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Effect of certain organic amendments and *Trichoderma* species on the root-knot nematode, *Meloidogyne incognita*, infecting pea (*Pisum sativum* L.) plants

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Abstract

This study was conducted to evaluate the effects of three fungal species, *Trichoderma harzianum* (*Th*), *T. viride* (*Tv*), and *T. vierns* (*Tvi*), and ground seeds of fennel (*Foeniculum vierns*) and caraway (*Carum carvi*), and powdered leaves of basil (*Ocimum basilicum*) as soil amendments against the root-knot nematode, *Meloidogyne incognita* on pea root, *Pisum sativum* under screen house conditions. The treatments were compared to a nematicide, carbofuran 10G, and untreated check. The tested materials significantly ($P \leq 0.05$) reduced *M. incognita* on pea as indicated by the numbers of galls, egg masses, and J_2 per root system in pots, either as single or combined treatments. The combined treatments caused higher average total percentages of nematode reduction than those achieved by single ones. The single treatments recorded the averages total percentages of nematode reduction ranged from 72.4 to 82.0%. Basil waste as single was superior in reducing nematode numbers of J_2 and egg masses in root system per plant, as it achieved the highest average total percentages of nematode reduction (77.9%) than 73. 9 and 72.4% caused by fennel and caraway, respectively. In combined treatments, the average total percentages of nematode reduction ranged from 72.4 to 86.4%. Basil + (Th) caused the highest reduction (86.4%), followed by caraway+Tvi (86.3%) and caraway+Th (86.2%). The nematicide, carbofuran was as effective as most as the treatments in reducing nematode parameters. Also, the treatments highly increased growth and yield criteria of pea infected with *M. incognita* as indicated by shoot length and fresh and dry weights and root fresh weight, number and fresh and dry weights of pods.

Keywords: *Pisum sativum*, *Meloidogyne incognita*, *Trichoderma* spp., Nematicides, Leaf and seed wastes

Background

Pea, *Pisum sativum* L. is one of the most important vegetable crops grown in cool season throughout the world. Peas are rich in starch and high in fiber, protein, vitamin A, vitamin B6, vitamin C, vitamin K, phosphorus, magnesium, copper, iron, zinc, and lutein (Pownall *et al.*, 2010). Among various pathogens attacking pea plants, the root-knot nematode, *Meloidogyne incognita*, is the most important one that attacks the roots (Anwar and Mcknery, 2010). Since nematicides are very expensive and cause serious environmental hazards, management of nematodes needs to be directed towards the use of natural products. Green manuring of plant parts has been carried out as a method for controlling the root-knot and other plant-parasitic nematodes by several investigators (Youssef and Lashein, 2013; El-Nagdi *et al.*,2017; Abd El-Khair *et al.*, 2018). *Trichoderma* spp. are basically mycoparasites (Papavizas, 1985), but in recent years, their suppressive effects against plant nematodes have also been reported (Siddiqui and Shaukat, 2004; Javeed *et al.*, 2016; Abd El-Khair *et al.*,2019). Soils rich in organic matter are generally colonized by some biocontrol agents such as *T. harzianum* that improve biocontrol activity. *Moringa oleifera* when combined with *T. harzianum* reduced significantly the reproduction, egg hatching, and juvenile numbers of *M. javanica* on eggplant (Murslain *et al.*, 2014). *T. harzianum*, when

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combined with *Lantana camara*, significantly reduced the root-knot nematode, *M. incognita*, population criteria on tomato which subsequently highly increased mean of fruit weight and total yield (Feyisa *et al.,* 2015).

The present study aimed to evaluate the efficacy of 3 species, *Trichoderma harzianum*, *T. viride*, and *T. vierns*, with certain medicinal plant wastes alone or in combination to manage the root-knot nematode infecting pea roots.

Materials and methods

Identification and inoculum of *M. incognita*

Population of *Meloidogyne*, raised from a single egg mass, was established and collected from eggplant cv. Baladi in a pure culture. Large numbers of egg masses were excised by sterilized forceps from the galled roots. Large numbers of newly hatched 2nd stage juveniles (J_2), used as an inoculum, were prepared by hatching the egg masses after being washed in distilled water, then placed on a sieve layered with a tissue paper at room temperature 23 °C. The sieve was placed over a funnel containing water. This step was repeated several times. The nematode suspension was collected from the plates, and the 2nd (J_2) was placed in a counting dish under stereomicroscope (Askary *et al.*, 2018). *Meloidogyne* species were identified to be *M. incognita*, using adult females, on the basis of the morphological and taxonomical characteristics of the female perineal pattern (Barker, 1985).

Preparation of *Trichoderma* spp. inocula

The inocula of *Th*, *Tv*, and *Tvi* were separately prepared using Sorghum to sand to water (2:2:1 v/v/v) medium. The sterilized medium was individually inoculated by each fungus, using fungal disc (1-cm diameter) obtained from 7-day-old culture. The inoculated medium for each fungal species was incubated at 30 ± 2 °C for 15 days. The resulting fungal inocula were applied in pots' experiment.

Screen house experiment

Seventy-six pots, each containing 2 kg of solarized sandy loam soil (1:1), were used. The soil was mixed with the tested materials thoroughly to ensure an equal distribution of materials in soil and for further decomposition of plant materials as follows: *Trichoderma harzianum* (*Th*), *T. viride* (*Tv*), and *T. vierns* (*Tvi*) inocula at the rate of 3% of soil weight (w:w) were applied in each pot. Soil in each pot was firstly inoculated in the 10th month of 2018 with *Trichoderma* spp. and plant materials. Seeds of fennel (*Foeniculum vierns*) and caraway (*Carum carvi*) were ground in a blender and added at the rate of 10 g/pot. Also, powdered leaves of basil (*Ocimum basilicum*) were added at the rate of 10 g/pot. These materials as indicated in Table 1 were used either alone or in combinations against the root-knot nematode, *M. incognita*, infecting pea roots. The nematicide, carbofuran 10% G (Furadan®) at the rate of 0.02 g/pot (equivalent to 1 kg a.i./feddan = 4200m^2), and carrier (Sorghum) were added in each pot under the screen house conditions.

Fifteen days later, pea cv. Concessa seeds were sown in each pot at the rate of 2 seeds per pot. In the 11th month of 2018, each pot was inoculated by 1000 newly hatched J_2 of *M. incognita* (in 4 holes made around the plant). The pots were watered when needed and were divided into 2 groups: (1) botanical treatments and (2) fungal treatments in single treatments. In combined treatments, they were divided into three groups according to their combinations. All pots were arranged according to a completely randomized design on a bench in the screen house with 5 replicates. Equal number of replicates was served as untreated check. All pots were inoculated with okadean (containing nitrogen-fixing bacterium namely, *Rhizobium leguminosarum*) at recommended rate.

Procedures of counting nematodes

Three months after inoculation, root system in each pot was cut into two halves. Numbers of egg masses as well as the number of galls were counted under binuclear in one half of roots. The numbers of J_2 in the 2nd half of roots were cut into small pieces. Then, J_2s were extracted by incubation method (Young, 1954) in tap water to help egg hatching from egg masses. Also, numbers of bacterial nodules were counted. Average total percentages' reduction or increase was calculated by dividing sum percentages of nematode reduction, plant growth, and yield increases of all parameters of each treatment/number of these parameters. This measurement was used to compare treatments within all groups. Also, average overall percentages reduction of nematode, plant growth, and yield increases were calculated by dividing sum average percentages reduction or increase of all treatments of all criteria in each group/number of parameters to compare among different groups.

Plant growth and yield parameters

The plant growth and yield parameters were recorded after 3 months of nematode's inoculation.

Interaction of data for mixtures

This was estimated for the average total percentages of nematode reduction according to Lempel's formula reported by Richer (1987) as follows:

$$E = (X + Y) - XY/100$$

where E = the expected effect of the mixture.

X = the effect due to component A alone.

Table 1 Effect of basil, caraway, fennel, *Trichoderma harzianum*, *T. viride*, and *T. vierns*, added alone on second-stage juveniles (J_2), egg masses, and galls of *Meloidogyne incognita* and number of bacterial nodules in the plant of pea root system under pot conditions

Treatments	Nematode and gall numbers/root system			No. of nodules/root system
	J_2 in roots	Egg masses	Galls	
Basil	120de	5bc	8bc	52a
Caraway	93f	7b	10b	38bc
Fennel	133 cd	6b	9bc	39bc
Trichoderma harzianum (Th)	120de	5bc	7bc	38bc
T. viride (Tv)	110ef	5bc	8bc	43b
T. vierns (Tvi)	175b	3c	6c	42b
Sorghum (carrier)	140c	7b	10b	43b
Carbofuran® 10%G	160b	5bc	8bc	51a
Nematode only (control)	1087a	15a	27a	35c

Values are averages of 5 replicates. Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$

Y = the effect due to component B alone.

The expected effect was compared to the actual one obtained experimentally from the mixture to determine the additive or synergistic and antagonistic effect according to the equation given by Mansour et al. (1966) as follows:

$$\text{Co–toxicity} = \frac{\text{Observed effect}(\%) - \text{Expected effect}(\%) \times 100}{\text{Expected effect }(\%)}$$

This factor was used to classify the results into 3 categories where a positive factor 20 or more was considered potentiation, a negative factor − 20 or more was considered antagonistic, and immediate values between − 20 and + 20 indicate additive.

Statistical analysis

Data were statistically analyzed on the basis analysis of variance (ANOVA) procedures. Duncan's multiple range test as reported by Snedecor and Cochran (1989) was applied for detecting variations among treatments at 5% level of probability. This was done by Computer Statistical Package (COSTAT) User Manual Version 3.03, Barkley Co.

Results and discussion

M. incognita-related parameters

Regarding nematode parameters, 2 measurements were used to compare among treatments and their groups. The first measurement was the average total percentages' reduction of nematode parameters among treatments of the tested materials within all groups. The second was the average overall percentages' reduction of nematode criteria among different groups of treatments. Results showed that *T. harzianum* (*Th*), *T. viride* (*Tv*), or *T. vierns* (*Tvi*) and amended with basil, caraway, or fennel plant wastes significantly ($P \leq 0.05$) reduced *M. incognita*. The numbers of galls, egg masses, and J_2 on the root system of pea were less than untreated check (Tables 1, 2, 3, 4, 5, 6, 7, 8, and 9).

In single treatments, the average total percentages of nematode reduction per root system of pea ranged from 72.4 to 82.0% (Tables 1 and 2), while in combined treatments, they ranged from 72.4 to 86.4% (Tables 5 and 6). The combined treatments caused higher average total percentages of nematode reduction than those achieved by single treatments. It is worthy to notice the basil waste, as soil amendment was superior to those of caraway and fennel in reducing nematode numbers of J_2 and egg masses in roots, as it achieved the highest reduction (77.9%) than (73.9 and 72.4%) caused by fennel and caraway, respectively. Reduction in the number of galls followed the same trend. Also, *T. vierns* was superior in reducing nematode numbers, as it recorded the highest reduction (82.0%) compared to 78.3% caused by basil + other 2 *Trichoderma* spp. The recorded percentages of reduction in the number of galls followed the same trend.

Using the average overall percentage, fungal treatments in one group recorded high averages overall percentages nematode and gall reductions (79.5 and 74.1%) and were higher than those recorded by the botanical treatments group (74.7 and 66.7%), respectively. Vice versa, the number of nodules behaved as an independent pattern as botanical treatments caused higher average overall percentages of increase (22.9%) than fungal treatments (17.2%) (Table 2).

The combined treatment of basil + *Th* caused the highest average total percentages' nematode reduction (86.4%), followed by caraway + *Tvi* (86.3%) and caraway + Th (86.2%) than the other combined treatments. By comparing different groups, using average overall

Table 2 % Effect of *Trichoderma harzianum*, *T. viride*, *T. vierns*, basil, caraway, and fennel, added alone on the root-knot nematode, *Meloidogyne incognita*, and number of nodules in pea root under pot conditions

Treatments	% Reduction in nematode numbers and gall numbers / root system				% Increase in no. of nodules/root system
	J_2 in roots	Egg masses	Average total percentages of nematode number reduction	Galls	
Basil	89.0	66.7	77.9	70.4	48.6
Caraway	91.4	53.3	72.4	63.0	8.6
Fennel	87.8	60.0	73.9	66.7	11.4
Average overall percentages	89.4	60.0	74.7	66.7	22.9
Trichoderma harzianum (Th)	89.9	66.7	78.3	74.1	8.6
T. viride (Tv)	89.9	66.7	78.3	70.4	22.9
T. vierns (Tvi)	83.9	80.0	82.0	77.8	20.0
Average overall Percentages	87.9	71.1	79.5	74.1	17.2
Sorghum (carrier)	87.1	53.0	70.2	63.0	22.9
Carbofuran® 10%G	85.3	66.7	75.9	70.4	45.7
Nematode only (control)	0.0	0.0	0.0	0.0	0.0

Values are averages of 5 replicates. Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$

percentage, the combined treatments of basil + fungal treatments in one group recorded the highest average overall percentage of nematode reduction (84.1%) than 83.9 and 79.1% recorded by caraway or fennel + fungal treatments, respectively. Also, the reduction in number of galls recorded the same trend as the highest average overall percentages of gall reduction (79.0%) was caused by the group of basil + fungal treatments than the other botanicals + fungal treatments. The percentages of increase in the number of nodules were nearly equal at different combined treatments, based on average overall percentages of the number of nodule increases. Then, nematicide, carbofuran, was as effective as most of the treatments in reducing the number of J_2 in roots and egg masses (75.9%) and root galling (70.4%) and recorded the highest percentage increase in number of nodules (45.7%) than the other single and combined treatments, except that caused by basil treatments. Sorghum carrier recorded the least average total percentages of nematode reduction (70.2%) (Table 6).

Co-toxicity factors for the 2 applied combined treatments of basil, caraway, or fennel, each with the 3 fungal species added at the same time, showed additive or synergistic interaction effects for the average total percentages nematode reduction on pea (Table

Table 3 Effect of basil, caraway, and fennel, *Trichoderma harzianum*, *T. viride*, and *T. vierns*, added alone on vegetative growth parameters of pea root infected by *Meloidogyne incognita*

Treatments	Plant growth parameters								Average total percentages of plant growth parameters increase
	Shoot length (cm)	% Inc.	Shoot fresh weight (g)	% Inc.	Shoot dry weight (g)	% Inc.	Root fresh weight (g)	% Inc.	
Basil	39cd	3.0	9.6de	14.3	4.6d	35.3	3.3a	73.7	31.6
Caraway	46ab	21.1	14.7ab	75.0	4.1e	20.6	2.6bc	36.8	38.4
Fennel	47ab	23.7	10.3cde	22.6	5.6c	64.7	2.1 cd	10.5	30.4
Average overall percentages increase		15.9		37.3		40.2		37.0	33.5
Trichoderma harzianum (Th)	47ab	23.7	15.6ab	85.7	7.2a	111.8	2.3bcd	21.1	60.6
T. viride (Tv)	48a	26.3	13.1bc	56.0	6.0b	76.5	2.9ab	52.6	52.9
T. vierns (Tvi)	43bc	13.2	9.2de	10.0	3.9ef	14.7	2.6bc	36.8	18.7
Average overall percentages increase		21.1		50.6		67.7		36.8	46.1
Sorghum (carrier)	42 cd	10.5	9.1de	8.3	3.7fg	9.0	2.5bc	31.6	14.9
Carbofuran® 10%G	47ab	23.7	17.3a	106.0	5.9bc	73.5	2.7bc	42.1	61.3
Nematode only (control)	38d	0.0	8.4e	0.0	3.4 g	0.0	1.9d	0.0	0.0

Values are averages of 5 replicates. Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$

Table 4 Effect of basil, caraway, fennel, *Trichoderma harzianum*, *T. viride*, and *T. vierns* on pod parameters of pea root infected by *Meloidogyne incognita*

Treatments	Pod parameters						Average percentages of pod parameters increase
	Number	% Inc.	Fresh weight (g)	% Inc.	Dry weight (g)	% Inc.	
Basil	1.4de	8.0	2.6e	–	1.0c	42.9	17.0
Caraway	3.3a	154.0	8.3a	168.0	2.9a	314.0	212.0
Fennel	2.3b	76.9	4.3b	38.7	1.6b	129.0	81.5
Average overall percentages increase		79.6		68.9		162.0	103.5
Trichoderma harzianum (Th)	1.8c	38.5	3.7 cd	19.4	1.6b	129.0	62.3
T. viride (Tv)	1.5cde	15.4	3.9bc	25.8	1.4b	100.0	47.1
T. vierns (Tvi)	1.5cde	15.4	3.4 cd	1.0	1.4b	100.0	38.8
Average overall percentages increase		23.1		15.4		109.7	49.4
Sorghum (carrier)	1.7 cd	30.8	3.6 cd	16.1	1.5b	114.3	53.7
Carbofuran® 10%G	1.5cde	15.4	3.7 cd	19.4	1.4b	100.0	44.9
Nematode only (control)	1.3e	0.0	3.1d	0.0	0.7c	0.0	0.0

Values are averages of 5 replicates. Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$. *Inc.* increase

7). Co-toxicity for the applied combined treatments showed this additive interaction effects reflecting on the highest percentages of nematode reduction on pea. Similarly, Radwan et al. (2004) indicated that combination between *Bacillus thuringiensis* and poultry manure, sawdust, grape marc, and chicken litter exhibited additive effect on *M. incognita* on tomato. Farrag (2011) reported that the combination of *Bacillus megaterium*, *Trichoderma album*, and *Ascophyllum nodosum* each with chamomile, chitosan, or oxamyl showed an additive effect in most cases against *M. incognita* on tomato. Youssef et al. (2015) found that *P. fluorescens* + a commercial product named phosphorine containing *B. megaterium* caused an additive effect in controlling *M. incognita* on green bean under field conditions.

Plant growth-related parameters

Regarding plant growth parameters, 2 measurements were used for comparison among treatments. The first measurement was average total percentages of plant growth parameter increases among treatments within all groups. The second was average overall percentages of plant growth criteria among different groups of treatments. On this basis, the pots treated with *Th*, *Tv*, or *Tvi*, and basil, caraway, or fennel plant wastes significantly ($P \leq 0.05$) increased length and fresh and dry weights of shoots and root fresh weight of pea

Table 5 Effect of basil, caraway, and fennel, added in combination with *Trichoderma harzianum*, *T. viride*, and *T. vierns* on *Meloidogyne incognita* and number of nodules in pea root system

Treatments	Nematode and gall numbers/root system			No. of nodules/ root system
	J2 in roots	Egg masses	Galls	
Basil + Th	79gh	3c	5d	44bc
Basil+ Tv	74 h	5bc	7 cd	40 cd
Basil + Tvi	88def	3c	5d	46ab
Caraway + Th	83efg	3c	5d	45ab
Caraway + Tv	90de	5bc	8bc	42bc
Caraway + Tvi	80fgh	3c	6 cd	44bc
Fennel + Th	93d	7b	10b	40 cd
Fennel + Tv	90de	5bc	8bc	44bc
Fennel + Tvi	92d	3c	5d	46ab
Sorghum (carrier)	140c	7b	10b	43bc
Carbofuran® 10%G	160b	5bc	8bc	51a
Nematode only (control)	1087a	15a	27a	35d

Values are averages of 5 replicates. Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$

Table 6 Effect of combined addition of basil, caraway, and fennel, each with *Trichoderma harzianum*, *T. viride*, and *T. vierns* on *Meloidogyne incognita* and number of nodules in pea root system

Treatments	% Reduction in nematode and gall numbers				% Increase in no. of nodules
	J2 in roots	Egg masses	Average percentages of nematode reduction	Galls	
Basil + Th	92.7	80.0	86.4	81.5	25.7
Basil+ Tv	93.2	66.7	80.0	74.1	14.3
Basil + Tvi	91.9	80.0	86.0	81.5	31.4
Average overall percentages	92.6	75.6	84.1	79.0	23.8
Caraway + Th	92.4	80.0	86.2	81.5	28.6
Caraway + Tv	91.7	66.7	79.2	70.4	20.0
Caraway + Tvi	92.6	80.0	86.3	77.8	25.7
Average overall percentages	92.2	75.6	83.9	76.6	24.8
Fennel + Th	91.4	53.3	72.4	63.0	14.3
Fennel + Tv	91.7	66.7	79.2	81.5	25.7
Fennel + Tvi	91.5	80.0	85.8	74.1	31.4
Average overall percentages	91.5	66.7	79.1	72.9	23.8
Sorghum (carrier)	87.1	53.3	70.2	63.0	22.9
Carbofuran® 10%G	85.0	66.7	75.9	70.4	45.7
Nematode only (control)	0.0	0.0	0.0	0.0	0.0

Values are averages of 5 replicates. Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$

infected with *M. incognita* than the untreated check(Tables 3 and 8).

In the single treatments, it is worthy to notice that caraway waste was superior to other plant wastes as it recorded the highest average total percentages of plant growth increase (38.4%) than basil or fennel and untreated check. Also, *Th* was superior to other fungal species depending upon average total percentages of plant growth increase (Table 3). The 3 *Trichoderma* spp. in one group caused higher average overall percentages of plant growth increases (46.1%) than that caused by another group of plant wastes (33.5%) (Table 3).

In the combined treatments, basil, caraway, or fennel + *Th* caused higher average total percentages of plant growth increases than the other treatments and untreated check. Basil waste + each of the 3 *Trichoderma* spp. in one group caused the highest plant growth increase (89.1%), followed by other groups of fennel waste + each of the 3 *Trichoderma* spp. (89.0%) and caraway waste + each of the 3 *Trichoderma* spp.(77.4%). Carbofuran registered intermediate value (61.3%). Sorghum recorded the least one (Table 8).

Yield (pod)-related parameters

As to pod parameters, the same 2 measurements mentioned above related to yield criteria were used for comparison among treatments and their groups.

Table 7 Type of interactions of basil, caraway, and fennel each combined with *Trichoderma harzianum*, *T. viride*, and *T. vierns* on the root-knot nematode, *Meloidogyne incognita*, infecting pea root system

Treatment	Effect for average total percentages of nematode reduction		Co-toxicity factor	Type of interaction
	Expected	Observed		
Basil + Th	95.2	86.4	− 9	Additive or synergistic
Basil+ Tv	95.2	80.0	− 16	Additive or synergistic
Basil + Tvi	96.0	86.0	− 14	Additive or synergistic
Caraway + Th	94.0	86.2	− 8	Additive or synergistic
Caraway + Tv	94.0	79.2	− 16	Additive or synergistic
Caraway + Tvi	95.0	86.3	− 8	Additive or synergistic
Fennel + Th	94.3	72.4	− 13	Additive or synergistic
Fennel + Tv	94.3	79.2	− 16	Additive or synergistic
Fennel + Tvi	95.3	85.8	− 10	Additive or synergistic

Table 8 Combined effect of basil, caraway, and fennel each with *Trichoderma harzianum*, *T. viride*, and *T. vierns* on certain vegetative growth parameters of pea infected by *Meloidogyne incognita*

Treatments	Plant growth parameters								Average percentages of plant growth parameter increase
	Shoot length (cm)	% Inc.	Shoot fresh weight (g)	% Inc.	Shoot dry weight (g)	% Inc.	Root fresh weight (g)	% Inc.	
Basil + Th	51ab	34.2	20.9b	148.8	10.3a	202.9	2.7de	42.1	107.0
Basil+ Tv	45 cd	18.4	21.9a	160.7	8.4b	147.1	3.0 cd	57.9	96.0
Basil +Tvi	53a	39.5	19.8c	135.7	5.3f	55.9	2.4ef	26.3	64.4
Average overall percentages		30.7		148.4		135.3		42.1	89.1
Caraway + Th	52a	36.8	19.1d	127.4	7.3c	114.7	3.5ab	84.2	90.8
Caraway + Tv	50ab	31.6	17.1e	103.6	6.1de	79.4	3.3bc	73.7	72.1
Caraway + Tvi	44 cd	15.8	20.5b	144.0	6.3de	85.3	2.5ef	31.6	69.2
Average overall percentages		28.1		125.0		93.1		63.2	77.4
Fennel + Th	52a	36.8	20.7b	146.4	6.5d	91.2	3.7a	94.7	92.3
Fennel + Tv	50ab	31.6	19.1d	127.4	7.6c	123.5	3.3bc	73.7	89.1
Fennel + Tvi	47bc	23.7	20.6b	145.2	8.6b	152.9	2.3f	21.1	85.7
Average overall percentages	49.7	30.7		139.7		122.5		63.2	89.0
Sorghum (carrier)	42de	10.5	9.1f	8.3	3.7 g	9.0	2.5ef	31.6	14.9
Carbofuran® 10%G	47bc	23.7	17.3e	106.0	5.9e	73.5	2.7de	42.1	61.3.
Nematode only (control)	38e	0.0	8.4 g	0.0	3.4 g	0.0	1.9 g	0.0	0.0

Values are averages of 5 replicates. Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$. *Inc* increase

On this basis, the pots treated with *Th, Tv*, or *Tvi*, basil, caraway or fennel plant wastes significantly ($P \leq 0.05$) increased number and fresh and dry weights of pods of pea infected with *M. incognita* than the untreated check (Tables 4 and 9).

In single treatments, caraway waste recorded higher average total percentages of pod parameters increase (212.0%) more than those caused by other botanical treatments. The fungus *T. harzianum* (*Th*) recorded a higher pod parameter increase (62.3%), than the other

Table 9 Effect of basil, caraway, and fennel, each combined with *Trichoderma harzianum*, *T. viride, and T. vierns*, on pod parameters of pea root infected by *Meloidogyne incognita*

Treatments	Pod parameters						Average total percentages of pod parameters increase
	Number	% Inc.	Fresh weight (g)	% Inc.	Dry weight (g)	% Inc.	
Basil + Th	3.3 cd	153.8	7.3c	135.5	3.5b	400.0	229.8
Basil+ Tv	3.5bc	169.2	8.5b	174.2	3.3b	371.4	238.3
Basil +Tvi	3.3 cd	153.8	7.2 cd	132.3	3.4b	385.7	223.9
Average overall percentages		158.9		147.3		385.7	230.7
Caraway + Th	3.5bc	169.2	7.3c	135.5	2.1d	200.0	168.2
Caraway + Tv	4.3a	230.8	5.5e	77.4	2.3 cd	242.9	183.7
Caraway +Tvi	3.8b	192.3	10.6a	241.9	4.0a	471.4	301.9
Average overall percentages		197.4		151.6		304.8	217.9
Fennel + Th	3.0d	130.8	6.5d	109.7	3.4b	385.7	208.7
Fennel + Tv	3.3 cd	153.8	4.3ef	38.7	3.8a	442.9	211.8
Fennel + Tvi	3.0d	130.8	5.4e	74.2	2.4c	242.9	149.3
Average overall percentages		138.5		74.2		357.2	189.9
Sorghum (carrier)	1.7e	30.8	3.6gh	16.1	1.5e	114.3	53.7
Carbofuran® 10%G	1.5ef	15.4	3.7fg	19.4	1.4e	100.0	44.9
Nematode only (control)	1.3f	0.0	3.1 h	0.0	0.7f	0.0	0.0

Values are averages of 5 replicates. Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$. *Inc.* increase

fungal treatments. Also, botanical wastes recorded a higher average pod parameters increase of 103.5% than the fungal treatments (49.4%) and untreated check (Table 4).

In combined treatments, the basil + *Th*, caraway + *Tvi*, and fennel + *Tv* caused higher percentages of pod parameter increase (229.8, 301.9, and 211.8%), respectively than other treatments and untreated check. When basil was combined with each of the 3 *Trichoderma* spp. in one group, it increased average overall percentages of pod parameters by (230.7%) higher than the groups of caraway or fennel + each of the 3 *Trichoderma* spp. and untreated check. Carbofuran registered intermediate value (44.9%) (Table 9).

Obtained results showed that some medicinal plant wastes had nematicidal effect against *M. incognita* parameters *viz*., J_2 in roots; galls and egg-masses in pea. In the other words, the tested powdered leaves and seeds, when used as soil amendments, caused considerable reduction of the root-knot nematode, *M. incognita*, infecting pea, and subsequently increased plant growth and yield criteria. These results agree with those obtained by Youssef et al. (2004), Youssef and Lashein (2013), and El-Nagdi et al. (2017). The exact mechanism of action of organic amendments may be due to that secondary products from their decomposition are directly toxic to nematodes (Mahmood and Saxena, 1992). Besides, it is possible that nematicidal activity, at least by nitrogenous by-products, should be the most evident when the C:N ratio of the amendment is less than 20:1 (Stirling, 1991). On this basis, basil waste as soil amendment was superior to those of caraway and fennel in reducing nematode numbers of J_2 and egg masses in roots. Montasser (1991) concluded that tannins may be responsible for the suppressive effect on *M. arenaria* and *M. javanica.* Also, essential oils have the ability to reduce certain phytonematodes (Abd-Elgawad and Omer, 1995).

Also, the present results showed that the *Tv*, *Th*, or *Tvr*, combined with basil, caraway, and fennel wastes had the highest nematicidal effect against the same *M. incognita* parameters. These results agree with those recorded by Olabiyi and Gbadamosi (2013) who stated that *T. harzanium* combined with composted materials reduced nematode population and gall index in sesame. *T. vierns* combined with the plant debris of oak forests also significantly decreased the number of galls of *M. javanica* on tomato roots (Moradi *et al.,* 2015). Greater sporulation and multiplication of *T. harzianum* were supported by decomposed leaves of neem as a medicinal plant as reported by Khan et al. (2012). Direct parasitism of *T. harzianum* on *M. javanica* in vitro was reported (Sharon *et al*., 2001). They showed that protease enzyme and chitinase were secreted by the fungus, *Trichoderma*, in the culture filtrate that may inhibit egg hatch. Nematicides provide very effective and faster results by reducing nematode numbers and reproduction rates, but they are not environmentally friendly and have many drawbacks (Abd-El-Khair *et al.,* 2019).

Conclusions

Combining *Th*, *Tv*, and *Tvi* fungi with basil, caraway, and fennel wastes provided satisfactory control of the root-knot nematode in pea. The integrated treatments of plant wastes and biocontrol agents not only did reduce suppressive effect of the nematodes, but also caused an additional increase in growth and pod production criteria of pea. The tested fungal species were isolated from Egyptian soil, and medicinal plants are commonly cultivated in Egypt and their leaves and seeds can be further collected and applied with *Trichoderma* spp. in the field for better root-knot nematode control in pea, improving soil fertility and subsequently, increasing plant growth and yield.

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Authors' contributions

The WMAEN and MMAY were equal to the design and execution of this experiment. The MMAY wrote the manuscript. The HAEK isolated and identified the tested fungal species. The MMMAE provided the facilities during this work. All authors approved the final manuscript.

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Availability of data and materials

The tested fungal species and plant wastes are available in Egyptian environment and were prepared 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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References

Abd-Elgawad MM, Omer EA (1995) Effect of essential oils of some medicinal plants on phytonematodes. Anz Schad, Pflanz Umwelt 68:82–84

Abd-El-Khair H, El-Nagdi WMA, MMA H (2018) Effect of olive and castor bean oil cakes singly or combined with *Trichoderma* spp. on *Fusarium solani* and *Meloidogyne incognita* infecting Eggplant. Middle East J Appl Sci 8(2):465–473

Abd-El-Khair H, El-Nagdi WMA, MMA Y, MMM A-E, Dawood MG (2019) Protective effect of *Bacillus subtilis*, *B. pumilus,* and *Pseudomonas fluorescens* isolates against root knot nematode *Meloidogyne incognita* on cowpea. Bull Nat Res Centre 43(64):1–7

Anwar SA, Mcknery MV (2010) Incidence and reproduction of *Meloidogyne incognita* on vegetable crop genotypes. Pak J Zool 42:135–141

Askary TH, Khalil A, Nazir N (2018) Resistance of grapevine cultivars to *Meloidogyne hapla* under polyhouse condition. Int J Curr Microbiol App Sci 7(08):2351–2359. https://doi.org/10.20546/ijcmas.2018.708.236

Barker TR (1985). Nematode extraction and bioassays. In: "An Advanced Treatise on *Meloidogyne*": Vol. II. Methodology (Barker TR, Carter CC, Sasser JN (eds). North Carolina State University, USA, 19-35 pp.

El-Nagdi Wafaa MA, Youssef MMA, Dawood MG (2017) Nematicidal activity of certain medicinal plant residues in relation to controlling root knot nematode, *Meloidogyne incognita* on cowpea. Appl Sci Reptr 20(2):35–38

Farrag Samia AA (2011) Studies on the management of root knot nematodes. *Meloidogyne* spp. infecting tomato.M.Sc.Thesis,Faculty of Agriculture. Alexandria Universty, 136 pp

Feyisa B, Lencho A, Selvaraj T,Getaneh G(2015). Evaluation of some botanicals and*Trichoderma harzianum* for the management of tomato root-knot nematode (*Meloidogyne Incognita* (Kofoid and White) Chit Wood]. Adv Crop Sci Tech 4:201. doi:https://doi.org/10.4172/2329-8863.1000201

Javeed MT, Al-Hazmi AS, MolanYY (2016) Antagonistic effects of some indigenous isolates of *Trichoderma* spp. against *Meloidogyne javanica*. Pak J Nematol 34(2):183–191

Khan MR, Mohiddin FA, Ejaz MN, Khan MM (2012) Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves. Turk J Biol 36:161–169

Mahmood I, Saxena SK (1992) Effect of green manuring with certain legumes on the control of plant parasitic nematodes. Pak J Nematol 10(2):139–143

Mansour NA, El-Dafrawi ME, Tappozada A, Zeid MI (1966) Toxicological studies on the Egyptian cotton leaf worm, *Proedenia litura*. Potentiation and antagonism of organophosphorus and carbaamate insecticides. J Econ Entomol 59:307–311

Montasser SA (1991) Effect of dry dregs as soil amendment on gall formation by *Meloidogyne javanica* and growth of sunflower (*Helianthus annus* L.). Pak J Nematol 9:17–20

Moradi R, Moradi F, Mirehki K, Abdollahi M (2015) Plant debris of oak forest as soil amendment, to improve the biocontrol activity of *Pseudomonas fluorescens* and *Trichoderma vierns* against *Meloidogyne javanica*, in tomato. J Crop Prot 4(3):373–384

Murslain M, Javed N, Khan SA, Khan HU, Abbas H, Kamran M (2014) Combined efficacy of *Moringa oleifera* leaves and a fungus, *Trichoderma harzianum* against *Meloidogyne javanica* on eggplant. Pak J Zool 46(3):827–832

Olabiyi TI, Gbadamosi AR (2013) The effect of four compost soil amendments based on *Trichoderma harzianum* on nematode pests of sesame. Int J Agro Plant Prod 4(5):3859–3863

Papavizas GC (1985) *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. Ann Rev Phytopathol 23:23–54

Pownall TL, Udenigwe CC, Aluko RE (2010) Amino acids composition and antioxidant properties of pea seed (*Pisum sativum*) enzymatic protein hydrolysate fractions. J Agric Food Chem 58:4712–4718

Radwan MA, Abu-Elamayem MM, ShMI K, Maadawy EK (2004) Management of root knot nematode by integration of *Bacillus thuringiensis* with either organic amendments or carbofuran. Pak J Nematol 22(2):135–142

Richer DL (1987) Synergism. a patent view. Pest Sci 19:309–315

Sharon E, Bar-Eyal M, Chet I, Herrera-Estrella A, Kleifeld O, Spiegel Y (2001) Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology 91:687–693. https://doi.org/10.1094/PHYTO.2001.91.7.687

Siddiqui IA, Shaukat SS (2004) *Trichoderma harzianum* enhances the production of nematicidal compounds *in vitro* and improves biocontrol of *Meloidogyne javanica* by *Pseudomonas fluorescens* in tomato. Lett Appl Microbiol 38:169–175

Snedecor GW, Cochran WG (1989) Statistical methods, 8th edn. Iowa State University Press, Ames, Iowa

Stirling GR (1991) Biological control of plant parasitic nematodes. Progress, problems and prospects. CAB International, Wallingford, UK

Young TW (1954) An incubation method for collecting migratory- endoparasitic nematodes. Plant Dis Reptr 38:794–795

Youssef MMA, El-Ghonaimy AM, El-Nagdi WMA (2015) Evaluation of some commercial bacterial biofertilizers and isolates against root knot nematode, *Meloidogyne incognita* infesting green bean, *Phaseolus vulgaris*. Sci Agric 10(1):49–54

Youssef MMA, El-Nagdi WMA, MFM E (2004) Effect of glycyrrhiza (*Glycyrrhiza glabra*) and henna (*Lawsonia inermis*) plant wastes on controlling the root knot nematode, *Meloidogyne incognita* on eggplant (*Solanum melongena*). Bull NRC, Egypt. 29:711–716

Youssef MMA, Lashein AMS (2013) Efficacy of different medicinal plants as green and dry leaves and extracts of leaves on root knot nematode, *Meloidogyne incognita* infecting eggplant. Eur J Agric Environ Med 2(1):10–14

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