Saudi Journal of Biological Sciences 28 (2021) 3759–3767

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# Evaluation of the effect of different concentrations of organic amendments and botanical extracts on the mortality and hatching of Meloidogyne javanica

Sukalpa Das [a,b], Abdul Wadud [c,d], Md. Atiqur Rahman Khokon [e,*]

[a] Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
[b] Department of Agricultural Extension, Bangladesh
[c] Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
[d] Bangladesh Agricultural Research Institute
[e] Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh

ARTICLE INFO

*Article history:*
Received 10 January 2021
Revised 9 March 2021
Accepted 10 March 2021
Available online 18 March 2021

*Keywords:*
*Meloidogyne javanica*
Vermicompost
Biogas digestate
Marigold
Cabbage
Mortality
Hatching

ABSTRACT

Organic amendments and botanical extracts are considered as some of the eco-friendly alternatives to chemical pesticide in suppressing plant pathogenic nematodes (PPN). Root-knot nematode (RKN) is the most important group of PPN distributed globally causing both qualitative and quantitative damage to many crops. Vermicompost and biogas digestate (BD) are two forms of organic amendments reported to have potential to limit RKN infestation. Likewise, marigold (*Tagates* spp.) and cabbage (*Brassica oleracea*) are two widely studied botanicals having shown their potential to control RKN. However, there was not much in vitro research related to organic amendments and botanicals targeting a particular species of RKN to observe their nematicidal effect. An in vitro experiment was undertaken to evaluate the effect of these organic amendments and botanical extracts at different concentrations (10.0%, 25.0%, 50.0% and 100.0%) on the hatching and mortality of *Meloidogyne javanica* at different time spans. Mortality of $J_2$ and inhibition of hatching of egg mass of *M. javanica* differed significantly ($p < 0.0001$) among the interaction effect of treatments and incubation time for both organic amendments and botanical extracts. Findings of this experiment indicated that potentiality for increasing mortality and inhibition of hatching was higher and steadier in botanical extracts than those of organic amendments.


## 1. Introduction

Plant pathogenic nematodes (PPN) are responsible for causing 12.6% of global crop loss which is equivalent to the estimated amount of 215.77 billion US dollars (Abu-Elgawad and Askary, 2015). PPN are worm-like pseudocoelomate, unsegmented animals comprising about 15% of all forms of nematodes (Decraemer and Hunt, 2006). They have different types of feeding behavior and are mostly subterranean in nature (Decraemer and Hunt, 2006). The symptoms expressed on plants by PPN are very similar to that with fungal attack, water stress or other physiological disorders which made them to be considered as the hidden enemy of farmers. Root-knot nematodes (RKN) belonging to the genus *Meloidogyne* are the most prominent group of PPN distributed worldwide. RKN can parasitize more than 3000 species of plant causing an estimated crop loss of worth 100 billion US dollar annually (Hunt and Handoo, 2009; Dejene, 2014). Out of 106 described species of RKN, 95% of infestations are caused by only 4 of them *viz*. *Meloidogyne incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Sasser et al., 1983; Karssen and Moens, 2006).

As an easy way out, farmers prefer to use synthetic chemical pesticides to control PPN infestation. Soil fumigants, organophosphate and carbamate groups of pesticides are some of the widely used chemical nematicides against PPN (Dejene, 2014). However, these broad spectrum pesticides are non-selective, detrimental for many non-target organisms, highly toxic to the environment and increase the production cost to a greater extent (Kepenekci et al., 2017). In addition to that, long term use of these chemicals

* Corresponding author.
*E-mail address:* atiq.ppath@bau.edu.bd (Md. Atiqur Rahman Khokon).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.03.041
1319-562X/

is giving rise to the emergence of resistance-breaking nematode pathotypes on many important crops, resulting in the prohibition or restrictions on various substances employed worldwide (Abu-Elgawad and Askary, 2015; Silva et al., 2017). Therefore, scientists are in a continuous quest for non-chemical and efficient alternative methods for the management of PPN (Huang et al., 2016).

Use of organic amendments are considered as one of the eco-friendly alternatives to chemical pesticide in suppressing PPN (Xiao et al., 2016). Moreover, soil organic amendments are available at low cost and develop a positive impact on plant growth, physical, chemical and biological properties of the soils (Davey, 1996). Vermicompost, composted in the presence of earthworm, is one of the important organic amendments benefiting the crop production by its high porosity, aeration, drainage, water-holding capacities and low C: N ratios (Edwards, 1998). It was reported that vermicompost significantly decreased the RKN-induced galls on plants and significantly increased root defense metabolite concentration (Xiao et al., 2016). According to many findings, abundant humic acid substances and different hormones such as IAA, cytokinins and gibberellins present in vermicompost could reduce nematode infestation (Oka, 2010). However, there was not much in vitro research carried out targeting a particular species of RKN to observe the nematicidal effect of vermicompost on it. Biogas digestate (BD) is another form of organic amendment reported to have potential to limit PPN infestation (Min et al., 2011). Due to the ever-exhausting reserve of fossil fuel and to reduce the negative impact of it on the environment, biodegradable organic waste is being considered as the alternative energy source (Koszel and Lorencowicz, 2015). Biogas plant is one of the environment-friendly energy producers that anaerobically decompose the organic waste to produce biogas and digestate as byproduct (Koszel and Lorencowicz, 2015). The solid form of the digestate can be used as a bio-fertilizer that improves soil fertility, crop quality and their immunity to biotic and abiotic agents (Koszel and Lorencowicz, 2015). The mechanism by which organic amendments suppresses PPN are still speculative (Arancon et al., 2002). It is reported that PPN are controlled by the stimulation of naturally occurring antagonists and by changing the soil nematode community structure (Min et al., 2011). Thus the nematicidal activity of organic amendment should be specifically assessed (Renco, 2013).

Bio-fumigation is a non-chemical approach to suppress soil borne pests and diseases by which parts of different plant species are incorporated into the soil (Berbegal et al., 2008). Various plants are important sources of compounds with nematicidal properties (Abo-Elyousr et al., 2010; Taye et al., 2012; da Silva et al., 2019). Marigold (*Tagates* spp.) and cruciferous plants of *Brassica* spp. are two widely studied groups of botanicals having shown their potential to control RKN (Zasada and Ferris, 2004; Abo-Elyousr et al., 2010; Youssef and Lashein, 2013). It was reported in several experiments that plants of *Tagates* spp. could immobilize second-stage juveniles ($J_2$) and reduce root galling and nematode reproduction (Abo-Elyousr et al., 2010; Tibugari et al., 2012; Taye et al., 2012). However, its effect on the population of RKN is highly variable depending on the combination of cultivar of *Tagates* spp. and species of RKN (Karssen and Moens, 2006). It is assumed that marigold plants reduce the RKN population by exerting an antagonistic effect against them because $J_2$ enters roots but fails to form giant cells (Karssen and Moens, 2006). On the other hand, mechanism of control of RKN by cabbage (*Brassica* spp.) is attributed to the release of glucosinolates which on hydrolysis produce isothiocyanate (ITC) that have toxic effect to certain nematode species (Zasada and Ferris, 2004). Successful management of PPN by ITC depends on the incorporation of appropriate amounts of glucosinolate-containing biomass and sensitivity of the target PPN species to ITC (Zasada and Ferris, 2004). Therefore, RKN species-specific further research is necessary.

$J_2$ and egg mass are two most targeted stages of RKN management, because $J_2$ moves through soil to infect the plant and egg mass remains outside the roots. There are few studies concerning the direct effect of organic amendments and bio-fumigants on hatchability of egg mass and mortality of $J_2$ of RKN. The aim of this in vitro experiment was to assess the efficacy of different concentrations of organic amendments (vermicompost and BD) and bio-fumigants (marigold and cabbage) on the hatching and mortality of *M. javanica* considering different time intervals. It was hypothesized that these two groups of bio-compounds would be successful in managing *M. javanica* by impacting the hatching and mortality of $J_2$. The most effective concentration of these compounds obtained in the experiment would help in predicting the application rate in the field. Moreover, the findings of the experiment would provide a fair basis of comparison between these two groups of non-chemical approaches concerning their affectivity in suppressing *M. javanica*.

## 2. Materials and methods

### 2.1. Preparation of marigold (Tagetes spp.) and cabbage (Brassica oleracea) leaf extract

Marigold and cabbage leaf extracts were prepared by the method described by Orisajo et al. (2007) with some modifications. Leaves of marigold and cabbage were chopped into 1–2 cm pieces. Chopped leaves were washed by tap and distilled water subsequently. Twenty five gram of fresh leaves was ground in 250 ml (1 g/10 ml basis) of distilled water in a blender for 3 min. The blended mixture was kept undisturbed for 72 h and filtered through white cotton cloth. The filtrate was centrifuged at 5000 rpm for 10 min and the supernatant was stored as a stock solution of 100.0% concentration. Other concentrations (10.0%, 25.0%, and 50.0%) of leaf extracts were prepared by diluting the stock solution with the required amount of double distilled water (DDW). Solutions of different concentrations of leaf extracts were considered as treatments.

### 2.2. Preparation of vermicompost tonic (VT)

Vermicompost was prepared by using different animal dung and agro/kitchen waste on vermibed (Nath and Singh, 2011). Earthworm (*Eisenia foetida*) was used as a composter. VT was prepared by dissolving 4 g of vermicompost in 100 ml water (Kumar et al., 2011). The solution was centrifuged at 5000 rpm for 10 min. The supernatant was stored as a stock solution of 100.0% concentration. Other concentrations (10.0%, 25.0%, and 50.0%) of tonic were prepared by diluting the stock solution with the required amount of double distilled water (DDW). Solutions of different concentrations of VT were considered as treatments.

### 2.3. Preparation of biogas digestate (BD) solution

Solid BD was obtained from the biogas plant at Mymensingh, Bangladesh. Cattle dung and agro/kitchen waste was used as a source of biogas production. Solution of BD was prepared by the method described by Huang et al. (2015) with some modifications. Solid BD was ground to powder of < 1 mm particles and 1.2 g of it was dissolved in 100 ml distilled water. The solution was centrifuged at 5000 rpm for 10 min and the supernatant was stored as a stock solution of 100.0% concentration. Other concentrations (10.0%, 25.0%, and 50.0%) of BD were prepared by diluting the stock solution with the required amount of DDW. Solutions of different concentrations of BD were considered as treatments.

### 2.4. Nematode inoculum

Egg masses and $J_2$ used in this experiment were randomly collected from previously characterized pure culture of *M. javanica*, maintained and raised in brinjal (*Solanum melongena* L.) plants at the net-house of the Seed Pathology Centre (SPC) of Bangladesh Agricultural University (BAU).

### 2.5. Mortality study

The brinjal plants inoculated with the egg masses of *M. javanica* were uprooted from soil and the root system was washed gently with running tap water to remove adhering soil. Egg masses of *M. javanica* were gently picked using forceps. Eggs were incubated for 48 h using Baermann funnel method (Baermann, 1917) to obtain $J_2$. Population density of $J_2$ was calculated from 5 replications of one ml aliquots of an inoculum suspension. Freshly hatched one hundred (48 h old) $J_2$ were put in a 2.5 cm diameter Petri plate containing 5 ml solution of each treatment. $J_2$ kept in tap water was treated as control. Plates were covered with a lid and incubated at room temperature (25 ± 2 °C) during the experiment period. Each treatment was replicated 3 times. Data on mortality was recorded every 3 days after incubation (DAI) and continued up to 9 DAI. Mortality of the $J_2$ was assessed by observing the mobility of the $J_2$ under stereo microscope (Zeiss, Carl Zeiss Microscopy GmbH, Germany) at 60X magnification and expressed as the percentage of the total population. The moribund and non-mobile $J_2$ were prodded using a 'fishing' needle to check for mobile responses (Das et al. 2011).

### 2.6. Assessment of hatching inhibition

Five egg masses of *M. javanica* were kept on a 48-µm sieve fixed at the perforated cap of an inverted eppendorf tube and immersed in 5 ml solution of each treatment in a small plastic bottle (Khokon et al., 2009). Egg masses kept in tap water were treated as control. Each treatment was replicated 3 times. The bottles were kept at room temperature (25 ± 2 °C). Number of hatched $J_2$ was counted in a counting dish under stereo microscope (Zeiss, Carl Zeiss Microscopy GmbH, Germany) and the solution of each treatment was replaced after every counting. Data was recorded at every 1 week interval and continued until 6th week. Percent egg hatch inhibition over control was calculated using the formula (Mahesha et al., 2017):

$$\text{Percent egg hatch inhibition} = (C\text{-}T)/C \times 100$$

where C = Number of hatched $J_2$ in control and T = Number of hatched $J_2$ in treatment.

### 2.7. Statistical analysis

Statistical analyses were done by Statistix 10 (© 1985–2013 Analytical Software, Miller Landing Rd, Tallahassee, FL 32312) and MS Excel. Two-way ANOVA was performed to determine the significance of the interaction effect of different concentrations of two types of bio-control agents (organic amendments and plant extracts) and time on the mortality and hatching inhibition. Tukey's HSD test was performed at 5% level of probability to find the significant difference among means.

## 3. Results

In this experiment, the influence of different concentrations of two organic amendments (vermicompost and BD) and two plant extracts (cabbage and marigold) on the hatching and mortality of $J_2$ of *M. javanica* was evaluated considering different incubation time. Four different concentrations (10.0%, 25.0%, 50.0%, and 100.0%) of organic amendments and plant extracts were used for both hatching and mortality experiments. Tap water was treated as control.

Mortality of $J_2$ of *M. javanica* differed significantly ($p < 0.0001$) among the interaction effect of treatments and incubation time for both organic amendments and plant extracts. Although mortality was significantly ($p < 0.0001$) higher than control in all treatments, the trend of mortality was different among organic amendments and plant extracts (Table 1, 2, 3 and 4). In VT, rate of mortality increased with the progress of incubation time in all concentrations and the highest percentage of mortality (60.0%) was recorded at lower concentration (10.0%) of it at nine days after incubation (DAI) (Table 1). Similar trend of mortality was observed for BD and the highest mortality, although it was<50%, was observed at 9 DAI in 50.0% concentration (Table 2). On the contrary, 100% mortality was observed at 3 DAI for 100.0% concentration of marigold extract and more that 50% of mortality was recorded for both 25.0% and 50.0% concentration at 6 DAI (Table 3). In cabbage extract, 100% mortality of $J_2$ was recorded at 6 DAI in 100.0% concentration and it reached to 79% and 88% in 25.0% and 50.0% concentration, respectively at 9 DAI. The irregular pattern of mortality and comparatively lower mortality rate of $J_2$ even at the end of the experiment period in different concentrations of two organic amendments suggested that these were less effective than botanical extracts in causing mortality of $J_2$ of *M. javanica*.

The hatching experiment was continued up to 6 WAI. In the experiment, the number of hatched $J_2$ was the highest in control at the second week after incubation (WAI) that declined gradually in the following weeks of the experiment (Figs. 1–4). Hatching of $J_2$ was lower than control at 1st and 2nd week after incubation (WAI) but no marked difference in the pattern of hatching was observed among the concentrations of organic amendments with the progress of time up to the end of the experiment (Figs. 1 and 2). Unlike organic amendments, in all concentrations of both marigold and cabbage extract, hatching of $J_2$ was lower than control throughout the experiment (Figs. 3 and 4).

In this work, inhibition of hatching of $J_2$ of *M. javanica* was found to be significantly ($p < 0.0001$) different among the interaction effect of concentration and time for both organic amendments and botanical extracts (Table 5–8). Although it was recorded in the

**Table 1**
Effect of different concentrations of vermicompost tonic (VT) and incubation time on the mortality of $J_2$ of *M. javanica*.

| Time | Treatment (%) | Mortality (%) |
|---|---|---|
| 3 DAI | 10.0 | 17.67 ± 2.02 (d-f) |
| | 25.0 | 24.33 ± 0.88 (c-e) |
| | 50.0 | 14.67 ± 2.02 fg |
| | 100.0 | 17.33 ± 1.85 ef |
| | Water | 2.33 ± 0.88 h |
| 6 DAI | 10.0 | 32 ± 1.15c |
| | 25.0 | 44 ± 1.15b |
| | 50.0 | 27.67 ± 2.18c |
| | 100.0 | 25.33 ± 1.2 cd |
| | Water | 4.66 ± 1.2 h |
| 9 DAI | 10.0 | 60 ± 1.53 a |
| | 25.0 | 53 ± 1.15 a |
| | 50.0 | 31.67 ± 1.45c |
| | 100.0 | 44 ± 1.73b |
| | Water | 7.33 ± 1.67 gh |
| Level of significance | * | |
| CV (%) | 9.79 | |

Values are the mean ± Standard Error of three replicates. Treatment means were compared by two-way ANOVA. Same letter in a column do not differ significantly according to Tukey's test at 5% probability.
DAI = Days after incubation *1% level of probability.

**Table 2**
Effect of different concentrations of biogas digestate (BD) and incubation time on the mortality of $J_2$ of *M. javanica*.

| Time | Treatment (%) | Mortality (%) |
|---|---|---|
| 3 DAI | 10.0 | 7.33 ± 0.66 (g-i) |
| | 25.0 | 12.33 ± 0.88 (f-h) |
| | 50.0 | 16.33 ± 2.72 fg |
| | 100.0 | 19 ± 2.65f |
| | Water | 2.33 ± 0.88 i |
| 6 DAI | 10.0 | 32 ± 2.08 e |
| | 25.0 | 32.67 ± 1.45 de |
| | 50.0 | 46.67 ± 2.72 ab |
| | 100.0 | 42.67 ± 0.88 (a-c) |
| | Water | 4.66 ± 1.2 hi |
| 9 DAI | 10.0 | 33.67 ± 2.02 (c-e) |
| | 25.0 | 38.33 ± 1.76 (b-e) |
| | 50.0 | 49 ± 2.51 a |
| | 100.0 | 42 ± 1.52 (a-d) |
| | Water | 7.33 ± 1.67 (g-i) |
| Level of significance | * | |
| CV (%) | 12.43 | |

Values are the mean ± Standard Error of three replicates. Treatment means were compared by two-way ANOVA. Same letter in a column do not differ significantly according to Tukey's test at 5% probability.
DAI = Days after incubation *1% level of probability.

**Table 3**
Effect of different concentrations of marigold extract and incubation time on the mortality of $J_2$ of *M. javanica*.

| Time | Treatment (%) | Mortality (%) |
|---|---|---|
| 3 DAI | 10.0 | 19.33 ± 1.76f |
| | 25.0 | 28.66 ± 1.2 e |
| | 50.0 | 25.56 ± 2.9 ef |
| | 100.0 | 100 ± 0 a |
| | Water | 2.33 ± 0.88 g |
| 6 DAI | 10.0 | 29 ± 0.57 e |
| | 25.0 | 60 ± 1.73 d |
| | 50.0 | 58 ± 0.57 d |
| | 100.0 | 100 ± 0 a |
| | Water | 4.66 ± 1.2 g |
| 9 DAI | 10.0 | 32 ± 2.08 e |
| | 25.0 | 71 ± 2.3c |
| | 50.0 | 86 ± 2.65b |
| | 100.0 | 100 ± 0 a |
| | Water | 7.33 ± 1.67 g |
| Level of significance | * | |
| CV (%) | 5.76 | |

Values are the mean ± Standard Error of three replicates. Treatment means were compared by two-way ANOVA. Same letter in a column do not differ significantly according to Tukey's test at 5% probability.
DAI = Days after incubation *1% level of probability.

experiment that VT inhibited the hatching of $J_2$, the pattern of inhibition was unsteady over the experiment period (Table 5). The lowest concentration of VT (10.0%) showed the highest percentage of inhibition (75%) at 1 WAI. Similarly, an irregular pattern of hatching inhibition was observed in different concentrations of BD during the experiment (Table 6). More than 80% of inhibition was observed in 25.0% and 100.0% concentration of BD at 1 WAI and 6 WAI respectively. Inhibition of hatching was above 75% at 1 WAI in all concentrations of marigold extract and it was 93% and 100% in 50.0% and 100.0% concentration respectively (Table 7). However, at the lower concentrations (10.0% and 25.0%) of marigold extract, inhibition of hatching was found irregular with the progress of time. Apart from some exceptions in 10.0% concentration at different time intervals, inhibition of hatching was above 75% from the 1 WAI which reached 100% in 100.0% concentration of cabbage extract at 4 WAI (Table 8). Findings of this experiment indicated that potentiality for inhibition of hatching was higher and steadier in botanical extracts than that of organic amendments.

**Table 4**
Effect of different concentrations of cabbage extract and incubation time on the mortality of egg mass of *M. javanica*.

| Time | Treatment (%) | Mortality (%) |
|---|---|---|
| 3 DAI | 10.0 | 2.33 ± 1.2 h |
| | 25.0 | 5.67 ± 2.19 h |
| | 50.0 | 19.33 ± 0.88 g |
| | 100.0 | 73.33 ± 2.60c |
| | Water | 2.33 ± 0.88 h |
| 6 DAI | 10.0 | 27.33 ± 2.6 g |
| | 25.0 | 43 ± 1.53f |
| | 50.0 | 54.33 ± 1.76 e |
| | 100.0 | 100 ± 0 a |
| | Water | 4.66 ± 1.2 h |
| 9 DAI | 10.0 | 63 ± 1.15 d |
| | 25.0 | 79.33 ± 0.88c |
| | 50.0 | 88 ± 2.08b |
| | 100.0 | 100 ± 0 a |
| | Water | 7.33 ± 1.67 h |
| Level of significance | * | |
| CV (%) | 6.13 | |

Values are the mean ± Standard Error of three replicates. Treatment means were compared by two-way ANOVA. Same letter in a column do not differ significantly according to Tukey's test at 5% probability.
DAI = Days after incubation *1% level of probability

## 4. Discussions

This experiment was conducted to evaluate the effect of different concentrations of two organic amendments (vermicompost and BD) and two plant extracts (marigold and cabbage) on the hatching of egg mass and mortality of $J_2$ of *M. javanica*, isolated and characterized from the soil of Bangladesh. Although it was found in the experiment that both mortality and hatching were affected by these two groups of bio-compounds, the effect differed among the compounds.

In VT, mortality of $J_2$ of *M. javanica* increased with the progress of incubation time in all concentrations and the highest mortality (60%) was observed at the lowest concentration (10.0%) at the end of the experiment period (9 DAI). Similarly, the highest inhibition of hatching (75%) of egg mass was observed at the lowest concentration (10.0%) of VT at 1WAI. This result partially matched with the findings of Liu et al. (2019). In an *in vitro* experiment, they observed the decreasing and increasing trend of hatchability of egg mass and mortality of $J_2$ of *M. incognita* of tomato, respectively, with the increasing concentration of vermicompost extract and incubation time. On the contrary, in their pot experiment, they did not observe the increasing pattern of inhibitory effect on RKN with the increasing proportion of vermicompost. Kumar et al. (2011) also noticed the increasing inhibition of hatching of egg mass of *M. incognita* with the increasing concentration of vermicompost extract. However, in their experiment, they used 3.0% of vermicompost extract as the highest concentration and they observed 45% of hatching inhibition in that. In both these experiments *M. incognita* was used. It was found that different species of RKN differ in their pathogenic ability to cause disease even on the same host. (Navas et al., 2001; Hesar et al., 2011; Bucki et al., 2017). Accordingly, different species of RKN might respond differently to the extracts of vermicompost as we have worked with M. *javanica* in this experiment. It was observed in our work that hatching of egg mass of *M. javanica* in different concentrations of VT did not differ significantly with control after 2 weeks of incubation and an unsteady pattern of inhibition of hatching was found during the experiment period. The inconsistent or variable suppressive effect of vermicompost on PPN was reported by many researchers as the nematicidal capacity of them depends on the raw material used, the type of composting process and the species of nematode present (Renco, 2013). In the plant-soil system, ver-

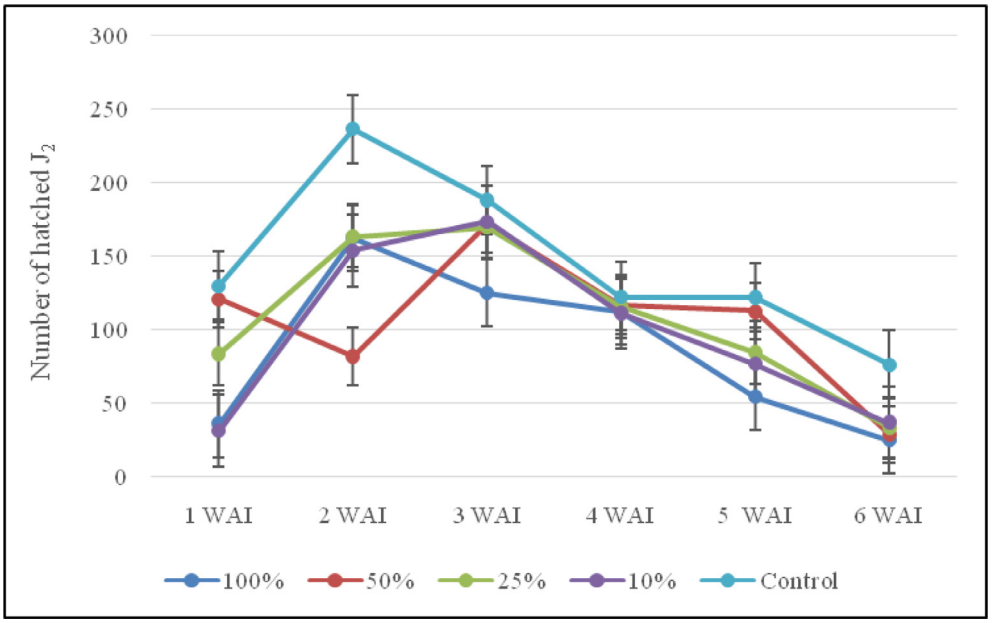


**Fig. 1.** Number of $J_2$ of *M. javanica* hatched at different weeks after incubation (WAI) in different concentrations of vermicompost tonic (VT). $J_2$ hatched in water was treated as control.

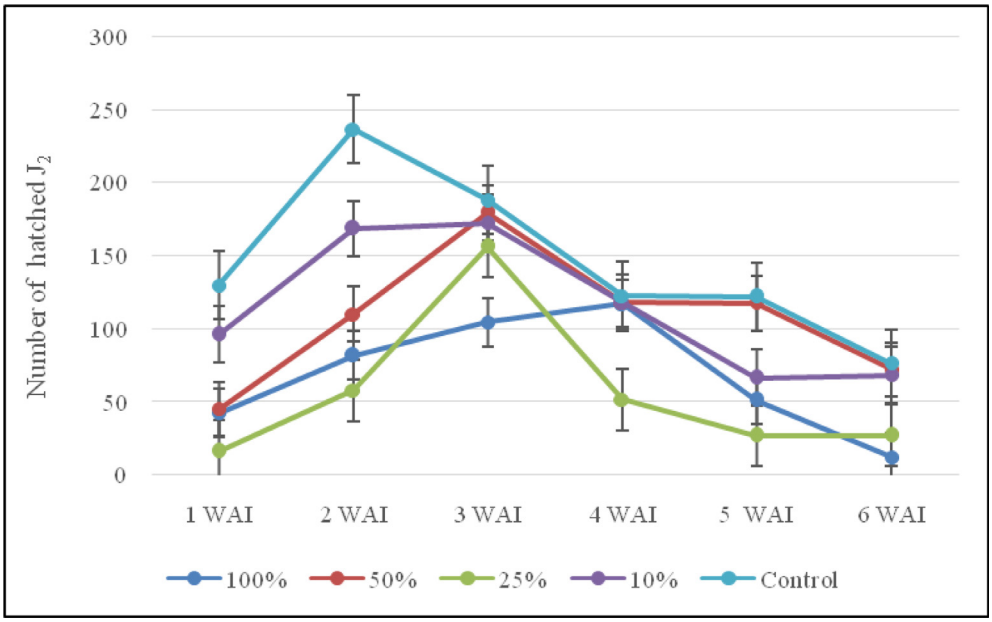


**Fig. 2.** Number of $J_2$ of *M. javanica* hatched at different weeks after incubation (WAI) in different concentrations of biogas digestate BD. $J_2$ hatched in water was treated as control.

micompost suppresses RKN by changing the soil properties, bringing positive shift in favour of the beneficial nematode community, increasing growth of plants, releasing nematicidal substances like humic acid and raising secondary defense compounds in roots (Oka and Yermiyahu, 2002; Xiao et al., 2016). Al-Hazmi et al. (2019) reported 17 to 60% of egg hatch inhibition and increasing $J_2$ mortality of *M. javanica* by exposing those in 0.25% to 1.0% of humic acid concentration. Humic acid might be present and activated at maximum in the 10.0% VT in our experiment as it was conducted in a controlled condition without soil and host.

Another organic amendment, BD, was used in this experiment to observe its direct impact on the hatching and mortality of *M. javanica* in different concentrations over the time. From the findings it was quite evident that mortality of $J_2$ was not much influenced by BD as the highest mortality was less than 50% at the end of the experiment period. In BD solutions there were different degrees of inhibition of hatching, but this did not follow any regular pattern. Thus any conclusive decision could not be made about their exact impact on the hatching of egg mass. There is very limited research finding available regarding the nematicidal potential of BD. The liquid fraction of the post-digestion matter, formed in a biogas plant by the process of fermentation of organic waste, is known as anaerobically digested slurry (ADS) (Koszel and Lorencowicz, 2015). In a pot experiment, Min et al. (2011) reported that damage index of tomato and radish by *M. incognita* and *Pratylenchus penetrans*, respectively, was significantly lower in the pots

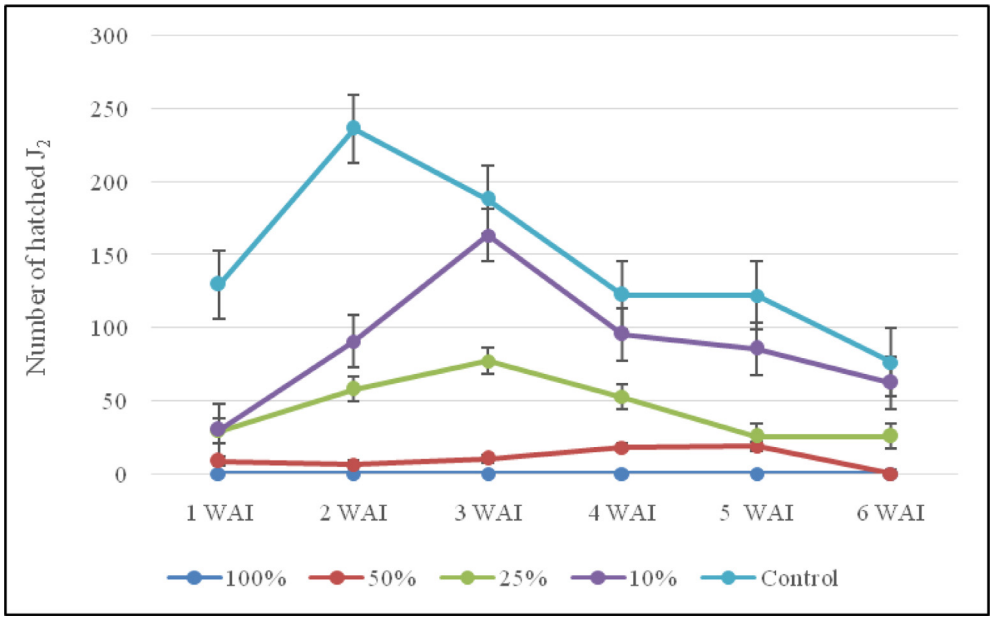


**Fig. 3.** Number of $J_2$ of *M. javanica* hatched at different weeks after incubation (WAI) in different concentrations of marigold extract. $J_2$ hatched in water was treated as control.

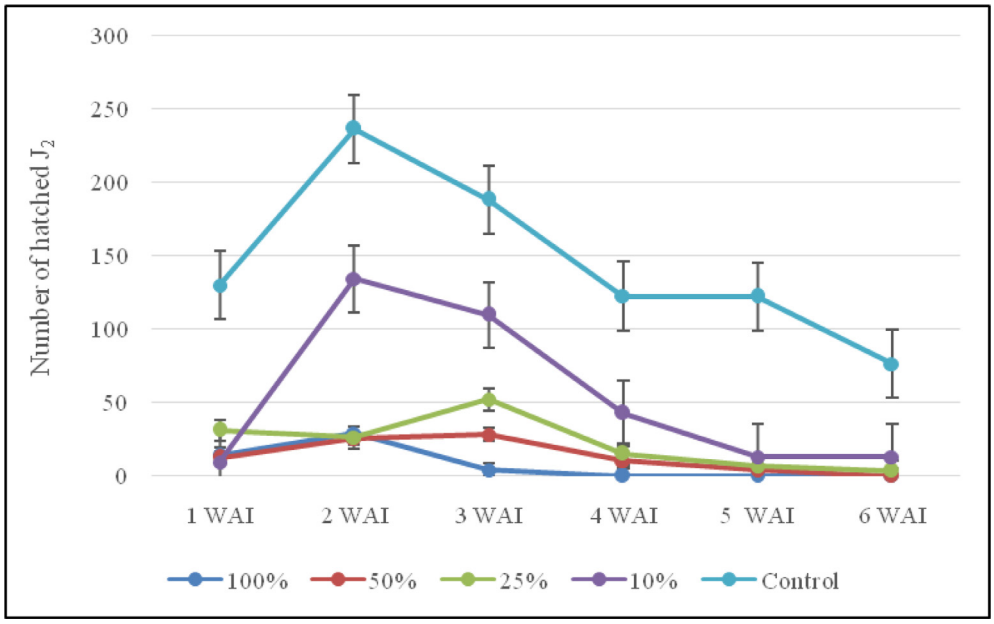


**Fig. 4.** Number of $J_2$ of *M. javanica* hatched at different weeks after incubation (WAI) in different concentrations of cabbage extract. $J_2$ hatched in water was treated as control.

treated with different rates of ADS, although they observed inconsistent results in field condition. ADS contains $NH_3$ and organic acids such as acetic acid, butyric acid and propionic acid that possess nematicidal activity (Akhtar and Malik, 2000; McBride et al., 2000; Chantigny et al., 2004). However, these compounds get activated in the soil–plant system and their performance is likely to vary in different experimental set-ups (Min et al., 2011). In our *in vitro* experiment, we used solutions of solid BD without any host and soil, in which nematicidal ingredients might be in less effective form to impact the biology of *M. javanica*. Biochar is the pyrolyzed carbon reached charred biomass produced through the process of thermal decomposition of organic matter carried out at temperature ranging from 200 to 900 °C in an oxygen deficient environment, is being gained increasing attention as an organic amendment for the sustainable nematode management (Kolton et al., 2011; Asif et al., 2017). The process of producing biochar is somewhat similar to BD. Biochar, singly or in combination with other bio-compounds, was found to reduce the infestation of *M. incognita*, whereas reports are also available that it had very little, indirect or no impact on suppression of nematode disease (Huang et al., 2015; Ebrahimi et al., 2016; Debode et al., 2020; Ansari et al., 2020). The result of these pot or field experiments are in line with

**Table 5**
Effect of different concentrations of vermicompost tonic (VT) and incubation time on the hatching of egg mass of *M. javanica.*

| Time | Treatment (%) | Inhibition of hatching (%) |
|---|---|---|
| 1 WAI | 10 | 75.96 ± 3.22 a |
| | 25 | 35.4 ± 3.4182 de |
| | 50 | 6.45 ± 2.29f |
| | 100 | 72.35 ± 3.47 ab |
| 2 WAI | 10 | 34.88 ± 1.57 de |
| | 25 | 30.79 ± 1.76 e |
| | 50 | 65.39 ± 1.01 (a-c) |
| | 100 | 31.21 ± 1.71 e |
| 3 WAI | 10 | 7.80 ± 1.79f |
| | 25 | 9.92 ± 2.61f |
| | 50 | 8.68 ± 2.55f |
| | 100 | 33.51 ± 2.92 e |
| 4 WAI | 10 | 9.01 ± 2.16f |
| | 25 | 5.19 ± 2.60f |
| | 50 | 4.64 ± 3.042f |
| | 100 | 8.19 ± 3.57f |
| 5 WAI | 10 | 37.15 ± 3.22 de |
| | 25 | 30.87 ± 2.85 e |
| | 50 | 7.92 ± 4.02f |
| | 100 | 55.73 ± 3.75 bc |
| 6 WAI | 10 | 51.75 ± 5.81 cd |
| | 25 | 56.57 ± 5.47 bc |
| | 50 | 62.28 ± 4.18 (a-c) |
| | 100 | 67.54 ± 4.64 (a-c) |
| Level of significance | * | |
| CV (%) | 16.88 | |

Values are the mean ± Standard Error of three replicates. Treatment means were compared by two-way ANOVA. Same letter in a column do not differ significantly according to Tukey's test at 5% probability.
WAI = Week after incubation *1% level of probability

**Table 6**
Effect of different concentrations of biogas digestate (BD) and incubation time on the hatching of egg mass of *M. javanica.*

| Time | Treatment (%) | Inhibition of hatching (%) |
|---|---|---|
| 1 WAI | 10 | 25.58 ± 3.66 g |
| | 25 | 87.59 ± 2.05 a |
| | 50 | 65.11 ± 2.49 (b-d) |
| | 100 | 67.18 ± 3.17 bc |
| 2 WAI | 10 | 28.67 ± 2.03 g |
| | 25 | 75.56 ± 1.83 ab |
| | 50 | 53.53 ± 2.19 (d-f) |
| | 100 | 65.39 ± 1.15 (b-d) |
| 3 WAI | 10 | 8.33 ± 2.68 hi |
| | 25 | 17.02 ± 1.91 gh |
| | 50 | 4.61 ± 1.24 hi |
| | 100 | 44.5 ± 1.85f |
| 4 WAI | 10 | 3.55 ± 1.19 i |
| | 25 | 57.92 ± 3.079 (c-e) |
| | 50 | 3.27 ± 1.41 i |
| | 100 | 4.09 ± 0.94 hi |
| 5 WAI | 10 | 45.62 ± 2.89 ef |
| | 25 | 77.86 ± 2.51 ab |
| | 50 | 3.82 ± 1.19 hi |
| | 100 | 58.19 ± 1.71 (c-e) |
| 6 WAI | 10 | 10.08 ± 4.18 hi |
| | 25 | 64.91 ± 3.89 (b-d) |
| | 50 | 5.26 ± 2.27 hi |
| | 100 | 84.21 ± 84.21 a |
| Level of significance | * | |
| CV (%) | 10.50 | |

Values are the mean ± Standard Error of three replicates. Treatment means were compared by two-way ANOVA. Same letter in a column do not differ significantly according to Tukey's test at 5% probability.
WAI = Week after incubation *1% level of probability

**Table 7**
Effect of different concentrations of marigold extract and incubation time on the hatching of egg mass of *M. javanica.*

| Time | Treatment (%) | Inhibition of hatching (%) |
|---|---|---|
| 1 WAI | 10 | 76.74 ± 2.23 cd |
| | 25 | 77.26 ± 2.69 cd |
| | 50 | 93.28 ± 1.81 ab |
| | 100 | 100 ± 0 a |
| 2 WAI | 10 | 61.58 ± 1.25 e |
| | 25 | 75.42 ± 0.88 cd |
| | 50 | 97.31 ± 0.37 a |
| | 100 | 100 ± 0 a |
| 3 WAI | 10 | 13.12 ± 2.34 g |
| | 25 | 58.86 ± 1.45 e |
| | 50 | 94.51 ± 0.46 ab |
| | 100 | 100 ± 0 a |
| 4 WAI | 10 | 21.85 ± 1.52 fg |
| | 25 | 56.83 ± 1.09 e |
| | 50 | 85.24 ± 1.89 bc |
| | 100 | 100 ± 0 a |
| 5 WAI | 10 | 29.78 ± 2.85f |
| | 25 | 78.96 ± 2.38c |
| | 50 | 84.69 ± 2.13 bc |
| | 100 | 100 ± 0 a |
| 6 WAI | 10 | 17.98 ± 5.71 fg |
| | 25 | 66.22 ± 5.38 de |
| | 50 | 100 ± 0 a |
| | 100 | 100 ± 0 a |
| Level of significance | * | |
| CV (%) | 5.04 | |

Values are the mean ± Standard Error of three replicates. Treatment means were compared by two-way ANOVA. Same letter in a column do not differ significantly according to Tukey's test at 5% probability.
WAI = Week after incubation *1% level of probability

**Table 8**
Effect of different concentrations of cabbage extract and incubation time on the hatching of egg mass of *M. javanica.*

| Time | Treatment (%) | Inhibition of hatching (%) |
|---|---|---|
| 1 WAI | 10 | 75.96 ± 3.22 a |
| | 25 | 35.4 ± 3.4182 de |
| | 50 | 6.45 ± 2.29f |
| | 100 | 72.35 ± 3.47 ab |
| 2 WAI | 10 | 34.88 ± 1.57 de |
| | 25 | 30.79 ± 1.76 e |
| | 50 | 65.39 ± 1.01 (a-c) |
| | 100 | 31.21 ± 1.71 e |
| 3 WAI | 10 | 7.80 ± 1.79f |
| | 25 | 9.92 ± 2.61f |
| | 50 | 8.68 ± 2.55f |
| | 100 | 33.51 ± 2.92 e |
| 4 WAI | 10 | 9.01 ± 2.16f |
| | 25 | 5.19 ± 2.60f |
| | 50 | 4.64 ± 3.042f |
| | 100 | 8.19 ± 3.57f |
| 5 WAI | 10 | 37.15 ± 3.22 de |
| | 25 | 30.87 ± 2.85 e |
| | 50 | 7.92 ± 4.02f |
| | 100 | 55.73 ± 3.75 bc |
| 6 WAI | 10 | 51.75 ± 5.81 cd |
| | 25 | 56.57 ± 5.47 bc |
| | 50 | 62.28 ± 4.18 (a-c) |
| | 100 | 67.54 ± 4.64 (a-c) |
| Level of significance | * | |
| CV (%) | 2.7 | |

Values are the mean ± Standard Error of three replicates. Treatment means were compared by two-way ANOVA. Same letter in a column do not differ significantly according to Tukey's test at 5% probability.
WAI = Week after incubation *1% level of probability.

our findings, although none of them tested the efficacy of biochar in affecting the hatching of egg mass or mortality of $J_2$ of RKN in laboratory condition.

Marigold extract was one of the two botanicals tested in the experiment. In contrast to organic amendments, marigold extract showed 100% mortality of $J_2$ at the shortest time of the experiment. Similarly, 100% inhibition of hatching was observed at the end of

the 1st week of incubation and hatching of egg mass was lower than control in all concentrations of marigold extract throughout the experiment period. Taye et al. (2012) reported pronounced reduction in the population density of RKN and root-knot index using 5.0% concentration of marigold leaf extract in the field condition. Suppression of RKN in soil and in laboratory condition by marigold was also reported in several findings (Abo-Elyousr et al., 2010; Hasabo and Noweer, 2005; Natarajan et al., 2006; Walia and Gupta, 1979). However, some contrasting findings regarding the effectiveness of marigold against PPN were also observed. Tibugari et al. (2012) compared the effectiveness of the aqueous extract of garlic (*Allium sativum*), castor beans (*Ricinus communis*) and marigold (*Tagetes erecta*) in the bio-control of RKN in tomato and observed the poorer performance of marigold than other two botanicals. Abo-Elyousr et al. (2010) also found that extract of garlic (*Allium sativum*) and neem (*Azadirachta indica*) were better in suppressing RKN in both in *vitro* and in the field condition. Marigold has been extensively used as biological nematicide because of its cultivability, availability and cheapness (Tibugari et al., 2012). Apart from the pungency which may be deterrent to RKN, marigold produces alpha-terthienyl that possesses nematicidal properties (Sivapalan, 1972; Alam et al., 1979).

Cabbage extract was also found highly effective in this experiment in impacting mortality and hatching of *M. javanica*. Mortality of $J_2$ reached 100% at the 100.0% concentration of cabbage extract and around 80% mortality was recorded in the lower concentrations of it during the experiment period. Similarly, inhibition of hatching of egg mass was 100% in 100.0% concentration of cabbage extract. Rate of hatching of egg mass was lower than control in all concentrations of cabbage extract all along the experiment. Our findings were in line with that of Youssef and Lashein, 2013 as they incorporated crushed cabbage leaves at different rates into the soil under greenhouse condition and observed that the higher the rate of residue, the higher the percentage of RKN reduction in tomato plant. Anita (2012) evaluated the effect of incorporating fresh crucifer residue on RKN inoculum density and root-knot disease development in celery yield, and found that bio-fumigation with sulphur containing cruciferous vegetable waste at the rate of 1 kg/5kg of soil reduced the incidence of root-knot disease with enhanced plant growth and yield of the crop. The *Brassica* genus is the most studied group of plants that decrease PPN population by releasing volatile organic compounds (VOCs) (Ploeg, 2008; Carboni and Ntalli, 2014). In an *in vitro* assay, da Silva et al. (2019) observed that water exposed to VOCs from broccoli shoots (*Brassica oleracea*) decreased the motility of $J_2$ of *M. javanica* by 0%.

From the findings of this experiment, it can be opined that two botanical extracts had a better impact on hatching and mortality of *M. javanica* compared to two organic amendments. With a view to test the another option of non-chemical approach in our earlier experiment, we found that 25.0% concentration of the culture filtrate of two rhizospheric bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* caused 100% of $J_2$ mortality and 100% inhibition of hatching of egg mass (Das et al., 2020). On the other hand, Abo-Elyousr et al. (2010) observed plant extracts as better performing in managing RKN than rhizospheric bacteria *P. flrorescens*. Nath and Singh (2011) demonstrated that vermicompost is more efficient in controlling PPN when it is applied with plant products. Therefore, an appropriate combination of several non-chemical methods can lead to the efficient management of RKN which we are going to experiment in the coming days based on the findings of these reports. Most research works on organic amendments have focused on their influence on RKN in field condition or in pots; little effort has been placed on to study their effect on the survival and hatchability in *in vitro* condition. This experiment provided the first report regarding the impact of BD on hatching and mortality of RKN. It was observed in the experiment that both organic amendments had an impact, but in an irregular pattern, on both mortality and hatching which revealed the existence of some toxic chemicals in those. Further characterization of those chemicals and their precise mode of action are necessary to decide the optimal application rate in the field. In applying organic amendments specific concentration and dosage should be maintained so that it controls RKN without hampering the plant growth (Liu et al., 2019). Similarly, to include botanical extracts in nematode management strategy, data on its chemical composition, lethal concentration values of plant derived chemicals for specific species must be known. In this experiment, we investigated the optimum extract dilution level of two locally available organic amendments and botanical extracts which will help in devising appropriate field application of organic amendments and botanicals. However, combined application of organic amendments and botanicals might give additional effects that can be focused on future research work. Further, other species of RKN and their economically important host can be considered for non-chemical approaches for nematode management for precision agriculture.

Declaration of Competing Interest

All authors declare that they have no conflict of interest.

## Acknowledgements

This work was a part of PhD research of the first author financially supported by the World Bank funded National Agricultural Technology Program, Phase – II (NATP - 2) Project (Project ID: P149553) in Bangladesh.

## References

Abo-Elyousr, K.A., Khan, Z., Award, M.E., Abedel-Moneim, M.F., 2010. Evaluation of plant extracts and *Pseudomonas* spp. for control of root-knot nematode. *Meloidogyne incognita* on tomato. Nematropica 40, 289–299.

Abu-Elgawad, M.M.M., Askary, T.H., 2015. Impact of Phytonematodes on Agriculture Economy. In: Askary, T.H., Martinelli, P.R.P. (Eds.), Bio-control Agents of Phytonematodes. CABI, Wallingford, pp. 3–49.

Akhtar, M., Malik, A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresour. Technol. 74, 35–47.

Alam, M.M., Khan, A.M., Saxena, S.K., 1979. Mechanism of control of plant parasitic nematodes as a result of the application of organic amendments to the soil. Indian J. Nematol. 7, 27–31.

Al-Hazmi, A.S., Al-Yahya, F.A., AbdelRafaa, O.A., Lafi, H.A., 2019. Effects of humic acid, *Trichoderma harzianum* and *paecilomyces lilacinus* on *Meloidogyne javanica*. Int. J. Agric. Environ. Biores. 4, 61–74.

Ansari, T., Asif, M., Khan, A., Tariq, F., Khan, F., Siddiqui, M.A. 2020. Effect of combined soil application of biochar and oilcakes on Melodogyne incognita infesting lentil (Lens culinaris cv. Desi). Indian Phytopathology. DOI: 10.1007/s42360-020-00206-1.

Anita, B., 2012. Crucifer vegetable leaf wastes as bio-fumigants for the management of root knot nematode (*Meloidogyne hapla* Chitwwod) in celery (*Apium graveolens* L.). JBiopest. 5, 111–114.

Arancon, N., Edwards, C., Lee, S., Yardim, E., 2002. Management of plant parasitic nematode populations by use of vermicomposts. Proc. Brighton Crop Protect.: Conf. Pests Diseases 2, 705–710.

Asif, M., Ahmad, F., Ansari, T., Khan, A., Khan, F., Tariq, M., Siddiqui, M.A., 2017. Biochar: a soil conditioner and disease suppressor. J. Agric. Soil Chem. 1, 11–16.

Baermann, G., 1917. Eine eifache Methode Zur Auffindung von Anklyostomum (Nematoden) larven in Erdproben. Geneesk. Tijdschr. Ned. -Indie. 57, 131–137.

Berbegal, M., Garcia-Jemenez, J., Armengol, J., 2008. Effect of cauliflower residue amendments and soil solarization on verticilium wilt control in Artichoke. Plant Dis. 92, 595–600.

Bucki, P., Paran, I., Ozalvo, R., Iverkleid, I., Ganot, L., Miyara, S.B., 2017. Pathogenic variability of *Meloidogyne incognita* population occurring in pepper-production greenhouses in Israel toward *Me1*, *Me3* and *N* pepper resistance genes. Plant Dis. 101, 1391–1401.

Carboni, P., Ntalli, N., 2014. Botanical Nematicides, recent findings. In: Gross, A.D., Coats, J.R., Duke, S.O., Seiber, N.J. (Eds.), Bio-pesticides: State of the Art and Future Opportunities. ACS Publications, Iowa, pp. 145–157.

Chantigny, M.H., Rochette, P., Angers, D.A., Masse, D., Cote, D., 2004. Ammonia volatilization and selected soil characteristics following application of anaerobically digested pig slurry. Soil Sci. Soc. Am. J. 68, 306–312.

Das, S., Wesemael, W.M.L., Perry, R.N., 2011. Effect of temperature and time on the survival and energy reserves of juveniles of *Meloidogyne* spp. Agric. Sci. Res. J. 1, 102–112.

Das, S., Wadud, M.A., Khokon, M.A.R., 2020. Functional Evaluation of culture filtrates of *Bacillus subtilis* and *Pseudomonas fluorescens* on the mortality and hatching of *Meloidogyne javanica*. Saudi J. Biol. Sci. https://doi.org/10.1016/j.sjbs.2020.11.055.

da Silva, J.C.P., Campos, V.P., Barros, A.F., Pedroso, L.A., Silva, M.F., de Souza, J.T., Pedroso, M.P., de Medeiros, F.H.V., 2019. Performance of volatiles emitted from different plant species against juveniles and eggs of *Meloidogyne incognita*. Crop Prot. 116, 196–203.

Davey, C.B. 1996. Nursery Soil Management - organic amendments, in: Landis, T.D., Douth, D.B. (Tech Coordinators), National Proceedings, Forest and Conservation Nursery Associations, General Technical Report PNW-GTR-389, USDA Forest Service PNWRS, pp. 6-18.

Decraemer, W., Hunt, D.J., 2006. Structure and Classification. In: Perry, R.N., Moens, M. (Eds.), Plant nematology. CABI, Wallingford, pp. 4–32.

Debode, J., Ebrahimi, N., D'Hose, T., Cremelie, P., Viaene, N., Vandecasteele, B., 2020. Has compost with biochar added during the process added value over biochar or compost to increase disease suppression. Appl. Soil Ecol. 153, 103571.

Dejene, T.A., 2014. Opportunities for biological control of root-knot nematodes in organic farming systems: a review. Int. J. Org. Agric. Res. Dev.. 9, 87–107.

Ebrahimi, N., Viaene, N., Vandecasteele, B., D'Hose, T., Debode, J., Cremelie, P., Tender, C.A.D., Moens, M., 2016. Traditional and new soil amendments reduce survival and reproduction of potato cyst nematodes, except for biochar. Appl. Soil Ecol. 107, 191–204.

Edwards, C.A., 1998. Breakdown of animal, vegetable and industrial organic wastes by earthworms. In: Edward, C.A. (Ed.), Earthworm Ecology. CRC Press/Lewis, Florida, pp. 237–354.

Hasabo, A.H., Noweer, E.M.A., 2005. Management of root-knot nematode *Meloidogyne incognita* on eggplant with some plant extracts. Egypt. J. Phytopathol. 33, 65–72.

Hesar, A.M., Mogadam, E.M., Maafi, Z.T., 2011. Morphometrical and genetic diversity of *Meloidogyne javanica* isolates from the north east of Iran. J. Nematode Morphol. Systemat. 14, 1–11.

Hunt, D.J., Handoo, Z.A., 2009. Taxonomy, Identification and Principal Species. In: Perry, R.N., Moens, M., Star, J.L. (Eds.), Root-knot nematodes. CABI, Wallingford, pp. 55–88.

Huang, W., Ji, H., Gheysen, G., Debode, J., Kyndt, T., 2015. Biochar-amended potting medium reduces the susceptibility of rice to root-knot nematode infections. BMC Plant Biol. https://doi.org/10.1186/s12870-015-0654-7.

Huang, W., Cui, J.K., Liu, S., Peng, D., 2016. Testing various bio-control agents against the root-knot nematode (*Meloidogyne incognita*) in cucumber plants identifies a combination of *Syncephalastrum racemosum* and *Paecilomyces lilacinus* as being most effective. Biol. Control 92, 31–37.

Karssen, G., Moens, M., 2006. Root-knot Nematodes. In: Perry, R.N., Moens, M. (Eds.), Plant nematology. CABI, Wallingford, pp. 59–88.

Kepenekci, L., Dura, O., Dura, S., 2017. Determination of Nematicidal Effects of some Bio-pesticides against Root-knot Nematode (*Meloidogyne incognita*) on Kiwifruit. J. Agric. Sci. Technol. 7, 546–551.

Koszel, M., Lorencowicz, E., 2015. Agricultural use of biogas digestate as a replacement fertilizer. Agric. Agric. Sci. Procedia 7, 119–124.

Khokon, M.A.M., Okuma, E., Rahman, T., Wesemael, W.M.L., Murata, Y., Moens, M., 2009. Quantitative analysis of the effects of diffusates from plant roots on the hatching of *Meloidogyne chitwoodi* from young and senescing host plants. Biosci. Biotechnol. Biochem. 73, 2345–2347.

Kolton, M., Harei, Y.M., Pasternak, Z., Graber, E.R., Elad, Y., Cytryn, E., 2011. Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl. Environ. Microbiol. 77, 4924–4930.

Kumar, K.R., Nattuhurai, N., Gurusami, R., 2011. Influence of vermicompost in root-knot management as a function of soil fortification. Elixir Agriculture. 38, 4210–4213.

Liu, D., Han, W., Zhang, Y., Jiang, Y., 2019. Evaluation of vermicompost and extracts on tomato root-knot nematode. Bangladesh J. Bot. 48, 845–851.

Mahesha, H.S., Ravichandra, N.G., Rao, M.S., Narasegowda, N.C., 2017. Bio-efficacy of different strains of *Bacillus* spp. against *Meloidogyne incognita* under in vitro. Int. J. Current Microbiol. Appl. Sci. 6 (11), 2511–2517.

McBride, R.G., Mikkelsen, R.L., Barker, K.R., 2000. The role of low molecular weight organic acids from decomposing rye in inhibiting root-knot nematode populations in soil. Appl. Soil Ecol. 15, 243–251.

Min, Y.Y., Toyota, K., Sato, E., Takada, A., 2011. Effects of anaerobically digested slurry on Meloidogyne incognita and Pratylenchus penetrans in tomato and raddish production. Appl. Environ. Soil Sci. https://doi.org/10.1155/2011/528712.

Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S., Dhakshnamoorthy, G., 2006. Cold aqueous extract of African marigold, *Tagates erecta* for control of tomato root-knot nematodes *Meloidogyne incognita*. Crop Prot. 25, 1210–1213.

Nath, G., Singh, K., 2011. Role of vermicompost as biofertilizer for the productivity of cauliflower (*Brassica oleracea*) and biopesticides against nematode (*Meloidogyne incognita*). World Appl. Sci. J. 12, 1676–1684.

Navas, A., Castagnone-Sereno, P., Blazquez, J., 2001. Genetic structure and diversity within local population of *Meloidogyne* (Nematoda: Meloidogynidae). Nematology 3, 243–253.

Oka, Y., Yermiyahu, U., 2002. Suppressive effect of compost against the root-knot nematode *Meloidogyne javanica* on tomato. Nematology 4, 891–898.

Oka, Y., 2010. Mechanisms of nematode suppression by organic soil amendments – a review. Appl. Soil Ecol. 44, 101–115.

Orisajo, S.B., Okeniyi, M.O., Fademi, O.A., Dongo, L.N., 2007. Nematicidal effects of water extracts of *Acalypha ciliate*, *Jatropha gosssypifolia*, *Azadiractha indica* and *Allium ascalonicum* on *Meloidogyne incognita* infection on cacao seedlings. J. Res. Biosci. 3, 49–53.

Ploeg, A., 2008. Bio-fumigation to manage Plant-parasitic nematodes. In: Ciancio, A., Mukherji, K.G. (Eds.), Integrated Management and Biocontrol of Vegetable and Grain Crops Nematode. Springer Netharlands, Dordrecht, pp. 239–248.

Renco, M., 2013. Organic amendments of soil as useful tools of plant parasitic nematodes control. Helminthologica. 50, 3–14.

Sasser, J.N., Eisenback, J.D., Carter, C.C., Triantaphyllou, A.C., 1983. The International Meloidogyne project - its goals and accomplishments. Annu. Rev. Phytopathol. 21, 271–288.

Silva, J.D.O., Santana, M.V., Freire, L.L., Ferreira, B.D.S., Rocha, M.R.D., 2017. Bio-control agents in the management of *Meloidogyne incognita* in tomato. Ciência Rural Santa Maria. 47, (10) e20161053.

Sivapalan, P., 1972. Nematode pests of tea. In: Webster, J.M. (Ed.), Economic Nematology. Academic Press, Newyork, USA, pp. 285–311.

Taye, W., Sakhuja, P.K., Tefera, T., 2012. Evaluation of plant extracts on infestation of root-knot nematode on tomato (*Lycopersicon esculentum*). J. Agric. Res. Dev. 2, 86–91.

Tibugari, H., Mombeshora, D., Mandumbu, R., Karavina, C., Parwada, C., 2012. A comparison of the effectiveness of the aqueous extracts of garlic, castor beans and marigold in the biocontrol of root-knot nematode in tomato. J. Agric. Technol. 8, 479–492.

Walia, K.K., Gupta, D.C., 1979. Management of root-knot nematode, *Meloidogyne incognita* on vegetable crops with *Tagates* sp. Indian J. Nematol. 27, 18–23.

Xiao, z., Liu, M., Jiang, L., Chen, X., Griffiths, B.S., Li, H., Hu, F. 2016. Vermicompost increases defense against root-knot nematode (Meloidogyne incognita) in tomato plants. Applied Soil Ecology. 105, 177-186.

Youssef, M.M.A., Lashein, A.M.S., 2013. Effect of cabbage (*Brassica oleracea*) leaf residue as a biofumigant, on root knot nematode, *Meloidogyne incognita* infecting tomato. J. Plant Protect. Res. https://doi.org/10.2478/jppr-2013-0040.

Zasada, I.A., Ferris, H., 2004. Nematode suppression with brassicaceous amendments: application based upon glucosinolate profiles. Soil Biol. Biochem. 36, 1017–1024.