



**Full Length Article**

## Effects of Fertilizers on Development of Root-Knot Nematode, *Meloidogyne javanica*

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### Abstract

Root-knot nematodes (*Meloidogyne* spp.) are known as one of the most important pests of summer crops. Although the use of synthetic nematicides is as a traditional effective method for their control, but heavy costs and adverse environmental effects are the concerns of this strategy. Hence, as an eco-friendly approach, an experiment was conducted to control *Meloidogyne javanica* infestation on tomato seedlings using four fertilizers including a chemical fertilizer (NPK), an organic fertilizer (Humic acid), a semi-organic fertilizer (Agro-health) and a bio-fertilizer (Azotobarvar-1). Nematicidal capacity of the fertilizers was evaluated on *M. javanica* populations *in vitro* and *in vivo* assays, as well as the effect of the fertilizers on plant growth properties were measured in a free-nematode assay. The fertilizers increased second instar juveniles ( $J_2$ s) mortality and decreased egg hatching *in vitro*. The highest  $J_2$ s mortality rate was found in Agro-health (64.4%), NPK (56.9%), Azotobarvar-1 (51.8%) and Humic acid (48.6%), respectively. Also, the highest reduction on nematode activity (galls, egg masses, and the reproduction factor) was observed in Agro-health (16.4%), NPK (23.8%), Azotobarvar-1 (26.7%) and Humic acid (30.0%) treatments, respectively, *in vivo*. According to the results, using Agro-health at the dose of 8 g kg<sup>-1</sup> soil led to an acceptable level of nematode control. Although the fertilizers were not as effective as synthetic nematicides in controlling *M. javanica*, some of them could be a good alternative to nematicides, because, along with the effect of repressive on the nematode, they also improved host plant growth indices. © 2020 Friends Science Publishers

**Keywords:** Alternative to pesticide; Eco-friendly strategy; Nematicidal potential; Soil amendment; Suppressive effect

### Introduction

Root-knot nematodes, *Meloidogyne* spp. (RKNs) are the most economically damaging plant parasitic nematodes (PPNs) and cause considerable horticultural and field crop losses worldwide (Hajji-Hedfi *et al.* 2018; Kiriga *et al.* 2018). Summer crops producers often use soil chemotherapy methods to control RKNs in Iran. Although synthetic nematicides provide a quick and effective control, but the heavy costs and the environmental concerns are their disadvantages. Hence, finding an effective and eco-friendly material or method has been considered to suppression of RKNs populations (Julio *et al.* 2017).

Research has shown that soil amendments, including micro- and macro-nutrients (Mansourabad *et al.* 2016), bacterial bio-fertilizers (El-Hadad *et al.* 2011), chemical fertilizers (Habash and Al-Banna 2011), composts (Hu and Cao 2008; Hu and Qi 2010; Ketabchi *et al.* 2016), plant extracts (Ntalli and Caboni 2012) and chopped plant tissues (Soheili and Saeedizadeh 2017), have repressive effects on populations of RKNs. Habash and Al-Banna (2011) found that phosphonate fertilizers, including

calcium phosphonate, magnesium phosphonate and potassium phosphonate were able to stop egg hatching and increased mortality of *M. incognita* and *M. javanica* infectious juveniles *in vitro*. These fertilizers also reduced root galling of *M. javanica* on host plants in a greenhouse. According to Soheili and Saeedizadeh (2017), treating the soil with Brassicaceous tissues [rapeseed (*Brassica napus* L. cv. Okapi); garden cress, (*Lepidium sativum* L. cv. local of Tehran); and cabbage (*B. oleracea* L. cv. Snow crown)], as green manures caused population suppression and decreased activity (galling and reproduction) of *M. javanica* in a greenhouse.

Despite these promising findings, the effective control of RKNs is still under investigation, and synthetic nematicides are widely used to tackle the menace. Since, the world's attention and approach to organic farming is on the rise, therefore, it has been considered the replacement of eco-friendly materials rather than synthetic pesticides. The current study focused on determining the suppressive effect of the chemical, organic, semi-organic and biological fertilizers on populations of RKN, *M. javanica* in Petri dish and pot experiments.

## Materials and Methods

### Preparation of *M. javanica* inoculum

The inoculum of *M. javanica* was prepared according to the single egg mass method. For this purpose, first, tomato roots (cv. Super Chief) with symptoms of RKN were collected from farms in Karaj, Iran. The roots were then washed gently in running tap water. Afterward, *M. javanica* was identified morphologically based on the perineal pattern of females using a microscope with 40x magnification (Cetintas and Cakmak 2016). Subsequently, in order to proliferate the nematode, the egg masses isolated from the galled roots were added to pots filled with steam-sterilized sandy soil and a tomato seedling, cv. Super Chief. The pots were then stored in a greenhouse ( $25 \pm 2^\circ\text{C}$  temperature, 12 h lighting). After two months, infected roots were harvested, washed, crushed, and disinfected with 1% NaOCl solution. The eggs suspension was then incubated in distilled water at  $25 \pm 2^\circ\text{C}$  for a week. Afterward, the obtained second-stage juvenile ( $J_2$ s) were collected, counted, and considered as *M. javanica* inoculum (Saeedizadeh 2016).

### In vitro assays

*In vitro* assays were arranged to determine the effect of the fertilizers; bio-fertilizer (Azotobarvar-1<sup>®</sup>, Granular 10%, containing Azotobacter spp., Green Biotech Co., Iran), semi-organic fertilizer (Agro-health<sup>®</sup>, Emulsifiable Concentrate 10%, Khorram Bahar Atis Co., Iran), chemical fertilizer (NPK, Powder 20-20-20, Zagross Co., Iran), and organic fertilizer (Humic acid, Emulsifiable Concentrate 10%, Zagross Co., Iran); on  $J_2$ s mortality and egg hatching rate (%) of *M. javanica* in Petri dish experiments. The egg hatching assay was conducted using eggs suspension as a population under test. The density of the eggs suspension was estimated by an inverted microscope (40x). The suspension was transferred to plastic Petri dishes (5 cm diameters) with 200 eggs as a replicate. The eggs were exposed to fertilizers at doses of 1, 2, 4 and 8 g L<sup>-1</sup> distilled water, individually. A treatment of a reference nematicide, Fenamiphos (Nemacur<sup>®</sup> Granular 10%, Bayer Crop Science AG, Germany) was arranged as a positive control at dosage of 0.5, 1, 1.5 and 2 g L<sup>-1</sup> distilled water. Also eggs in distilled water (no fertilizer and no nematicide) were considered as a negative control. The eggs suspensions were incubated at  $25 \pm 2^\circ\text{C}$ , relative humidity 80% and without light for eight days. Egg hatching rate (%) was determined based on the number of hatched eggs by using an inverted microscope (40x) on the second, fourth, sixth, and eighth days from the start of the assay. Each treatment had three replicates. To check recovery, the eggs were transferred in fresh water and incubated after eighth day of exposure in order to ensure the nematocidal or nematostatic effect of the fertilizers (Habash and Al-Banna 2011).

To evaluate the effect of the fertilizers on  $J_2$ s mortality

rate (%) of *M. javanica*, an assay was arranged using  $J_2$ s suspension. A suspension of eggs was incubated at  $25 \pm 2^\circ\text{C}$  for a week. Then, the emerged  $J_2$ s were collected and counted as a population under test.  $J_2$ s population was set to 200  $J_2$ s per plastic Petri dish (with 5 cm diameter). The  $J_2$ s were exposed to the fertilizers at four doses of 1, 2, 4 and 8 g L<sup>-1</sup> distilled water, and the nematicide, Fenamiphos with four doses of 0.5, 1, 1.5 and 2 g L<sup>-1</sup> distilled water at  $25 \pm 2^\circ\text{C}$ , relative humidity 80% and without light. A negative control was also considered (no fertilizer and no nematicide). To estimate  $J_2$ s mortality rate (%) the number of dead  $J_2$ s was determined every day up to four days using stereomicroscope (40x). Each treatment had three replicates (Petri dishes) (Habash and Al-Banna 2011).

### In vivo assays

A pot assay was conducted to evaluate suppressive effect of the fertilizers (Azotobarvar-1, Agro-health, NPK and humic acid) on *M. javanica* infestation at the greenhouse. The soil required for the assay was collected from the same field as the inoculum, because it was a previously suitable environment for the growth and proliferation of nematode. The soil texture was a loamy sand with 85% sand, 11% silt, 4% clay, 0.3% organic matter, and a pH of 7.2. The collected soil, approximately 366 kg, was mixed, sieved through a screen (5 mesh) and autoclaved. Then, the suspension of the nematode inoculum was thoroughly mixed in the soil (10  $J_2$ s g<sup>-1</sup> soil). The nematode-inoculated soil was divided to 61 masses (5 amendments  $\times$  4 doses  $\times$  3 host plants) + 1 control (dose 0) in quantities of 6 kg (2 kg for a pot  $\times$  3 replicates). The soil masses were thoroughly mixed with the fertilizers (at doses 1, 2, 4, and 8 g kg<sup>-1</sup> soil), according to the treatments, individually. It was arranged a treatment of Fenamiphos (at doses 0.5, 1, 1.5 and 2 g kg<sup>-1</sup> soil) as a positive control; and a negative control (dose 0 or no fertilizer no nematicide). Then, the treated soils were transferred to plastic pots in quantities of 2 kg. After that, a four-leaf seedling of the host plant, previously grown in a sterile sandy substrate, was planted in the pots, according to treatments, individually. Host plant was tomato, *Lycopersicon esculentum* Mill. cv. Super Chief. It was considered three replicates for each dose. A pot containing one plant was represented as a replicate. The pots were kept in the greenhouse for two months; and were sufficiently irrigated.

In the pot assay, the studied traits of the nematode activity on the host plants included the number of galls and egg masses per root, and reproduction factor (RF). Two months after planting, the roots were collected and washed gently in running tap water. Then, the number of galls per root was counted. To determine egg mass numbers per root, the roots were crushed into pieces of 2 to 3 cm and exposed to a solution of Floxin B, 0.15 g L<sup>-1</sup>. Then, highlighted egg masses were counted using a stereomicroscope (20x). Also, RF was calculated as an indicator of reproduction and pathogenicity of *M. javanica* as follows:  $\text{RF} = \text{Pf.Pi}^{-1}$ , where

Pf is the final population (eggs, females, J<sub>3s</sub> and J<sub>4s</sub> into the plant's root system plus J<sub>2s</sub> and males in the soil) and Pi is the inoculum's initial population (Maleita *et al.* 2012; Soheili and Saedizadeh 2017). To determine of the nematode population in the soil, a 100 g subsample of well mixed soil from each replicate (pot) was processed according to centrifuge or sugar flotation method. The extracted nematode population was counted using a stereomicroscope (40x) and then it was applied to estimate the population of nematodes per 1 kg soil (pot). To estimate the number of females and juveniles (J<sub>3s</sub> and J<sub>4s</sub>) into the roots, 2 g subsample of well mixed chopped roots was macerated in a Warring blender, and then, the nematode population was counted using a stereomicroscope (40x) in the obtained suspension. The numbers of nematodes present in a root were calculated by multiplying the numbers of nematodes present in the subsample of the root, considering the total weight of the root (Soheili and Saedizadeh 2017).

In the second pot assay, the fertilizers effect on plants seedling growth properties were evaluated under the similar conditions to the first pot assay and free of the nematode inoculum. In this assay, after soil treatment with the amendments (according to the doses) and transferring to the pots, three seeds of the host plant (tomato, Super Chief cv.) were sowed in each pot. After germination, the seedlings were thinned so that in each pot remained a seedling. The pots were kept in the greenhouse; and the plants were irrigated as needed. The pot containing one plant was represented a replicate. Two months after sowing seeds, plants were taken, rewetted, weighed fresh root and shoot, and measured length of stem, as host plants growth properties (Soheili and Saedizadeh 2017).

### Data analysis

The data was analyzed using S.A.S. software version 9, SAS Institute Inc., Cary, N.C., USA. Charts were drawn using Microsoft Excel 2013 software. Three replicated pots per treatment were arranged in a completely randomized design.

## Results

### *In vitro* assays

The most suppressive effect of the fertilizers was observed on egg hatching rate (%) of *M. javanica* by Agro-health (16.4%), NPK (23.8%), bio-fertilizer (26.7%) and Humic acid (30.0%) treatments, respectively. The egg hatching decreased by increasing the dosage of the fertilizers at the same exposure times (Fig. 1; 2). Also, the use of the fertilizers increased the mortality rate of J<sub>2s</sub> population. So that, the highest level of J<sub>2s</sub> mortality was resulted in Agro-health (64.4%), NPK (56.9%), bio-fertilizer (51.8%) and Humic acid (48.6%) treatments, respectively. The dead J<sub>2s</sub> population increased as a result of increasing the dose and exposure time (Fig. 3; 4). The J<sub>2s</sub> mortality rate reached the highest level (100%) in treatments of Agro-health and NPK

at a final dose (8 g L<sup>-1</sup> d.H<sub>2</sub>O), however, the treatments in this dose could not stop the egg hatching completely on the last exposure time (eighth day) (Fig. 1; 3).

### *In vivo* assays

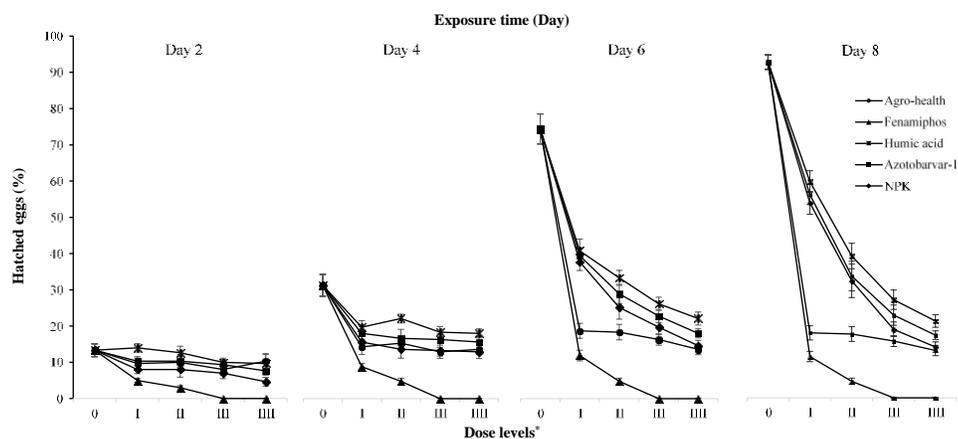
All treatments significantly ( $P \leq 0.05$ ) reduced the pathogenicity and reproduction indices (the number of gall and egg mass per root and RF) of *M. javanica*. The suppressive effect of Agro-health on *M. javanica* was ranked second after the nematicide, Fenamiphos. Moreover, its nematode controlling effect was more than that of the bio-fertilizer, Azotobarvar-1, NPK and Humic acid, respectively. The concentration of 8 g kg<sup>-1</sup> soil of Agro-health and Azotobarvar-1 was found to have the highest suppression effect, whereas the application of Fenamiphos at 2 g kg<sup>-1</sup> of soil highly significantly ( $P \leq 0.05$ ) reduced the galling and reproduction of *M. javanica* (Fig. 5).

Notably, the highest values of plant growth parameters were obtained when the seedlings were treated with both Azotobarvar-1 and Agro-health, while Fenamiphos did not improve plant growth compared to the control plants. Moreover, no significant ( $P \leq 0.05$ ) difference was found for shoot and root fresh weight between those pots treated with Azotobarvar-1 and Agro-health (Fig. 6).

## Discussion

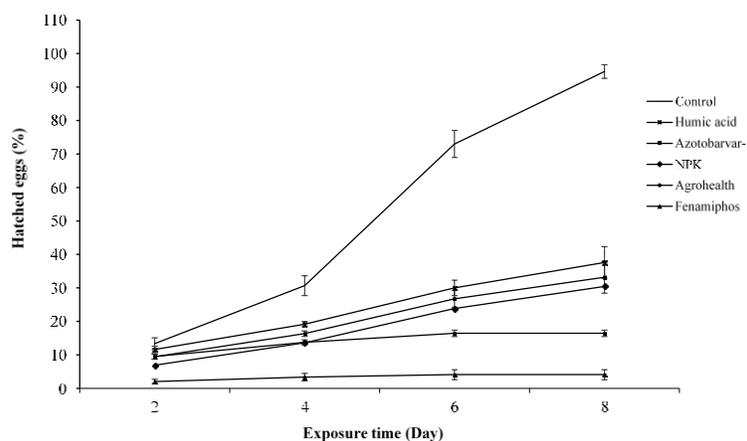
The acceptance of organic and semi-organic fertilizers for controlling PPNs is considerably increasing, since most old synthetic nematicides have been put aside due to application heavy costs and their environmental concerns. The results of the current study are supported by findings of others in the field of non-chemical control of phytonematodes, through a soil amendment by plant-based and biological fertilizers, which show the use of non-chemical strategies is the remarkable solution to manage PPNs (Hu and Qi 2010; Oka 2010; Mcorley 2011; Tranier *et al.* 2014; Mansourabad *et al.* 2016; Kepenekci *et al.* 2017; Soheili and Saedizadeh 2017). Furthermore, application of plant extracts has been effective in controlling these nematodes *in vitro* and *in vivo* (Ntalli and Caboni 2012; Aissani *et al.* 2013; Sardari *et al.* 2015). Likewise, the results of the current work showed that Agro-health, as a fertilizer based on herbal extracts, has a considerable potential to contribute as a successful environmental-friendly fertilizer in the management of *M. javanica*.

Active ingredient of Agro-health contains a mixture of herbal essential oils; castor-oil (*Ricinus communis*), cinnamon (*Cinnamomum verum* J. Presl); acetic acid and nitrate fertilizer. It is expected the suppressive effect of Agro-health on the RKN is related to the presence of plant and non-herbal compounds in the product. The anti-nematode ability of castor-oil, cinnamon, nitrate and acetic acid have been previously reported (Kong *et al.* 2007; Dong *et al.* 2012; Pan *et al.* 2015; Ntalli *et al.* 2016). Ozdemir and Gozel (2017)

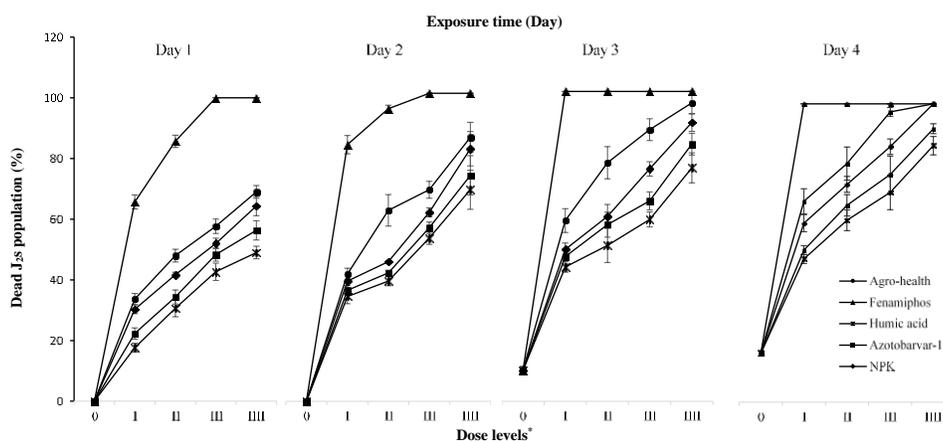


**Fig. 1:** Effect of amendments (NPK, Humic acid, Agro-health, Azotobarvar-1 and Fenamiphos) and their dose levels on egg hatching of *M. javanica* at exposure times

\* Defined doses at the X axis (0, I, II, III and IIII) respectively correspond to 0, 1, 2, 4 and 8 g L<sup>-1</sup> distilled water for the fertilizers; and 0.5, 1, 1.5, 2 and 2.5 g L<sup>-1</sup> distilled water for the nematicide (Fenamiphos)

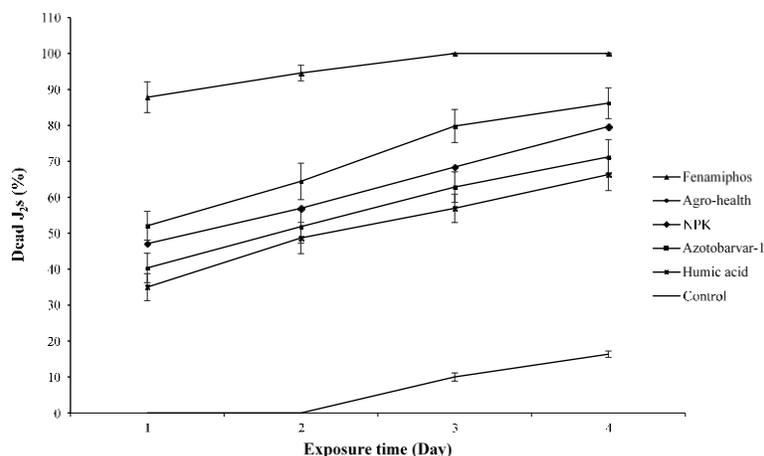


**Fig. 2:** Effect of amendments (NPK, Humic acid, Agro-health, Azotobarvar-1 and Fenamiphos) on egg hatching of *M. javanica* at exposure times

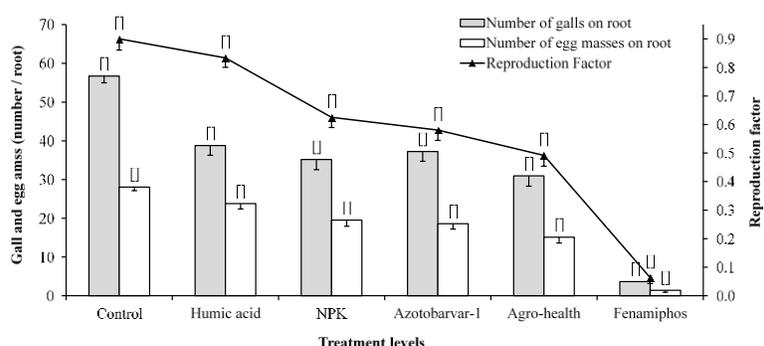


**Fig. 3:** Effect of amendments (NPK, Humic acid, Agro-health, Azotobarvar-1 and Fenamiphos) and their dose levels on J<sub>2</sub>s mortality of *M. javanica* at exposure times

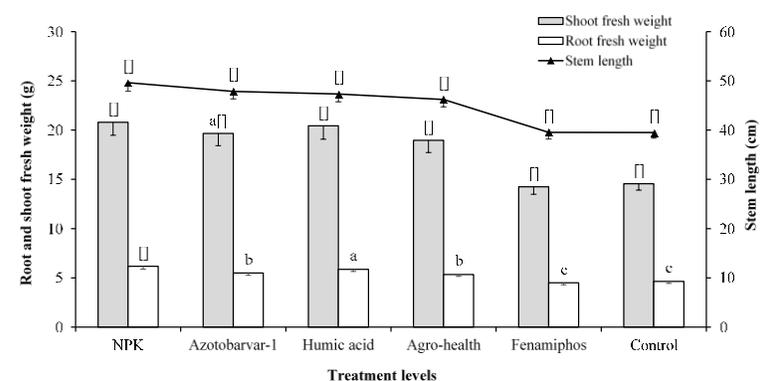
\* Defined doses at the X axis (0, I, II, III and IIII) respectively correspond to 0, 1, 2, 4 and 8 g L<sup>-1</sup> distilled water for the fertilizers; and 0.5, 1, 1.5, 2 and 2.5 g L<sup>-1</sup> distilled water for the nematicide (Fenamiphos)



**Fig. 4:** Effect of amendments (NPK, Humic acid, Agro-health, Azotobarvar-1 and Fenamiphos) on J<sub>2</sub>s mortality of *M. javanica* at exposure times



**Fig. 5:** Effects of treatments of soil amendments (NPK, Humic acid, Agro-health, Azotobarvar-1 and Fenamiphos) on the number of galls, egg masses, and reproduction factor of *M. javanica* on the root of tomato plants



**Fig. 6:** Effect of soil amendments (NPK, Humic acid, Agro-health, Azotobarvar-1 and Fenamiphos) on growth properties (fresh weight of root and shoot and length of stem) of tomato seedlings in the free-nematode treatments

found that herbal essential oils have had a high potential to become an alternative control strategy against PPNs. In a pot experiment by using urea coated with different doses, Wani and Bhat (2012) reported soil amendment with castor-oil reduced *M. incognita* populations, and developed plant growth properties and increased chlorophyll content of host

plant leaves. Also, the intercropping of castor-oil with cucumber (as a main crop) reduced the pathogenicity activity of *M. incognita* by 71.54% (Dong *et al.* 2012). Kong *et al.* (2007) reported that cassia [*Cinnamomum cassia* (L.) J. Presl] and cinnamon oil compounds have suppressive activity on pinewood nematode,

*Bursaphelenchus xylophilus* (Sterner and Buhrer) Nickle. Cassia and cinnamon oils and test compounds described merit further study as potential nematicides or leads for the control of pine wilt disease caused by *B. xylophilus*. The results of current study showed that, in addition to controlling *M. javanica* galling and reproduction, Agro-health also caused a significant promotion in plant growth indices which is due to the existence of a significant amount of nitrate in it. It has been reported about the potential nematicides nitrogenous compounds (Farahat *et al.* 2012; Pan *et al.* 2015). The use of organic compounds such as acetic acid, leading to structural deformities J<sub>2</sub>s of *M. incognita*. Obviously, an increase in the number of damaged and dead J<sub>2</sub>s will reduce their penetration and pathogenicity (Ntalli *et al.* 2016).

It seems the application of the semi-organic fertilizers; a mixture of essential oils of plants and chemical fertilizers; could be sound alternative strategy of RKNs management as it may lessen the problems of application of synthetic nematicides therewith improve the net income product. Although the active ingredients in Agro-health are not known as a reference nematicide, but they reduced the pathogenicity and reproduction indices of the RKN to an acceptable level and hence this product could be a suitable alternative to chemical nematicides because of the significant improvement in plant growth indices as well as its eco-friendly properties. It means, they have not been able to control the severe infestations of PPNs on field soils, but they are able to inhibit the development of PPNs populations by repressive properties.

Since short-term non-chemical strategies do not help to control of RKN, long-term use of Agro-health will certainly have more effective results. However, the effect of organic and semi-organic fertilizers has not been as much as synthetic nematicides in RKNs control, but the fertilizers will not lead to ecological concerns. In addition to developing crop performance, the fertilizers have positive results on nutrition, physical conditions and microbial populations of the soil. It seems these fertilizers need time to demonstrate their nematicidal ability and to be introduced as a real nematicide. More research is needed for better understanding of semi-organic fertilizers, and their nematicidal capacity. However, soil amendment with the fertilizers is recommended to control RKNs due to the positive effect of the fertilizers on growth of plants, and also the suppressive capacity of these materials on soil-inhabitant PPNs populations.

## Conclusion

The semi-organic fertilizer, Agro-health could be a suitable alternative to synthetic nematicides, with regard to the significant improvement of plant growth indices as well as its eco-friendly properties. However, further studies are needed for a better understanding of the effects of non-chemical nematicides on the activity of RKNs. Because of

the contribution to plant growth and due to the lack of destructive effects on the population of soil saprophytic microbes, the use of the fertilizer would have a positive feedback in the integrated management of RKNs in the future.

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