

# Evaluation of the effects of earthworm *Eisenia fetida*-based products on the pathogenicity of root-knot nematode (*Meloidogyne javanica*) infecting cucumber

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Received: 21 April 2013 / Accepted: 3 December 2013 / Published online: 10 April 2014  
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## Abstract

**Background** Biocontrol of nematode agents to decrease the hazardous impacts of chemical pesticide application including problems of public health and environmental pollution is a priority. In this study, solid (Vermicompost) and liquid products (Liquid Vermicompost, Vermiwash and Coelomic fluid) of the earthworm species *Eisenia fetida* were tested against root-knot nematode, *Meloidogyne javanica* in vitro and greenhouse conditions.

**Results** Results showed that Liquid Vermicompost, Coelomic fluid and Vermiwash had the greatest effect on egg hatching inhibition, respectively, and Coelomic fluid, Vermiwash and Liquid Vermicompost had the highest effect on mortality of larvae (J<sub>2</sub>), respectively, in vitro. All earthworm-based products were added to the cucumber pots and then a root-knot disease as well as plant growth indices was recorded. Results showed that all products could reduce the number of nematode juveniles and gall index in greenhouse conditions. The best combination for controlling disease was Vermicompost + 10 % Liquid Vermicompost and the highest rates of growth related to plants were treated with Vermicompost + 10 % Vermiwash.

**Conclusions** It is concluded that earthworm products have a remarkable potential as control agents against root-knot nematode and improving host plant health.

**Keywords** *Eisenia fetida* · *Meloidogyne javanica* · Liquid Vermicompost · Coelomic fluid · Vermiwash · Vermicompost

## Abbreviations

VC Vermicompost  
LVC Liquid Vermicompost  
VW Vermiwash  
CF Coelomic fluid

## Introduction

The root-knot nematodes, *Meloidogyne* spp. infect a wide range of agricultural products including vegetables, horticultural and field crops and weeds as well (Jepson 1987). Due to the world distribution, host range and the interaction with other plant disease causing agents in the disease complexes, they are considered as one of the most important plant pathogens which threaten world food resources. The species *Meloidogyne javanica* (Treub) Chiwood is taken into account as one of the dangerous species of this genus causing economic damages in the world and Iran also (Akhiani et al. 1984). Because of the importance of economic damages by this nematode, great efforts have been carried out to manage it including biological control practices and in the last decades, researchers have tried to manage the nematode populations using organic fertilizers (Addabdo 1995). One of the effective

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organic fertilizers is Vermicompost (VC). Vermicomposting is a simple biotechnological process of composting, in which certain species of earthworms e.g., *Eisenia fetida* are used to enhance the process of waste conversion and produce better products. As VC utilization improves the soil microbial populations, the soil fertility is also augmented (Ketterings et al. 1997). Many reports regarding the control of soil-born pathogenic agents e.g., *Fusarium*, *Plasmodiophora*, *Rhizoctonia*, *Phytophthora* and *Verticillium* by VC are available (Chaoui et al. 2002). Natural antibiotics and Actinomycetes which are present in the VC composition are responsible for eliminating of some plant pathogens (Singh 1993). Microbial observations revealed that the most of microbes in VC belong to classes of Actinomycetes and Proteobacteria (Yasir et al. 2009). The microbial populations in VC are important due to converting complex molecules into simpler ones such as cellulose, lignin and lignocellulose through vermicomposting process (Lacey 1997). On the other hand, Dash et al. (1980) reported that in earthworm-containing soils the plant parasitic nematodes populations are remarkably reduced. The reason behind this reduction is the existence of fungivorous and bacterivorous nematodes in the VC composition (Arancon et al. 2002). In another investigation, the reports indicate that utilization of VC at amount of 1 kg/m<sup>2</sup> could decrease the extent of damages by *M. incognita* in tobacco (Swathi et al. 1998). Morra et al. (1998) recommended the utilization of VC in tomato-squash rotation for lowering damages by nematode *M. incognita*. Meanwhile, it is well documented that the result of VC addition to soils reduces the gall numbers and egg mass of *M. javanica* (Ribeiro et al. 1998). Earthworm's products increase the activity of microorganisms which suppress nematode infestation by lowering soil pH (Garg et al. 2006).

The aim of the present investigation is to explore the impact of earthworm's (*Eisenia fetida*) solid (Vermicompost) and liquid (Liquid Vermicompost, Vermiwash, and Coelomic fluid) products on the root-knot nematode, *M. javanica* under in vitro and greenhouse conditions infecting cucumber.

## Materials and methods

### Collection and inoculum of *M. javanica*

For nematode inoculum preparation, few plant roots infected with root-knot nematode were collected from greenhouses in Chaharmahal va Bakhtiari province and egg masses on them cultured on the tomato plant variety Rutgers using single egg mass culture technique. After identification of nematode species, using eggs and larva, the target nematode population was cultured on the same

tomato plants. For making enough nematode inoculum, eggs of *M. javanica* were extracted from the infected roots using sodium hypochlorite (Hussey and Barker 1973).

### Preparation of earthworm-based products

#### Earthworms

Earthworms *Eisenia fetida* taken were from Soroosh Mohit Sabz (SMS) CO., Shahrekord, Iran. The earthworms were identified morphologically using an identification key (Lee 1985).

#### Vermicompost (VC)

Fresh VC was also purchased from the same place.

#### Liquid Vermicompost (LVC)

0.5 kilogram VC bag was immersed into a 20 l chloride-free water bucket for at least 6 days at room temperature. Then, in favor of microorganism proliferation, the water bucket was aerated vigorously for at least 2 days using an ordinary air pump.

#### Vermiwash (VW)

Earthworms were added to 500 ml warm water and kept for 30 min at room temperature and irritated by a glass stick for 3 min. Secreted materials from earthworm's skin were then centrifuged to remove the insoluble parts at 3,000 rpm for 10 min. The filtrate was made cell free using 0.2 µl membrane filtration.

#### Coelomic fluid (CF)

Earthworms were put in a petri-dish containing physiologic serum and received an electric shock every 3 s using a 9 V battery. Coelomic fluid was excreted through nephridiopores into petri-dish and used as pure sample throughout the experiments.

### In vitro ovicidal and larvicidal experiment using various concentration of liquid earthworm products

For the study of larvicidal and ovicidal effects of earthworm liquid products, 200 eggs and 100 larva (J<sub>2</sub>) were separately added to the 6 cm diameter petri-dishes and various liquid earthworm products with concentrations of 1, 10 and 100 % (undiluted) were added and distilled water was taken as control. After 24, 48 and 72 h, the dead larva as well as the unhatched eggs were counted. The factorial experiment based on completely randomized design was



**Table 1** Analysis of variance for the effects of different concentrations of earthworm *E. fetida* products (CF, LVC, VW) on the inhibition of egg hatching of *M. javanica* under laboratory conditions in all times

Source of variation	Df	Mean squared
Type of product	2	13,737.14**
Time	2	42,237.19**
Concentration	3	14,816.53**
Type of product × time	4	2,680.29**
Type of product × concentration	6	832.59**
concentration × time	6	659.7**
concentration × time × type of product	12	202.39 <sup>ns</sup>
Error	108	182.97

Cv 10.09, LSD 15.86, ns no significant difference

\*\* Significant differences  $p \leq 0.01$

conducted with four replicates. Statistical analysis software (SAS 1985) was used to test variations between treatments, and the least significant difference (LSD) at 5 % probability was used to determine differences between treatment means.

Investigation on the effects of the earthworm products on nematode pathogenicity in the greenhouse

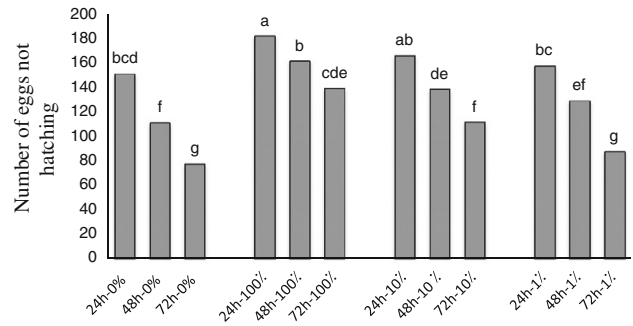
On the basis of in vitro experiment, various treatments of liquid as well as solid products and their combination were used in the greenhouse trial (Table 3). Cucumber seeds var. Beit Alpha 902, were grown in 2 kg sterilized soil. In the treatments having VC, pots incorporated with 200 g of this compost homogeneously mixed with soil. After plants reached at two leaf stage, to study the effects of liquid products (LVC, VW, and CF) they added to the pots (500 cc) along with the irrigation water every week and after of 4 leaf stage, 5,000 nematode eggs and larva inoculated to the cucumber host plants. 90 days after nematode inoculation, plant and nematode growth indices were separately measured and compared. The experiment was conducted based on completely randomized design having four replicates.

**Results and discussion**

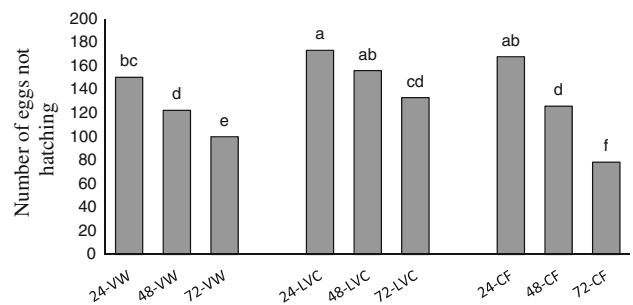
In vitro experiments of evaluation of liquid earthworm products on the *M. javanica*

*Nematode ovicidal effects of liquid earthworm products*

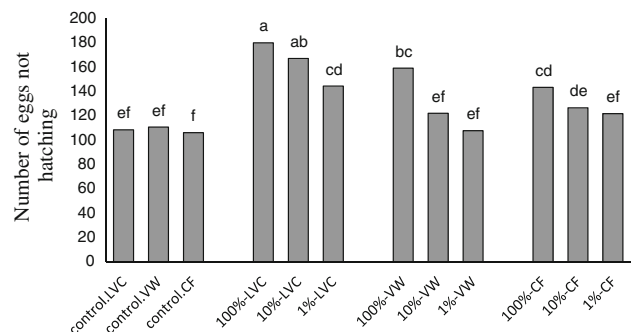
Results of the nematode egg hatching rate in the presence of the earthworm liquid products (Table 1) showed that



**Fig. 1** Effect of time and concentration factors of earthworm products (CF, LVC, VW) on egg hatching of *M. javanica* under laboratory conditions. Data are expressed as mean of four replicates in each treatment. Numbers with the same letters are not significantly different at 5 % level, LSD test

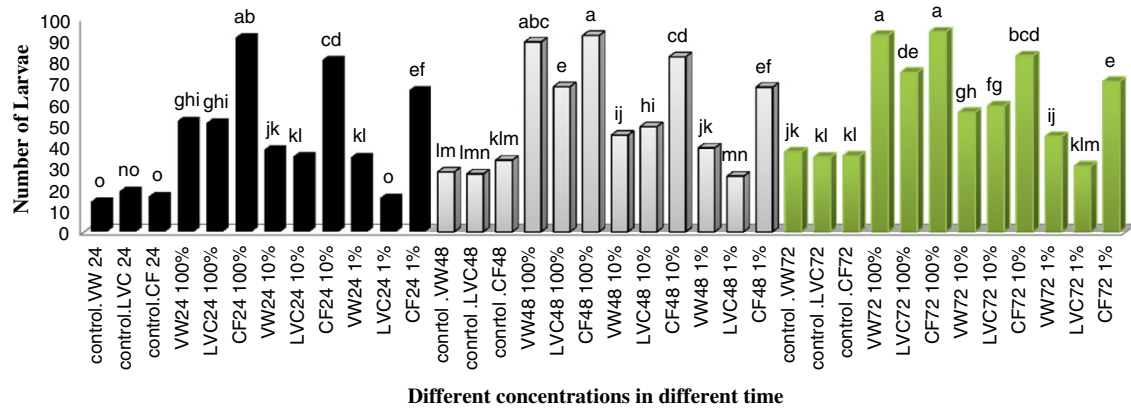


**Fig. 2** Effect of time factor and type of earthworm products (CF, LVC, VW) on egg hatching of *M. javanica* under laboratory conditions. Data are expressed as mean of four replicates in each treatment. Numbers with the same letters are not significantly different at 5 % level, LSD test



**Fig. 3** Effect of concentration factor and type of earthworm *E. fetida* products (CF, LVC, VW) on egg hatching of *M. javanica* under laboratory conditions. Data are expressed as mean of four replicates in each treatment. Numbers with the same letters are not significantly different at 5 % level, LSD test

there is not a significant difference among the three factors namely concentration, time and the kind of products at the same time at 5 % level. But using the analysis of the variance table, it is understood that among the three factors,



**Fig. 4** Effect of different concentrations earthworm products (CF, LVC, VW) on mortality of *M. javanica* larvae ( $J_2$ ) under laboratory conditions in all times. Data are expressed as mean of four replicates in each treatment

**Table 2** Analysis of variance for the effect of different concentrations earthworm products (CF, LVC, VW) on mortality of *M. javanica* larvae ( $J_2$ ) under laboratory conditions in all times

Source of variation	Df	Mean squared
Type of product	2	12,395.04**
Time	2	3,309.84**
Concentration	3	16,840.75**
Type of product $\times$ time	4	181.72**
Type of product $\times$ concentration	6	692.75**
Concentration $\times$ time	6	143.23**
Concentration $\times$ time $\times$ type of product	12	186.34**
Error	108	41.22

Cv 12.33

\*\* Significant differences  $p \leq 0.01$

there is a pairwise significant difference. 100 % concentration of the liquid products during 24 h and 1 % concentration after 72 h showed max. and min. effects on nematode egg hatching rate, respectively (Fig. 1). LVC during 24 h and after that CF after 24 h, LVC after 48 h, then VW after 24 h showed the max. effects and CF after 72 h resulted in the min. effects on the nematode egg hatching (Fig. 2). Results indicated in the Fig. 3 show that 100 % concentration of LVC and then 10 % LVC and also 100 % VW had the greatest egg hatching inhibition effects and 1 % VW had the lowest effect.

#### Nematode larvicidal effects of liquid earthworm products

There are significant differences among the three factors, time–concentration and kind of the products in the 3 time intervals on the nematode death ( $J_2$ ), and the results (Fig. 4; Table 2) indicated that 100 % concentration of CF during 24, 48, and 72 h then 100 % VW during 48 and

72 h showed the greatest effects in comparison to the other products. Lowest effect was recorded from LVC after 24 h.

Results showed in Fig. 4 indicated that CF in all the concentrations had the greatest nematode larvicidal effects and VW and LVC are on the next stages.

Research results by various scientists indicate that liquid earthworm products possess compounds with nematicidal effects. Our results agree with results recorded by Morra et al. (1998), they used VC for reducing *M. incognita* population on tomato. Edwards et al. (2007) reported that *E. fetida* LVC caused reduced nematode plant root invasion. These products reduce the environmental pH and caused increased microorganisms activity and microbial populations which is an important factor combating the nematodes (Garg et al. 2006). Microbial studies indicate that most of bacteria in the Vermicompost composition belong to the Actinomycetes and Proteobacteria (Yasir et al. 2009) and Actinomycetes was known as major microfloral component of most of the composts. They are important due to their capability of complex molecule degradations like cellulose, lignocellulose during compost formation (Jang and Chen 2003). Some of the Actinomycetes isolates can liquify lignin substrate up to 40 %, and they form three fourth of the total known antibiotics in the composts (Anderson and Wellington 2001). And also Actinomycetes was considered as the greatest producers of secondary metabolites, antifungal and antibacterial compounds (Bredhold et al. 2008) which is the most probably used for disintegration of parasite cell wall and finally death occurs. Following the same results, it was reported that some of bacterial populations in the earthworm products secrete chitinase which causes cell wall degradation of many pathogenic fungi and few parasite body surfaces (Yasir et al. 2009). Also, it is reported that VC possesses enzymes such as protease, lipase, amylase, ligninase and chitinase which play a role in organic compounds



degradation (Zaller 2007). From earthworm's intestine, new *Streptomyces* isolate has been reported which showed antimicrobial activity against pathogenic fungi and bacteria (Aruna et al. 2008) and also few Actinomycetes isolates with chitinase and proteinase activity (Gopalakrishnan et al. 2011), and the presence of these biological factors in liquid products derived from the VC invariably has an important role in enzyme degradation of root-knot nematode cuticle and the egg wall. Azotobacteria as well as Pseudomonases have been reported in these compounds (Nakkeeran et al. 2006) and these bacteria producing various metabolites inhibit nematode egg hatching (Sikora and Hoffmann-Hergarten 1993).

In total, earthworm products are the very active biological compounds of bacteria and enzymes and can be considered as nematode ovicidal as well as larvicidal factors, and at the same time there are some other unknown factors which need to be explored.

### Greenhouse trial

#### *Effects of earthworm-derived products on cucumber host plant growth indices infested with nematode*

Results of the effects of earthworm-derived products on cucumber host plant growth indices and its comparison with the healthy plants show that stem and root fresh weight have no significant differences at 5 % level. All the treatments in comparison with the nematode infected plants show significant difference in case of root length. Plants treated with 10 % VW and VC, 100 % VW, VC and VC along with LVC resulted in the max. means and caused root length expansion. After that, 10 % CF and 10 and 100 % LVC treatment are there. In stem length comparison, all treatments except 100 % LVC and 10 % CF resulted in significant difference with nematode only infected plants. From the stem length of view, plants treated with VW and LVC showed the max. and min. length, respectively. 100 % LVC and 10 % CF treated plants in comparison with the nematode only treated plants show no significant differences in case of dried stem weight at 5 % level. 10 % VW along with VC, 100 % VW, LVC with VC, and VC plus 10 % LVC increased dried stem weight, respectively, and showed significant difference with nematode alone treatment. In general, using the Table 3, it is understood that VW increased plant growth and simultaneous application of this product with the VC has synergistic effect on the host plant. 100 % LVC application reduced plant growth indices up to some extent (Table 3). Mean comparison for dried stem weight as well as stem length in between treated plants with CF and nematode infected host plants alone showed no significant differences at 5 %, hence this product has no effective role

**Table 3** Effect of earthworm-based products (*E. fetida*) on the growth of cucumber plants in infested soil by *M. javanica*

Treatment	Parameters		
	Length root (cm)	Length shoot (cm)	Dry weight shoot (g)
Nem.	12/5 e	32/75 c	2/08 d
VC + Nem.	22 abc	54/25 ab	6/17 abc
VC + LVC 10 % + Nem.	21 abcd	49/75 ab	7/67 ab
VC + VW 10 % + Nem.	26 a	55/75 a	8/19 a
LVC 100 % + Nem.	16/75 d	43/25 abc	4/76 cd
LVC 10 % + Nem.	17/5 cd	46/5 ab	5/34 bc
VW 100 % + Nem.	23/5 ab	55/25 ab	8 ab
CF 10 % + Nem.	18/75 bcd	40/25 bc	4/03 cd

Data are expressed as mean of four replicates in each treatment. Numbers with the same letters in each column are not significantly different at 5 % level, LSD test

in plant growth. Earthworm-derived products and specially VC having specific enzymes, convert insoluble soil nutrients like phosphorus into soluble and useful element for the host plant (Satchel and Martin 1984). Nutrient stress in plants when soil nutrient is less than the amount needed may be folded up and these conditions may result from original nutrient deficiency or immobility of that soil element in the plant. Among soil nutrients, phosphorus as one of the microelements, has remarkable role in plant growth and its resistance against pest and pathogens. Taking into the account the role of phosphorus in leaf emergence speed and leaf surface area, Gutierrez-boem and Thomas (1998) reported that phosphorus deficiency may result in reducing leaf emergence speed and by inhibiting cell development reduces cell surface, hence earthworm-based products, by supporting nutrients like phosphorus to the host plants can compensate nematode damage by providing enough leaf photosynthesis area.

Results on cucumber roots show that host root growth treated with earthworm-derived products increased. Studies on the availability of the plant hormones in these products indicate that they have cytokinin and oxine and they lead in increasing the stem and root length and initiation of the new roots (Selvaraj 2011). Tomatiet al. (1988) reported that giberline which is incorporated to the compost and then to soil by the earthworm causes increased plant height and provides favorable photosynthesis condition for the plant. From the other hand, organic acids which are exerted from the worm body, act as plant growth inducers in such a way that they have positive role in seed germination, root growth and plant tissue consistency (Kale 1998). These products have essential plant growth nutrients as well. Our finding supports the effect of earthworm-derived products on the plant growth indices by other researchers.



### Effects of earthworm-derived products on root-knot nematode indices

The results of the products effects on nematode indices are shown in Tables 4 and 5. These results indicated that gall No. in all products treatments in comparison to the nematode only treatment has meaningful differences at 5 % level. LVC along with VC had meaningful difference with 10 % CF, 100 % VW and other treatments are in between the two in gall No.

In case of no of egg masses in one gram of root, 100 % LVC and then LVC along with VC had the min. No. of egg masses and 10 % CF resulted in the max. No.

About No. of eggs in an egg mass, LVC along with VC treatment resulted in the least No. All the earthworm-derived products plus nematode treatments in comparison with nematode only treated plants had meaningful differences but among themselves resulted in no difference in case of no. of J<sub>2</sub> in 200 gram of soil (Table 4).

Table 5 shows that plants treated with earthworm-derived products and nematodes, in comparison to the nematode only treated plants, resulted in the min. final

nematode population, reproductive factor and reproduction percentage.

All the treatments are listed in Table 5, they showed differences at 5 % with nematode only treated plants but among themselves there were no meaningful differences.

About the effects of various earthworm-derived products on the nematodes not sufficient research has been carried out and most of the work is on the effects of VC against the nematodes.

Our results agree with the results reported by Gorakh et al. (2011) and Serfoji et al. (2010). These researchers also demonstrated potential of VC to control plant parasitic nematodes and reported nematode population reduction and eggs on the treated plants. Their exact mode of action is not fully known but in some reports induction of plant defense mechanism in such a way that brings about the plant resistance against plant pathogens such as fungi and nematodes has been reported (Arancon et al. 2002). Also, by the help of toxins, hydrogen sulfide, ammonium and nitrates and Rhizobacteria accumulation around the plant roots, nematode population may get reduced after appli-

**Table 4** Effect of earthworm-based products (*E. fetida*) on the reduction of infestation of *M. javanica* on cucumber plants

Treatment	Parameters			
	No. of gall/1 g root	No. of egg mass/1 g root	No. of eggs/egg mass	J <sub>2</sub> soil population/ 200 g soil
Nem.	16.31 a	12.27 a	344.50 a	389.5 a
VC + Nem.	4.32 bcd	2.12 bc	93.75 bc	44.5 b
VC + LVC 10 % + Nem.	2.02 d	1.45 c	89.25 c	50 b
VC + VW 10 % + Nem.	4.5 bc	2.63 bc	133.5 bc	36.75 b
LVC 100 % + Nem.	3 cd	1.62 c	112.5 bc	20.5 b
LVC 10 % + Nem.	4.55 bc	2.35 bc	107.25 bc	28 b
VW 100 % + Nem.	5.8 b	2.72 bc	133.5 bc	36.75 b
CF 10 % + Nem.	6.3 b	3.35 b	142.75 b	48 b

Data are expressed as mean of four replicates in each treatment. Numbers with the same letters in each column are not significantly different at 5 % level, LSD test

**Table 5** Effects of earthworm-based products (*E. fetida*) on the population factor of *M. javanica* in greenhouse condition

Treatment	Parameters			
	Final population (PF)	Reproductive factor (RF)	Multiplication rate (Mr %)	Nematode control %
Nem.	76,234 a	15.24 a	100 a	0 b
VC + Nem.	1,619 b	0.32 b	2.12 b	97.88 a
VC + LVC 10 % + Nem.	878 b	0.17 b	1.11 b	98.85 a
VC + VW 10 % + Nem.	8,258 b	1.64 b	10.83 b	86.75 a
LVC 100 % + Nem.	2,191 b	0.43 b	2.87 b	96.5 a
LVC 10 % + Nem.	6,361 b	1.26 b	8.34 b	91.5 a
VC 100 % + Nem.	12,443 b	2.48 b	16.32 b	86.5 a
CF 10 % + Nem.	5,926 b	1.18 b	7.76 b	92.25 a

Data are expressed as mean of four replicates in each treatment. Numbers with the same letters in each column are not significantly different at 5 % level, LSD test.  $Rf = \frac{Pf}{Pi}$



cation of earthworm-derived products (Rodriguez-Kabana 1986). VC may enhance and disperse predatory fungi and they trap the nematode larva and finally reduce the nematode population in the soil (Edwards and Fletcher 1988).

## Conclusion

Treatments with LVC while controlling the nematodes in the soil, reduced root infected indices to nematodes such as gall and egg No. and gall index in the root and at the same time, cucumber growth indices in treatments having VW get increased which indicated that nematode damage on the host plant has been compensated. It is worthy to say that the aim of nematode control is to bring the nematode below the economic threshold and in this context we can conclude that all these products could achieve reducing economic losses in the greenhouse conditions. According to our results, VC and VW resulted in remarkable effects on host plant growth indices and on the other hand, integration of VC with LVC reduced nematode population indices to a great extent.

In case we integrate these earthworm products, it is possible to achieve the nutrient supply for the host plant as well as toxin for the nematode and approach for the organic food production.

**Conflict of interest** The author declares that he has no competing interests.

**Authors' contributions** All authors read and approved the final manuscript.

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