-promoting bacteria (PGPB) could more likely have a greater variety of traits responsible for suppressing root-knot nematode over a wide range of environmental conditions.

Different fungal strains isolated from nematodes have shown to produce substances that inhibit nematode egg hatching or kill nematodes (Nitao et al. 2002). Moreover, soil-inhabiting fungi, such as *Paecilomyces lilacinus*, *Trichoderma harzianum*, *Verticillium chlamydosporium*, *Pochonia* spp., *Fusarium* spp., and *Penicillium* spp., have been proved as effective tools in killing eggs, juveniles, and females of nematodes and reduced the densities of parasitic nematodes in soil (Khan et al. 2020), particularly RKNs.

Plant extracts are compounds prepared by solvent-based extraction of all parts or particular parts of the plant releasing bioactive secondary metabolites and are therefore viewed as an option for controlling plant pathogens (Rodriguez et al. 2005).

Although botanicals have an environmentally and toxicologically safe, selective, and efficacious nematicidal potential (Aoudia et al. 2012), they are generally considered to be non-persistent under field conditions and readily transformed by light, oxygen, or microorganisms into less toxic products; thus, fewer residues are expected to result from the use of these natural products (Caboni et al. 2012).

The objective of this study was to evaluate the efficacy of application of different biocontrol agents, i.e., commercial rhizobacteria, egg parasitic fungus, and botanical extracts in comparison with chemical nematicides singly or in combination as antagonistic tools used for the control of the RKN *M. incognita* on tomato plants.

Methods

Source and inoculum of root-knot nematode, *Meloidogyne incognita*

Identified pure inoculum of *M. incognita* was maintained in the greenhouse on the tomato susceptible cultivar Super Strain B for use by a single egg mass to establish a nematode population and then used as a source of inoculum. The culture was maintained in the greenhouse on the tomato, *Solanum lycopersicum* L., susceptible cultivar Super Strain B.

Preparation of *Purpureocillium lilacinum* spores (inoculation of *P. lilacinum*)

Identified culture of *Purpureocillium lilacinum* AUMC 10620 was obtained from the Mycological Centre (AUMC), Assiut University, Egypt. *P. lilacinum* was subcultured in potato dextrose agar media. The culture plates were incubated at 25 °C for 7 days for full growth. The spore suspension was prepared by scrapping the mycelial mat with a sterile glass slide using sterile water. Two layers of muslin cloth were used to filter the sporal suspension, according to Yang et al. (2015).

Aqueous extract preparation

Fresh leaves of tested *Citrullus colocynthis* (Colocynth), *Tagetes erecta* L. (marigold), and *Moringa oleifera* (moringa) were obtained from their natural habitats at Sharkia Governorate, Egypt. The collected leaves were washed, shade dried, ground to a fine powder in a mill, kept in cool dark conditions until extraction with distilled water using a magnetic stirrer at room temperature (*ca*. 25 °C), and then filtered via filter paper (Whatman No. 1). The extraction ratio was 1 W: 10 V (plant: solvent). The extraction period was 2 h. The filtration was stored at − 20 °C for further usage. Then, each extract was arbitrarily termed as a standard solution (S).

Laboratory experiments

Effect of rhizobacteria, botanicals, commercial bionematicide, and chemical nematicide on M. incognita egg hatching

Five healthy and homogenate egg masses of *M. incognita* were hand-picked with fine forceps from small galls and surface sterilized in 1:500 (v/v) aqueous solution of sodium hypochlorite (Clorox) for 5 min (Haseeb et al. 2005) and added to 10 ml of prepared concentrations of commercial rhizobacteria (BECTO Grow Roots® included *Serratia marcescens* and *Pseudomonas putida*/*fluorescens*) and abamectin (Vertemic® 1.8% EC), fungus, three tested botanicals, and one chemical nematicide (emamectin Benzoate). Rhizobacteria, abamectin, and chemical nematicide (emamectin benzoate) were obtained from Agricultural Research Center, Giza, Egypt. Five egg masses were added to a 10-ml suspension of commercial rhizobacteria and abamectin at a concentration of 10^8 CFU/ml in Petri dishes (10 cm in diameter). The control treatment was similarly prepared in 10 ml distilled water only. Similarly, five egg masses were added to a 10-ml suspension of *P. lilacinum* fungus at a concentration of 10^6 CFU/ml in Petri dishes (10 cm in diameter), and the same protocol was followed with three botanicals *C. colocynthis*, *M. oleifera*, and *T. erecta*. Each treatment was replicated 5 times. All treatments were incubated at 22 ± 3 °C. Numbers of newly hatched juveniles were counted periodically using a research microscope (× 100 magnification) after 1, 2, 3, 5, 7, and 10 days of treatment. The hatching inhibition percentage was calculated in comparison with the control treatment, according to the following equation:

$$\text{Egg hatching inhibition } (\%) = \frac{\text{Control} - \text{treatment}}{\text{Control}} \times 100.$$

Effect of rhizobacteria, botanicals, commercial bionematicide, and chemical nematicide on the survival of M. incognita juveniles

In vitro, to test the toxicity of rhizobacteria, fungus (*P. lilacinum*), botanicals (*C. colocynthis*, *M. oleifera*, and *T. erecta*), and chemical nematicides against *M. incognita* J2, 10 ml of the recommended rate of abamectin, emamectin benzoate, stock solution of tested botanicals, rhizobacteria, and abamectin at a concentration of 10^8 CFU/ml, and *P. lilacinum* fungus at a concentration of 10^6 CFU/ml were evaluated. Moreover, 0.1 ml containing about 100 J2 was pipetted into each Petri dish. The control treatment consisted of the 100 IJs maintained in 10 ml distilled water alone. Each treatment was replicated 5 times, and the dishes were kept at 22 ± 3 °C as optimum temperature for IJ survival (Dunphy and Webster 1986). All dishes were sealed tightly with parafilm to avoid vaporization of the solution. The periodical examination was conducted by pipetting a 0.5-ml treatment solution into a Hawksley counting slide and examined with the aid of a research microscope at × 100. The nematode juveniles showing inactive straight posture or inactive (S) posture or not showing any movement after prodding were considered dead, and any other types of movement were scored as alive (Nardo and Grewal 2003). The juvenile's mortality was recorded after 1, 2, 3, and 4 days, and the mortality percentage was calculated by the following equation:

$$\text{Mortality } (\%) = (\text{No.of dead juveniles/total number of juveniles}) \times 100.$$

Greenhouse experiment

Under greenhouse conditions, plastic pots 20 cm in diameter filled with 1700 g mixture of sterilized sandy soil (73.1% sand, 12.5% clay, 8.1% silt), 120 g peat moss, and 3 mg urea fertilizer per kilogram of soil were used. After 4 weeks of sowing, tomato (*Solanum lycopersicum* L.) plants Elisa cultivar seedlings were thinned to three plants per pot. After 3 days, every plant seedling was inoculated with a mixture of 1000 eggs and newly J2 hatched juveniles of *M. incognita* by pipetting 4 ml of the inoculum suspension into four pencil holes around the root system. Immediately, pots of tomato plants were treated with 15 ml of rhizobacteria, *P. lilacinum*, *C. colocynthis*, *M. oleifera*, and *T. erecta*. Moreover, 0.2 ml of abamectin or emamectin benzoate mixed with 15 ml water was applied around infected seedlings of tomato by RKN.

The single, dual, and triple combinations of tested materials were used to conduct the current experiment in a completely randomized block design: (1) negative control (NC) and (2) positive control (PC) infected with *M. incognita* J2 and treated with (3) rhizobacteria (*Serratia* and *Pseudomonas*) (r), (4) *T. erecta* L. (Te), (5) *C. colocynthis* (Cc), (6) *Moringa oleifera* (Mo), (7) fungus (*P. lilacinum*) (PL), (8) abamectin (ab), (9) emamectin benzoate (eb), (10) rhizobacteria + *P. lilacinum* (r + Pl), (11) *C. colocynthis* + *M. oleifera* + *T. erecta* L. (CC + Mo + Te), (12) abamectin + emamectin benzoate (ab + eb) (13), abamectin + *P. lilacinum* (ab+Pl), (14) abamectin + rhizobacteria (ab+r), (15) emamectin benzoate + *P. lilacinum* (eb + Pl), (16) emamectin benzoate + rhizobacteria (eb + r), (17) botanical extracts (*C. colocynthis* + *M. oleifera* + *T. erecta* L.) *P. lilacinum* (Cc + Mo + Pl), and (18) botanical extracts (*C. colocynthis* + *M. oleifera* + *T. erecta* L.) + rhizobacteria (Cc + Mo + Te + r).

The pots were irrigated with water as needed and kept at an air temperature of 22 ± 3 °C on a glasshouse bench. Each treatment was replicated 5 times. Seventy-five days after inoculation, plants were removed carefully from pots and soaked in distilled water for 1 h. Then,

the roots were stained in phloxine B and total root-knot gall index and egg masses index (RGI and EI) were evaluated (Bridge and Page 1980). Data on plant growth parameters, including fresh root and shoot weight (g), leaves fresh weight (g), stem diameter (mm), and plant height (cm), were measured. Moreover, galling and nematode development parameters, gall diameter, and the number of egg masses/plant roots; the number of IJs/100 g; and reproduction factor (RF) (final population/initial population) were assessed. The RGI was evaluated using the following scale: 0 = no galling; 1 = 1: 2 galls; 2 = 3: 10 galls; 3 = 11: 30 galls; 4 = 31: 100 galls; and 5 = more than 100 galls (Taylor and Sasser 1978). Gall diameter was also measured at its greatest diameter. Furthermore, samples of 100 g soil were processed for nematode extraction using a combination of sieving and Baermann trays technique (Hooper, 1986). The parameters changing the percentage of increase or decrease imputed to "negative or positive" control values and the current equations were used:

$$\text{Reduction}\ (\%) = ((\text{Control}-\text{Treated})/\text{Control}) \times 100$$

$$\text{Increase}(\%) = ((\text{Treated}-\text{Control})/\text{Control}) \times 100$$

Statistical analysis

Statistically, the fully randomized design was implemented for laboratory experiments, while experimental units were organized in the greenhouse experiment in a randomized full block design. Data were subjected to statistical analysis using MSTAT version 4, where analysis of variance and means was compared using Duncan's multiple range test at $p \leq 0.05$ probability.

Results

In vitro assay of the tested botanicals, chemical nematicide, rhizobacteria, abamectin, and *Purpureocillium lilacinum* on egg mass hatching

The ovicidal effects of the stock solution 10% (*w/v*) of tested plant extracts (marigold, moringa, and colocynth) and recommended rate (RR) of chemical nematicide (emamectin benzoate), rhizobacteria (BECTO Grow Roots® included *Serratia* and *Pseudomonas*), recommend rate of abamectin (Vertemic® 1.8% EC), and *P. lilacinum* fungus on egg masses of *M. incognita* egg at different time intervals were evaluated (Table 1). Moreover, all of the tested materials were significantly ($p \leq 0.05$) reduced J2 emerging from egg masses with a highly ovicidal effect compared to distilled water (DW). In vitro pathogenicity tests illustrated different levels of ovicidal effect at different times of exposure.

Table 1 In vitro tests of abamectin, rhizobacteria, botanicals, fungus, and emmectin benzoate against hatching of *Meloidogyne incognita* egg masses

Treatments	Number of J2 emerged and inhibition (%) in egg masses hatching of *Meloidogyne incognita* after different time intervals (days)					
	One	Two	Three	Five	Seven	Ten
Negative control (NC), distilled water	222.60a	428.40a	618.20a	945.20a	1192.20a	1354.20a
Abamectin	8.20e	13.40d	22.80d	48.40d	98.20cd	145.60d
	(96.32)	**(96.87)**	**(96.31)**	**(94.88)**	**(91.76)**	**(89.25)**
Emmectin benzoate	9.60de	17.20d	26.00d	56.20d	102.80cd	166.40cd
	(95.69)	**(95.99)**	**(95.79)**	**(94.05)**	**(91.38)**	**(87.71)**
Colocynth, *Citrullus colocynthis*	20.40cde	45.60c	68.80c	148.40bc	210.00bc	382.20bc
	(90.84)	**(89.36)**	**(88.87)**	**(84.30)**	**(82.39)**	**(71.78)**
Moringa, *Moringa oleifera*	35.60b	74.80b	85.40b	199.80b	220.60b	425.20b
	(84.01)	**(82.54)**	**(86.19)**	**(78.86)**	**(81.50)**	**(68.60)**
Marigold, *Tagetes erecta*	33.40bc	58.60bc	81.00bc	184.20bc	192.20bc	388.00b
	(85.00)	**(86.32)**	**(86.90)**	**(80.51)**	**(83.88)**	**(71.35)**
Rhizobacteria, *Serratia* and *Pseudomonas*	24.40bcd	48.20c	89.40b	120.20cd	144.80bc	153.20d
	(89.04)	**(88.75)**	**(85.54)**	**(87.28)**	**(87.85)**	**(88.69)**
Fungus, *Purpureocillium lilacinum*	14.60de	19.20d	36.40d	52.40d	22.80d	13.60d
	(93.44)	**(95.52)**	**(94.11)**	**(94.46)**	**(98.09)**	**(99.00)**

Reported numbers represent means of 5 replicates
Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water
Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan's multiple range test
Egg hatching inhibition $(\%) = \frac{\text{Control}-\text{treatment}}{\text{Control}} \times 100$

After 2 and 2 days of exposure, abamectin (96.32, 96.87) and emamectin benzoate (95.69, 95.99) had the highest ovicidal effect and the least numbers of emerging J2. The percentages of egg hatching inhibition were 93.44, 95.52; 90.84, 89.36; 89.04, 88.75; 85.00, 86.32 and 84.01, 82.54 after 1 and 2 days of exposure to *P. lilacinum*, colocynth, rhizobacteria, marigold, and moringa. After 3 days of exposure, abamectin, chemical nematicide (emamectin benzoate), and *P. lilacinum* fungus were the most effective than the botanicals. The numbers of emerging J2 were 22.80, 26.00, and 36.40, respectively.

After 5, 7, and 10 days of treatment, *P. lilacinum*, followed by rhizobacteria (*Serratia* and *Pseudomonas*), possessed potential nematicidal activity against egg masses of RKN, *M. incognita*. Egg hatching inhibition increased gradually to reach 94.46, 98.09, and 99.00% at 5, 7, and 10 days post-treatment, respectively. On the other hand, the parallel values with abamectin and rhizobacteria were 94.88, 87.28; 91.76, 87.85, and 89.25; and 88.60, respectively than (94.05), (91.38), and (87.71) in Petri dishes treated with RR of emamectin benzoate.

A reversible trend was observed 10 days post-treatment, and the number of emerging *M. incognita* J2 increased reached 48.40, 98.20, and 145.60 in RR of abamectin compared with 52.40, 22.80, and 13.60 in Petri dishes treated with *P. lilacinum* 5, 7, and 10 days post-treatment.

As shown in Table 2, the mortality of second-stage juveniles of *M. incognita* was significantly increased ($p \leq 0.05$) after being treated with chemical nematicide, plant extracts, rhizobacteria (BECTO Grow Roots®), abamectin (Vertemic® 1.8% EC), and *P. lilacinum* than with J2 found in DW (control). However, abamectin as well as emamectin benzoate achieved the higher mortality numbers 2 days post-treatment followed by rhizobacteria colocynth and moringa. After 3 days of treatment, there was a corresponding increase in J2 mortality in a progressive manner, and both abamectin and *P. lilacinum* gave the best results. It was evident that *P. lilacinum* treatment increased *M. incognita* J2 mortality from day 7 until 10 with non-significant differences with abamectin, surpassing emamectin benzoate and rhizobacteria, whereas plant extracts had the least J2 mortality. The results presented in Table 2 revealed also that the highest percentage of J2 mortality was found in *P. lilacinum* (100%), followed by abamectin (96.80) and rhizobacteria (91.60).

In vitro nematicidal activities of such bioagents revealed that *P. lilacinum*, abamectin, and rhizobacteria were the most effective in killing second-stage juveniles of RKN, *M. incognita*. On the other hand, colocynth plant extract was more ($p \leq 0.05$) effective in killing nematodes than moringa and marigold (Table 2).

Greenhouse experiments

Tomato plant growth

Changes in tomato plant growth parameters (fresh root weight, shoot weight, the weight of leaves, stem diameter, and plant height) as a result of bio-agents application against *M. incognita* are presented (Table 3). The 3 plants extract (*C. colocynthis*, 15.22 g; *T. erecta* L., 15.18 g; *M. oleifera*, 14.92 g) in fresh root weight. The curative application of abamectin, emamectin benzoate, *C. colocynthis*, *T. erecta* L., *M. oleifera*, *Serratia* and *Pseudomonas*, and *P. lilacinum* significantly promoted fresh root and shoot weight than the control. Abamectin (16.68) and emamectin benzoate resulted in a significantly induced higher plant root and shoot weight than that other tomato plant treatments, and a non-significant difference was observed ($p \leq 0.05$) between the treatment of *P. lilacinum* and roots of healthy plants

Table 2 Percentage of mortality in second-stage juveniles of *Meloiodogyne incognita* after exposure to abamectin, emmectin benzoate, colocynth, marigold, rhizobacteria, and fungus for different time intervals

Treatments	Percentage mortality in *Meloiodogyne incognita* J2 after different time intervals (days)					
	One	Two	Three	Five	Seven	Ten
Negative control (distilled water)	0.20f	1.20e	1.80f	2.40e	2.80e	4.20e
Abamectin	26.80a	57.00a	75.20a	83.40a	91.60a	96.80a
Emmectin benzoate	19.60b	37.20b	46.00c	76.20b	82.80b	91.40b
Colocynth, *Citrullus colocynthis*	16.80bc	27.00c	45.20c	73.40b	80.80b	89.80bc
Moringa, *Moringa oleifera*	13.40cd	20.00d	38.80de	53.20d	71.60c	87.40c
Marigold, *Tagetes erecta*	12.20d	19.00d	33.20e	51.40d	63.40d	81.40d
Rhizobacteria, *Serratia* and *Pseudomonas*	17.60b	29.00c	42.60cd	68.60c	84.20b	91.60b
Fungus, *Purpureocillium lilacinum*	4.60e	19.20d	56.20b	82.40a	97.20a	100.00a

Reported numbers represent the means of 5 replicates

Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan's multiple range test

Mortality $(\%) = \frac{\text{Dead juveniles}}{\text{Total number of juveniles}} \times 100$

Table 3 Changes in tomato plant growth parameters after treatment with abamectin, emmectin benzoate, colocynth, marigold, moringa, rhizobacteria, and *Purpureocillium lilacinum* in comparison with plants infected with *Meloiodogyne incognita* under greenhouse conditions

Treatments	Fresh root weight (g) (% increase)	Fresh shoot weight (g) (% increase)	Fresh weight of leaves (g) (% increase)	Stem diameter (mm) (% increase)	Plant height (cm) (% increase)
Healthy plants	17.16a	21.87a	15.00a	11.71a	29.80a
Positive control infected with RNK *M. incognita*	14.85e	16.62f	7.80f	8.10e	20.90f
***M. incognita* + abamectin**	16.68b	20.98b	13.80b	11.60a	27.70b
	(12.32)	**(26.23)**	**76.92**	**(43.20)**	**(32.53)**
***M. incognita* + emmectin benzoate**	15.70c	18.81c	11.80c	10.60bc	27.70b
	(5.72)	**(13.17)**	**51.28**	**(30.86)**	**(32.53)**
M. incognita* + colocynth, *Citrullus colocynthis	15.22de	17.15ef	9.00e	8.80d	22.10e
	(2.49)	**(3.18)**	**15.38**	**(8.64)**	**(5.74)**
M. incognita* + marigold, *Tagetes erecta	15.18de	17.87d	9.40e	8.80d	23.60e
	(2.22)	**(7.52)**	**20.51**	**(8.64)**	**(12.91)**
M. incognita* + moringa, *Moringa oleifera	14.92de	16.80ef	8.60ef	8.50d	21.10f
	(0.47)	**(1.08)**	**10.25**	**(4.93)**	**(0.95)**
M. incognita* + *Serratia* and *Pseudomonas	15.28d	18.34cd	10.60d	10.10c	24.80d
	(2.89)	**(10.34)**	**35.89**	**(24.69)**	**(18.66)**
M. incognita* + *Purpureocillium lilacinum	16.84ab	17.65e	13.20b	11.20b	26.40c
	(13.40)	**(6.19)**	**69.23**	**(38.27)**	**(26.31)**

Each value is the mean of five replicates
Means in each column followed by the same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range test
Increase $\% = \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$

(Table 3). Likewise, remarkable increases in stem diameter and plant height were recorded with all tested bio-agents compared with emmectin benzoate and healthy plants.

The results revealed that all treatments significantly ($p \leq 0.05$) reduced galling (as indicated by the number of galls and root gall index, RGI) and reproduction (as indicated by the number of egg masses on roots and number of juveniles in 100 g soil) than the plants inoculated with *M. incognita* alone. Pots treated with abamectin surpassed those treated with emmectin benzoate, botanicals, and 2 bioagents in the suppression of IJs of *M. incognita*/100 g soil and number of galls. On the other hand, pots treated with the fungus, *P. lilacinum* ranked the next, followed by emmectin benzoate in par with rhizobacteria whereas botanicals showed the lowest nematicidal effect on the number of IJs/100 g soil and root galling and moringa being the best with non-significant differences ($P < 0.05$) were indicated compared to other extracts. Similarly, a number of egg masses and galls ≥ 4 mm were significantly diminished by all treatments and abamectin *P. lilacinus* being the best. Among the tested materials, the 3 bio-agents gave promising results and significantly suppressed *M. incognita* development and reproduction, followed by moringa plant extract whereas colocynth showed the lowest nematicidal effect.

Root and soil parameters

Root parameters (number of galls, root gall index, and number egg masses) were significantly ($p \leq 0.05$) decreased in treated pots. Individual and combined inoculations of certain bio-agents, i.e., abamectin, *P. lilacinum*, and rhizobacteria and emmectin benzoate, significantly decreased ($P \leq 0.05$) the number of galls, root gall index, and egg mass numbers than the positive control (Tables 4 and 5) Moreover, fewer egg masses (18.20) were produced on the root systems of tomato plants grown in pots filled with soil treated with ab+Pl, followed by eb + Pl (20.40) and ab + r (23.80), compared with tomato plants grown in pots filled with untreated soil (Table 5) with non-significant differences. On the other hand, the interaction botanical extracts were less effective but significantly decreased the number of galls (38.40), egg masses (46.00), and J2 density/100 g soil than the untreated plants. Interacting *P. lilacinum* fungus and 3 botanical extracts gave better results in reducing the number of galls, egg masses, and J2 density in soil than did rhizobacteria with such botanicals. Plants treated with abamectin + *P. lilacinum* or plus

Table 4 Individual effects of abamectin, emmectin benzoate, colocynth, marigold, moringa, rhizobacteria, and *Purpureocillium lilacinum* on galls and reproduction of the root-knot nematode *Meloidogyne incognita* in tomato plants under greenhouse conditions

Treatments	*Meloidogyne incognita* reproduction and galling parameters				
	No. of IJs/100 g soil (% reduction)	No. of galls (% reduction)	RGI	No. egg masses (% reduction)	Large size galls (≥ 4 mm)
Healthy plants	0.00g	0.00g	0.00f	0.00f	0.00f
Positive control infected with RNK *M. incognita*	352.00a	48.20a	4.00a	53.80a	23.20a
***M. incognita* + abamectin**	88.20f	10.80f	2.40e	15.80e	2.00f
	(74.94)	**(77.59)**		**(70.63)**	
***M. incognita* + emmectin benzoate**	195.00d	27.20e	3.00cd	32.60d	14.20bc
	(44.60)	**(43.56)**		**(39.40)**	
M. incognita* + colocynth, *Citrullus colocynthis	252.60b	40.20b	4.00a	48.20b	15.60bc
	(28.23)	**(16.59)**		**(10.40)**	
M. incognita* + marigold, *Tagetes erecta	284.80b	36.40bc	3.80a	42.40cd	12.20cd
	(19.09)	**(24.48)**		**(21.18)**	
M. incognita* + moringa, *Moringa oleifera	287.80b	34.60cd	3.60ab	41.60cd	16.20b
	(18.23)	**(28.21)**		**(22.67)**	
M. incognita* + *Serratia* and *Pseudomonas	210.00c	32.80d	3.20bc	34.80d	17.60b
	(40.34)	**(31.95)**		**(35.31)**	
M .incognita* + *Purpureocillium lilacinum	144.40e	12.60f	2.60de	15.40e	7.00e
	(58.97)	**(73.85)**		**(71.37)**	

0, no galls or egg masses; 1, 1–2 galls or egg masses; 2, 3–10 galls or egg masses; 3, 11–30 galls or egg masses; 4, 31–100 galls or egg masses; and 5, more than 100 galls or egg masses, according to the scale given by Taylor and Sasser (1978)
Figures in parenthesis refer to the percentage of reduction
Each value is the mean of five replicates
Means in each column followed by the same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range tests
Reduction% $= \frac{\text{Control–treatment}}{\text{Control}} \times 100$

Table 5 Evaluation of simultaneous exposure and interaction among chemical nematicide, bacteria, botanicals, and fungi against the root-knot development of *Meloidogyne incognita* in tomato plants under greenhouse conditions

Treatments	Root parameters			Soil parameters	
	No. of galls	No. of egg masses	Root gall index (RGI)	Nematode population density (J_2) 100 g soils/plant/pot	
				Final population (% reduction)	Reproduction factor (RF = Pf/Pi)
Infected tomato plants	48.20a	53.80a	4.00	456.00	2.28
Rhizobacteria + *Purpureocillium lilacinum*	21.20e	34.80cd	3	304.20cd (33.28)	1.52
Citrullus colocynthis* + *Moringa oleifera* + *Tagetes erecta	38.40b	46.00b	4	339.60b (25.52)	1.69
Treated plants with chemical nematicides: abamectin + emmectin benzoate	32.80c	39.40c	2.6	344.00b (24.56)	1.72
Abamectin + *Purpureocillium lilacinum*	6.60g	18.20e	2.00	116.60 h (74.42)	0.58
Emmectin benzoate + *Purpureocillium lilacinum*	11.40fg	20.40e	2.40	326.00bc (28.50)	1.63
Abamectin + Rhizobacteria	15.60f	23.80e	2.40	209.00f (54.16)	1.04
Emmectin benzoate + Rhizobacteria	25.60de	31.80d	3.00	246.00e (46.05)	1.23
Botanical extracts (*Citrullus colocynthis* + *Moringa oleifera* + *Tagetes erecta*) + Rhizobacteria	31.40cd	42.60c	3.60	354.00b (22.36)	1.78
Botanical extracts (*Citrullus colocynthis* + *Moringa oleifera* + *Tagetes erecta*) + *Purpureocillium lilacinum*	29.20cd	37.80c	3.20	246.00e (46.05)	1.23

Each value is the mean of five replicates
Means in each column followed by the same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range tests
Reduction% $= \frac{\text{Control–treatment}}{\text{Control}} \times 100$

rhizobacteria significantly diminished the final population of *M. incognita* (74.42%, 54.16%) and reproduction factor equal to 0.58 and 1.04, respectively.

Discussion

From the obtained results, botanicals, *P. lilacinum*, abamectin (a natural fermentation product of Streptomyces avermitilis), and rhizobacteria affected egg hatching of *M. incognita* and juvenile mortality. Under greenhouse conditions, tested materials have potentially controlled RKN *M. incognita*. Abmectin and *P. lilacinum* were superior to other treatments. *P. lilacinum* together with abamectin gave the best results in decreasing root galls, egg masses of RKN, *M. incognita,* and soil nematode population as well. Moreover, *P. lilacinum* together with rhizobacteria was better than the mixture of botanicals gave satisfactory results in suppressing the previous criteria.

The antimicrobial activity of *P. lilacinum* against RKNs was less effective on juveniles than on eggs (Holland et al. 1999). Several mechanisms for the biological management of plant-parasite nematodes by *P. lilacinum* have been proposed. The main mechanism of action is direct infection during the egg stage. In addition, *P. lilacinum* was observed to produce leucine toxins, chitinases, proteases, and acetic acid to promote the infection (Khan et al. 2004).

Paecilomyces lilacinus fungus, strain 251 decreased J2 hatching in vitro and in pot experiments. *P. lilacinum* significantly suppressed J_2, eggs, or egg masses of *M. incognita* as well as stimulated plant growth parameters. Moreover, combining *P. lilacinus* with other antagonistic or biocontrol agents is able to kill J2 of *M. incognita* and increase control effectiveness in infested fields with nematodes.

Numerous attempts have been made to use antagonistic fungi, rhizobacteria, and botanicals to control RKNs, but biocontrol agents displayed lower efficacies when used alone than chemical nematicides (Ding et al. 2020). Therefore, more than one application or repeated applications at the most suitable time are necessary to achieve long-term control of RKN and obtain effective biological control (Anastasiadisa et al. 2008).

Several studies on the combination treatments with various practices (soil solarization, chemical nematicides, botanicals, and biological agents) were conducted to effectively control RKNs (Anastasiadisa et al. 2008 and; El-Ashry et al. 2020). Based on the present study, the combination of antagonistic or biocontrol agents reduced the number of galls, egg masses, and *M. incognita* population as equally effective to the chemical emamectin benzoate. So far, abamectin (*S. avermitilis*), *P. lilacinum* fungus, and rhizobacteria were the most effective biocontrol agents used against phytonematodes in vitro and under greenhouse and field conditions (Khairy et al. 2021).

The present findings are consistent with the results of Wen-Kun et al. (2016). They revealed that the combination of *Syncephalastrum racemosum* and *P. lilacinus* recorded the highest ovicidal activity and reduced egg hatching and mortality rate in J_2 of *M. incognita* than the control. Moreover, under the pot conditions, the shoot length and plant weight were significantly stimulated and galls were significantly reduced compared to those of the untreated control of cucumber plants.

Based on the findings, the bio-agent abamectin surpassed *P. lilacinum* in the suppression of IJs of *M. incognita* in soil. The combination of abamectin plus *P. lilacinum*, followed by *P. lilacin* and rhizobacteria, has potentially controlled *M. incognita* under greenhouse conditions, and it possibly induced the systemic resistance of plants (Sharma et al. 2020) besides their influence on nematode reproduction.

Botanical pesticides have nematicidal properties, including repellence and denaturation of proteins, depending on the type of botanical compound and pest. In plant-parasitic nematodes such as *M. incognita*, the mode of action includes egg hatching inhibition and suppression of the nematode population. Their mode of action includes influencing embryonic development, killing the eggs, dissolving the egg masses (Asif et al. 2013), and affecting the presence of compounds such as isothiocyanates, thiophenes, glucosides, alkaloids, phenolics, tannins, and fatty acids (Akhtar 2000).

Jidere and Oluwatayo (2018) showed that the leaf extract of moringa decreased egg hatching and increased juvenile mortality of *M. incognita*. Marigold extract showed high hatching inhibition on treated egg masses (Guo et al. 2020). Under greenhouse conditions, moringa suppressed galling and reproduction of *M. incognita* and enhanced the length and fresh weight of shoots of eggplant (Khairy et al. 2021). Inhibition of egg hatching and increasing juvenile mortality of *M. incognita* in vitro and reduction of galling and reproduction of such nematode in vivo strongly suggest the presence of compounds that possess ovicidal and larvicidal properties.

Conclusion

Soil drenching with abamectin (*S. avermitilis*), *P. lilacinus*, and *rhizobacteria* provided a significant level of control of *M. incognita* close to that of chemical nematicide (emmitate benzoate). The use of rhizospheric microbes, such as abamectin, rhizobacteria, and fungi, is more environmentally friendly for managing phytonematode populations than chemical nematicides. The combined use of 2 different PGPB and antagonistic fungus *P. lilacinum* may represent a new biocontrol strategy for IPM programs to control plant-parasitic nematodes in

organic agricultural systems. Moreover, this study may provide new insights into understanding the effectiveness of the mixture of bioagents (abamectin, rhizobacteria, and *P. lilacinum*) and botanicals against *M. incognita* under greenhouse conditions.

Abbreviations

RKN: Root-knot nematodes; PGPR: Plant growth-promoting rhizobacteria; TE: *Tagetes erecta* L.; CC: *Citrullus colocynthis*; Mo: *Moringa oleifera*; PI: *Purpureocillium lilacinum*; r: Rhizobacteria; AB: Abamectin; EB: Emmectin benzoate; AUMC: Assiut University Mycological Centre

Acknowledgements

The authors are thankful to the Assiut University Mycological Centre (AUMC) for providing us the culture of *Purpureocillium lilacinum* AUMC 10620 to conduct this study.

Authors' contributions

AA designed and achieved the planning of the experiments and wrote the manuscript. AA and ER carried out the experiments. AA and AE have done the analysis of the data. ER had done the sample collection for experiment purposes. MA and AE helped in the planning of the experiment and critically revised the article. The authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt. [2]Plant Pathology Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt.

Received: 1 March 2021 Accepted: 7 June 2021
Published online: 16 June 2021

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