Controlling of Meloidogyne incognita (Tylenchida: Heteroderidae) using nematicides, Linum usitatissimum extract and certain organic acids on four peppers cultivars under greenhouse conditions

Ahmed M. Eldeeba, Ahmed A. Gh. Faraga, Muhammad S. Al-Harbib, Hosny Kesba, Samy Sayed, Ahmed E. Elesawye, Mohamed A. Hendawia, Elsayed M. Mostafa, Ahmed A.A. Aiouba

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**A B S T R A C T**

Organic acids and plant extracts, which have a nemacidal action and may be used instead of nematicides that pollute the environment, are one way for controlling the pepper root-knot nematode. We provide in this study for a first time a new strategy for management Meloidogyne incognita (Kofoid and White) by using organic acids and plant extract compared to nematicides on four peppers cultivars (Super amarr, Super mard, Super noura and Werta) under greenhouse conditions compared to nematicides. This study aimed to evaluate 0.1% of organic acids (humic and salicylic acid) and 0.1% of Linum usitatissimum extract on plant parameters of pepper varieties (Super amarr, Super mard, Super noura and Werta) and control of M. incognita under greenhouse conditions compared to four nematicides (Oxamyl 24% SL, Fosthiazates 75% EC, Ethoprophos N40% EC and Fenamiphos 40% EC). Our data obtained four nematicides were more effectiveness than other treatments in reduced galls and egg masses of M. incognita. Whilst, humic and salicylic acids have remarkably higher nemacidal activity than L. usitatissimum in all lines of pepper. Therefore, plant extract and organic acids may be used a best alternative of nematicides to control PPNs and caused the longitudinal growth of plant. Also, ultimately reduce environmental risk from nematicide pollution.

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1. Introduction

Pepper (Capsicum annum) is a major vegetable crop in Egypt, both for domestic use and export (Wesolowska et al., 2011) and it is a member of the solanaceous vegetables group. Peppers are high in phenolics and flavonoids (Bae et al., 2012), as well as carotenoids (Ha et al., 2007), vitamin C, vitamin E (García-Closas et al., 2004), and alkaloids (Srinivas et al., 2009), all of which are beneficial to human health. Throughout its growing stage, pepper output is seriously challenged by a variety of pests and diseases, with nematodes being one of the most troublesome pests, inflicting extensive root damage and considerable financial losses (Djian-Caporalino et al., 2007). Plant-parasitic nematodes (PPNs) are a serious agricultural concern, producing with an estimated annual decreased productivity of 12.3% ($157 billion) over the world (Singh et al., 2015), considerably more than invading insects (about US$70 billion; Bradshaw et al., 2016).

Meloidogyne spp., root knot nematodes (RKNs), is one of the most devastating nematode pests in agricultural producing regions, inflicting significant economic damage worldwide (Rehman et al., 2012). RKN-infected plants display symptoms both above and belowground. Aboveground, infected plants show poor development, fewer and small pale green leaves that wilt in high temperature. The symptoms emerge as large galls on the roots that are two to several times the diameter of a healthy root and interfere with the plant's ability to...
absorb and translocate water and dissolved nutrients. The worms prey on roots, injuring them and allowing other soil-borne illnesses to enter (Agrios, 2005). Escobar et al., (2015) reported that Meloidogyne incognita infection has a significant impact on pepper root development, resulting in root galls, yellowed and stunted leaves, and potentially the loss of entire plants.

Chemical toxins, resistant cultivars, crop rotation, and biological management are some of the approaches used to manage and control this nematode, and these methods are sometimes combined (Katooli et al., 2010). As population expansion necessitates increased agricultural output, the traditional method of nematode management is completely reliant on nematicides. However, the loss of pesticides as a result of EU rules (EC No1107/2009) has increased the need for effective nematode resistance, which state that they are dangerous to human health and a pollutant to the environment (Zhang et al., 2017). As a result, there is a critical need for a nematode control option that is both ecologically acceptable and effective, such as Linum usitatissimum extract (Ismail et al., 2014), organic acids like humic acid (HA) (Nagachandrabose and Baidoo, 2021) and salicylic acids (SA) (Martínez-Medina et al., 2017). Many crops have been displayed to benefit from HA, a byproduct formed during the breakdown of organic waste (Khatatb et al., 2012; Khan et al., 2013). In vitro, HA impacts the hatching of eggs and the survival of M. incognita second stage juveniles (Seevisavan and Senthilnathan, 2017). Salicylic acid (SA) is a phytohormone that controls many aspects of plant development and resilience to different stressors, including herbivores that feed on phloem. It governs many aspects of plant development and resilience to different challenges, including phloem-feeding herbivores, and causes a wide spectrum of metabolic and physiological responses in plants (Kawazu et al., 2012). Moreover, SA increases plant tolerance to a variety of biotic and abiotic stresses (Horváth et al., 2007).

The main hypothesis of this research is that the plant extract and organic acids would control PPNs and ultimately reduce environmental risk from nematode infection. This study aimed to evaluate Linum usitatissimum extract and organic acids (humic acid and salicylic acids) on plant parameters of pepper varieties (Super amarr, Super mard, Super noura and Werta) and control of Meloidogyne incognita under greenhouse conditions compared to four nematicides (Oxamyl 24% SL, Fosthiazate 75% EC, Ethoprophos N40% EC and Fenamiphos 40% EC).

2. Material and methods

2.1. Preparation of Root-knot Nematode, Meloidogyne incognita

Culture:

According to (El-Asoby et al., 2021), susceptible tomato cultivar (Super Strain B) was used to propagation of M. incognita by using a single egg mass of previous identified pure inoculum of M. incognita under greenhouse conditions. Infected tomato roots were cut to small parts then eggs of M. incognita extracted by using 0.5% sodium hypochlorite (180 ml water +20 ml Clorox). Collected eggs were immediately washed by distilled water and incubated in Petri dishes at 26 ± 2 °C until hatching. A micropipette was used to gather newly hatched juveniles.

2.2. Pesticides

The recommended application rates of four commercial formulations of registered nematicides (Oxamyl 24% SL, Fosthiazate 75% EC, Ethoprophos 40% EC and Fenamiphos 40% EC) (Table 1) were used to conducted current experiments to compare with co-friendly materials in controlling RKN. The registered nematicides were purchased from the Central Laboratory of Pesticides, Dokki, Giza, Egypt. As well as, a pot experiments were conducted in the Plant Protection Department, Faculty of Agriculture, Zagazig University.

2.3. Preparation of tested organic acids and plant extract:

Two organic acids (salicylic and humic) were obtained from were obtained from Gomhoria company, Egypt, besides linseed oil extract or flaxseed oil is made from the flax plant’s dried, matured seeds (Linum usitatissimum). Linseed oil is dried oil that reacts with oxygen in the air to polymerize into a solid state (Brendel, 2013).

Two organic acids (salicylic and humic) besides plant extract, L. usitatissimum were separately prepared by using 1 g from two organic acids and 1 ml of stock solution of L. usitatissimum that added to 1 L distilled water, respectively.

2.4. Greenhouse experiment setup and procedures

Plastic pots 13 cm in diameter were employed in the greenhouse, filled with 1300 g sterilized sandy soil (72.5% sand, 12.5% clay, 12.1% silt), 120 g peat moss, and 3 mg urea fertilizer per kilogram of soil. After one week of planting pepper (Capsicum annum L.) cultivars (Super mard, Super amarr, Super noura and Werta). The experimental pots were put in a random pattern with nine different treatments: (1) plants without infection with M. incognita (negative control), (2) plants infected with M. incognita (positive control), (3) plants infected with M. incognita + 10 ml of RD from oxamyl (24% SL), (4) plants infected with M. incognita + 10 ml of RD from Fosthiazate (75% EC), (5) plants infected with M. incognita + 10 ml of RD from Ethoprophos (40% EC), (6) plants infected with M. incognita + 10 ml of RD from Fenamiphos (40% EC), (7) plants infected with M. incognita + 10 ml of stock solution of plant extract L. usitatissimum, (8) plants infected with M. incognita + 10 ml of humic acid, (9) plants infected with M. incognita + 10 ml of Salicylic acid. Every plant seedling in treatments (2--9) was inoculated with a temperature of 25 °C. The test was ended after sixty days of nematode inoculation, and pepper plants were carefully removed from pots and bathed in clean water for 30 min. Then, plants were watered by tissue papers to conducted the following plant and nematode parameters. Plant growth parameters data, involving fresh and dry shoot weight (g) and root length (cm) were estimated. Moreover, number of galling/plant and number of egg masses/plant were assessed. Nematode extraction was performed on 100 g soil samples using a combination of sieving and the Baermann trays approach (Hooper, 1986). Reproduction factor (RF) was assessed according to the following equation:

\[ RF = \frac{FP}{FI} \]

whereas, FP is final population and IF is an initial population.

The present equations and the percentage of decrease or rise in the parameters imputed to “negative or positive” control values were used:

\[ \text{Reduction} \% = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100 \]
Increase (%) = \frac{Treated - Control}{Control} \times 100

2.5. Statistical analysis

Statistically, the fully randomized design was implemented for laboratory experiments. The data were analyzed using MSTAT version 4, and the analysis of variance and means were compared using Duncan’s multiple range test at a probability of ≤0.05.

3. Results

Effects of four nematicides, plant extract *L. usitatissimum* and organic acids (humic and salicylic acids) on reproduction of *M. incognita* and plant growth parameters in four lines of pepper.

3.1. Soil and root parameters

Humic and salicylic acids with nematicides exhibited strong nematicidal activity against *M. incognita* J2 in pots soils, number of galls/plant and number of egg masses/plant after 60 days of application by recommended rates and stock solution of tested materials in soil of all tested lines of *C. annuum* L (Table 2). In Super Mard Cultivar, The treatment with 4 ml/plant of each organic acids (humic and salicylic acid) and 40 μL/plant of linseed oil extract, *Linum usitatissimum*, showed comparable nematicidal activity with 75.28%, 38.85%, 59.51%; 80.83%, 47.92%, 68.46%; 65.00%, 19.83%, 52.86% galls, egg masses and number of J2/100 g per plant, respectively. Whilst, tested four nematicides were more effectiveness against *M. incognita* reproductive parameters than organic acids. Number of galls, egg masses and J2 number were 92.22%, 76.86%, 84.04%; 85.28%, 54.54%, 77.95%; 81.39%, 52.88%, 76.01% and 88.61%, 67.76%, 81.15% with oxamyl, fosthiazate, ethoprophos and fenamiphos, respectively. Moreover, in Super Noura cultivar, high numbers of J2/100 g soil were found in soil pots treated with humic acid, salicylic acid, and *L. usitatissimum*. Gall numbers, egg masses and J2/100 g soil were 38.00, 36.00, 41.00; 26.33, 29.00, 32.33 and 20.00, 24.66, 21.88 with *L. usitatissimum*, humic and salicylic acids, respectively. Whereas the same parameters in pots treated with oxamyl, fosthiazate, ethoprophos and fenamiphos were 11.00, 18.66, 8.66; 19.66, 11.33, 16.00; 22.00, 21.66, 21.33 and 20.00, 24.66, 21.88 with *L. usitatissimum*, humic and salicylic acids, respectively. Whereas, the same parameters in pots treated with oxamyl, fosthiazate, ethoprophos and fenamiphos were 11.00, 18.66, 8.66; 19.66, 11.33, 16.00; 22.00, 21.66, 21.33 and 13.66, 14.66, 11.33, respectively. Whereas, Oxamyl achieved the maximum effect in reducing number of gall (6.66) by 93.88%, number of egg masses (8.00) by 76.91% and J2/100 g soil (6.68) by 89.28%, respectively in Werta cultivar. The equivalent values in treatments of humic and salicylic acids were 24.33 by 77.67, 24.66 by 28.85%, respectively in Werta cultivar. The equivalent values in treatments of humic and salicylic acids were 24.33 by 77.67, 24.66 by 28.85%, respectively in Werta cultivar. The equivalent values in treatments of humic and salicylic acids were 24.33 by 77.67, 24.66 by 28.85%, respectively in Werta cultivar. The equivalent values in treatments of humic and salicylic acids were 24.33 by 77.67, 24.66 by 28.85%, respectively in Werta cultivar. The equivalent values in treatments of humic and salicylic acids were 24.33 by 77.67, 24.66 by 28.85%, respectively in Werta cultivar. The equivalent values in treatments of humic and salicylic acids were 24.33 by 77.67, 24.66 by 28.85%, respectively in Werta cultivar. The equivalent values in treatments of humic and salicylic acids were 24.33 by 77.67, 24.66 by 28.85%, respectively in Werta cultivar. The equivalent values in treatments of humic and salicylic acids were 24.33 by 77.67, 24.66 by 28.85%, respectively in Werta cultivar.
Table 2

| Treatment               | Control - Treated | Oxamyl  | Fosthiazate | Ethoprophos | Fenamiphos | Humic acid | Salicylic acid | Linum usitatissimum |
|-------------------------|-------------------|---------|-------------|-------------|------------|------------|----------------|---------------------|
| Root parameters (% reduction) | No. of galls/plant | No. of egg masses/plant | No. of J2/plant | No. of egg masses/plant | No. of J2/plant | No. of egg masses/plant | No. of J2/plant | No. of egg masses/plant | No. of J2/plant |
| Healthy plants         | 0.00              | 0.00    | 0.00        | 0.00        | 0.00       | 0.00       | 0.00           | 0.00               | 0.00               |
| Control + M. incognita | 57.67             | 137.00  | 9.37        | 40.33       | 120.00     | 71.33      | 41.00          | 126.66             | 62.33              |
| Oxamyl                 | 12.02             | 13.00   | 10.66       | 10.90       | 9.33       | 9.33       | 8.66           | 18.66              | 11.00              |
| Fosthiazate            | 15.00             | 25.66   | 23.00       | 15.06       | 18.33      | 17.66      | 16.00          | 21.33              | 19.66              |
| Ethoprophos            | 17.66             | 27.66   | 23.66       | 16.39       | 19.00      | 22.33      | 21.33          | 22.00              | 13.33              |
| Linum usitatissimum    | 36.66             | 47.33   | 26.00       | 32.21       | 32.33      | 42.00      | 41.00          | 36.00              | 38.00              |
| Salicylic acid         | 26.00             | 26.66   | 25.00       | 21.55       | 21.00      | 23.00      | 21.88          | 24.66              | 22.00              |

Each value is a mean of five replicates. Means with the same letter are not significantly different (p < 0.05). 

Table 4 displayed that humic and salicylic acid overwhelmed L. usitatissimum in fresh and dry weight of pepper plants. Moreover, two organic acids surpassed four nematicides in enhancement of plant growth parameters. For instance, the increasing percentage of fresh and dry shoot weight for Super Mard cultivar treated with humic, salicylic acids and L. usitatissimum were 40.22%, 20.49%, 40.59%, 29.65% and 31.64%, 7.91% compared with 8.58%, 11.49%, 32.46%, 7.91%; 32.83, 9.62% and 34.47%, 8.54% and fenamiphos for oxamyl, fosthiazate and ethoprophos, respectively. Furthermore, in positive control pots (infected pepper plants), the parallel values of fresh and dry shoot weight were 13.40 and 8.44 g, respectively. These data indicated that humic and salicylic acids have remarkably higher nematocidal activity than plant extract and while four nematicides were the most efficiency. Whilst, fresh and dry weight of pepper shoot Super Amarr cultivar treated with two organic acids were 18.96, 8.20; 16.12, 6.72; 14.83, 7.90 g with humic, salicylic acid and linseed oil compared with 15.00, 7.71; 14.68, 7.53; 14.90, 7.69; 18.01, 7.06 g with oxamyl, fosthiazate, ethoprophos and fenamiphos, respectively. Furthermore, parallel values of fresh and dry shoot weight in infected pepper plants with M. incognita (positive control) were 14.41 and 6.52 g, respectively. Current data indicated that four nematicides were more effectiveness than humic and salicylic acids in reduced galls and egg masses of M. incognita whereas extract L. usitatissimum was the least effective one. The type of organic acids and the application of nematicides affected the pepper shoot weights at each of recommended concentration in Super Noura whereas, Application of humic and salicylic acids resulted in enhance in fresh and dry shoot weight as compared with positive control treatment. However, compared to the non-treated Super Noura cultivar (positive control), adding humic and salicylic acids always resulted in a higher shoot weight more than those of nematicides. Fresh and dry pepper shoot in treatments of humic, salicylic acids and L. usitatissimum were 18.85, 7.66; 18.86, 7.98 and 17.82, 7.61, whilst, in treatments of oxamyl, fosthiazate, ethoprophos and fenamiphos were 18.47, 7.59; 18.06, 7.33; 17.95, 7.15 and 18.38, 7.48, respectively compared with 17.42 and 6.15 in infected pepper plants. Moreover, fresh and dry weight of pepper shoot in treatments received two organic acids were 19.43 g (23.60%), 7.89 g (37.45%) and 20.97 g (33.39%), 8.39 g (46.16%) with humic and salicylic acid respectively 19.26 g (22.51%), 8.00 g (39.37%); 18.38 g (16.92%), 7.85 g (36.75%); 17.93 g (14.05%) 7.71 g (34.32%) and 18.87 g (33.39%), 7.59; 18.06, 7.33; 17.95, 7.15 and 18.38, 7.48, respectively. These data indicated that four nematicides were more effectiveness than humic and salicylic acids which achieved considerable reduction of root galls, egg masses numbers and J2 numbers in pots soils. 

3.2. Plant growth parameters
Changes in tested lines of pepper plant growth parameters after treatment four nematicides (Oxamyl, Fosthiazate, Ethoprophos and Fenamiphos) plant extract (*Linum usitatissimum*) and organic acids (humic and salicylic acid) on final population and reproduction factor of *Meloidogyne incognita* in different pepper cultivars.

| Treatment | Super Mard cultivar | Super Amaar cultivar | Super Noura cultivar | Werta cultivar |
|-----------|---------------------|----------------------|----------------------|---------------|
|           | Finial population   | % reduction          | RF (FP/IP)           | Finial population | % reduction | RF (FP/IP) | Finial population | % reduction | RF (FP/IP) | Finial population | % reduction | RF (FP/IP) |
| Healthy plants | 0.00                | 0.00                 | –                    | 0.00            | 0.00        | –           | 0.00            | 0.00        | –           | 0.00            | 0.00        | –           |
| Control + *M. incognita* 1000 IJs | 749.71              | 0.00                 | 888.29               | 0.00           | 927.29      | 0.00        | 810.29         | 0.00        | –           | 0.00            | 0.00        | –           |
| Oxamyl | 156.26              | 79.15                | 0.028                | 141.70         | 84.04       | 0.016       | 112.58         | 87.85       | 0.121       | 86.84            | 89.28        | 0.107       |
| Fosthiazate | 195.00              | 73.98                | 0.260                | 195.78         | 77.95       | 0.220       | 208             | 77.56       | 0.224       | 151.58           | 81.29        | 0.187       |
| Ethoprophos | 229.58              | 69.37                | 0.306                | 219.70         | 75.26       | 0.247       | 277.29         | 70.09       | 0.299       | 173.29           | 78.61        | 0.213       |
| Fenamiphos | 164.58              | 78.04                | 0.219                | 167.44         | 81.15       | 0.188       | 147.29         | 84.11       | 0.158       | 171.00           | 85.56        | 0.144       |
| *Linum usitatissimum* | 476.58              | 36.43                | 0.635                | 418.73         | 52.86       | 0.471       | 452.57         | 57.04       | 0.574       | 398.58           | 50.81        | 0.491       |
| Humic acid | 398.58              | 46.83                | 0.531                | 359.58         | 59.51       | 0.404       | 420.29         | 54.67       | 0.453       | 281.58           | 65.24        | 0.347       |
| Salicylic acid | 338.00              | 54.91                | 0.450                | 280.15         | 68.46       | 0.315       | 284.44         | 69.32       | 0.306       | 242.58           | 70.06        | 0.299       |

RF = Reproduction factor, FP = final population, IP = initial population. Reduction (%) = Control - Treated x 100

Table 4

Changes in tested lines of pepper plant growth parameters after treatment four nematicides (Oxamyl, Fosthiazate, Ethoprophos and Fenamiphos) plant extract (*Linum usitatissimum*) and organic acids (humic and salicylic acid) on galls, egg masses and J2 of *Meloidogyne incognita* under greenhouse condition.

| Treatment | Super Mard cultivar | Super Amaar cultivar | Super Noura cultivar | Werta cultivar |
|-----------|---------------------|----------------------|----------------------|---------------|
|           | Plant growth parameters (% increase) |                   |                     |               |
|           | Fresh shoot weight (g) | Dry shoot weight (g) | Fresh shoot weight (g) | Dry shoot weight (g) | Fresh shoot weight (g) | Dry shoot weight (g) | Fresh shoot weight (g) | Dry shoot weight (g) |
| Healthy plants | 20.40               | 8.66                 | 20.75                | 8.93           | 20.60         | 8.78           | 21.97            | 9.19           |
| Control + *M. incognita* 1000 IJs | 13.40               | 6.44                 | 14.41                | 6.52           | 17.42         | 6.15           | 15.72            | 5.74           |
| Oxamyl | 14.55               | 7.18                 | 15.00                | 7.71           | 18.47         | 7.59           | 19.26            | 8.00           |
| Fosthiazate | 17.75               | 6.95                 | 14.68                | 7.53           | 18.06         | 7.31           | 18.38            | 7.85           |
| Ethoprophos | 17.80               | 7.06                 | 14.90                | 7.69           | 17.95         | 7.13           | 17.93            | 7.71           |
| Fenamiphos | 18.02               | 6.99                 | 18.01                | 7.06           | 18.38         | 7.48           | 18.87            | 7.88           |
| *Linum usitatissimum* | 34.47               | 8.54                 | 24.98                | 8.28           | 5.51          | 21.62          | 20.03            | 37.28           |
| Humic acid | 31.64               | 7.91                 | 29.11                | 7.90           | 17.82         | 7.61           | 18.01            | 7.77           |
| Salicylic acid | 18.79               | 7.76                 | 18.96                | 8.20           | 18.85         | 7.66           | 19.43            | 7.89           |

Each value is a mean of five replicates. Means with the same letter are not significantly different (p ≤ 0.05). Increase (%) = Treated - Control x 100

4. Discussion

Noteworthy aspects of these experiments were that both organic acids (humic and salicylic acids) and tested nematicides effectively reduced the galls formation, egg masses and number of J2 in pots soil; only oil plant extract (*Linum usitatissimum*) was tended to be less effective versus RKN (*M. incognita*).

These results suggest that eco-friendly materials particularly humic and salicylic acids besides *Linum usitatissimum* may play varying roles in reducing reproduction of *M. incognita* across pots soils that are favorable to specific pepper cultivars. Nevertheless, it would be valuable to study the effects of resistance or highly susceptible pepper cultivars as main option on RKN, *M. incognita*, reproduction in the absence and presence of eco-friendly materials to select proper cultivars of pepper plants.

HA and SA have higher nematocidal activity contra *M. incognita* J2 and eggs than oil of plant extract, *L. usitatissimum*. Current results are harmony with El-Sherif et al. (2015), used HA and SA at two concentration 0.1& 0.05% compared to oxamyl versus *Meloidogyne incognita* infecting tomato cv.9065FI and found that salicylic acid outperformed other treatments in terms of percentage increases in total plant fresh weight and shoot dry weight, as well as the lowest percentage reductions in final nematode population and number of egg masses.

The SA elicitor controls the activity of enzymes like polyphenol oxidase and peroxidase, The SA elicitor controls the activity of enzymes like polyphenol oxidase and peroxidase, that are key part of plant defenses versus biotic and abiotic stressors (Hayat et al., 2009; Zhao et al., 2009; War et al., 2011). Increase tested plants resistance treated with organic acids because of the enhancement.
activity of phenylalanine ammonialyase and this reaction enhances
phenolic production, which is linked to increased plant defense
resistance (Zinovieva et al., 2011; Diaz-Rivas et al., 2018; Khoshfarman-Borji et al., 2020). Moreover, Abd El-Kareem, 2007
mentioned to increased chitinase activity aids HA in combating
the negative effects of chocolate spot and rust infections in faba
bean plants, as well as enhanced plant growth indices and nutrient
and water absorption (Chen et al., 2004).

Nemours studies evaluated essential plant oils efficacy against
RKN, M. incognita, in vitro (eggs and infective juveniles) and
in vivo on vegetable plants (such as tomato, pepper, and cucumber).
Previous research has shown that essential oils comprising major
components have fungicidal toxicity that varies depending on
the chemical makeup of the plant species. The nematocidal
mechanisms of essential oils of medicinal plants as nematicides
are not clear. Various plant extracts exhibited acetylcholinesterase
inhibition. Others maybe cause disruption in cell membrane of
nematodes and affected its permeability (Kayani et al., 2012, Abd
El-Aal et al., 2021). Refaat et al. (2020) in vitro experiments on egg hatching and
juvenile mortality of the root-knot nematode Meloidogyne incognita
revealed that the oils investigated had a nematicidal effect
on eggs masses, free eggs, and juveniles of M. incognita. El-Ashyry
et al. (2021) reported that researchers employed a mixture of
abemectin, Purpureocillium lilacinum, rhizobacteria, and botanicals to
control Meloidogyne incognita on tomato compared to oxamyl.
Various applications by stock solution of seed oils and botanicals
only maybe achieve proper phytomolecules control management
as displayed from current study under greenhouse conditions
which agreement with El-Ashyry et al., 2021 and Refaat et al., 2020.

Organic acids have low molecular weights and are found in low
quantities in the soil, and low molecular weight organic acids tend
to vanish quickly (McBridge et al., 2000). As a result, the transitory
character of organic acids appears to impair their efficiency in
preventing plant pathogen-caused illnesses (Tabarant et al., 2011; Seo
and Kim, 2014; El-Sheref et al., 2015). As a result, the use of organic
acids to combat RKNs is not actively encouraged.

Given the amount of organic acids used in our study, the high
control cost of using a mixture of organic acids would be roughly
equal to the cost of a low-effective agrochemical nematicide, which
could result in economic disadvantages for organic acids and plant
extracts, which have generally failed to provide adequate nema-
tode control (Upadhyay and Rai, 1988, Aioub, et al., 2021).

5. Conclusion

Humic and salicylic acid were found to reduce gall formations,
eggs masses and number of J2 of M. incognita. When employed in
correct proportions with good climatic circumstances and resistant
plant cultivars, it can be regarded a viable alternative to chemical
nematicides, as well as the creation of stable formulations of active
organic acids for RKN control. However, further RKN control stud-
ies in the field are needed to establish the effectiveness of organic
acids or plant extracts, as well as the right practical concentration
and control cost, as compared to nematicides (oxamyl, fenthiazate,
ethoprophos, and fenamiphos).

Declaration of Competing Interest

The authors declare that they have no known competing finan-
cial interests or personal relationships that could have appeared
to influence the work reported in this paper.

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References

Abd El-Kareem, F., 2007. Induced Resistance in Bean Plants against Root Rot
And Alternaria leaf spot Diseases Using Biotic and Abiotic Inducers Under Field
Conditions. Res. J. Agric. and Biol. Sci. 3 (6), 767–776.
Abd El-Aal, E.M., Shahen, M., Sayed, S., Kesha, H., Ansari, M.J., El-Ashyry, R.M., Aioub,
A.A., Salma, A.S., Eldredge, A.M., 2021. In vivo and in vitro management of
Meloidogyne incognita (Tylenchida: Heteroderidae) using rhizosphere bacteria,
Pseudomonas spp. and Serratia spp. compared with oxamyl. Saud J. Biol. Sci. 28
(9), 4876–4883.
Aioub, A.A.A., El-Ashyry, R.M., Hashem, A.S., 2021. Compatibility of
entomopathogenic nematodes with insectsicides against the cabbage white
butterfly, Pieris rapae L. (Lepidoptera: Pieridae). Egypt J. Pest Control 31, 153.
https://doi.org/10.1186/s41938-021-00498-z.
Brendel, E., 2013. The effect of lindane oil on rose scale Aulacaspis rosae (Bouché)
(Hemiptera: Diaspididae) on greenhouse grown rose crops as an alternative pest
management strategy. Gesunde Pflanzen 65, 73–77. https://doi.org/10.1007/
s10343-012-0296-3.
Chen, Y., De Nobili, M., Aviad, T., 2004. Stimulatory effect of humic substances on
plant growth. In: Magdoff, F., Weil, R.R. (Eds.), Soil Organic Matter in Sustainable
Agriculture. FL, Boca Raton, pp. 103–130.
Diaz-Rivas, J.O., Gonzalez-Laredo, R.F., Chavez-Simental, J.A., Montoya-Ayón, J.B.,
Moreno-Jiménez, M.R., Gallegos-Díazante, J.A., Rocha-Guzmán, N.E., 2018.
Comparative characterization of extractable phenolic compounds by UPLC-
PDA-ESI-QqQ of Buddleja scordioides plants elicited with salicylic acid. J. Chem.,
10, https://doi.org/10.1155/2018/4536970.
El-Ashyry, R.M., Ali, M.A.S., Elshobi, A.E.A., Aioub, A.A.A., 2021. Integrated
management of Meloidogyne incognita on tomato using combinations of
abamectin, Purpureocillium lilacinum, rhizobacteria, and botanicals compared
with nematicide. Egypt J. Biol. Pest Control. 31, 93, https://doi.org/10.1186/
s10343-021-00435-x.
El-Sherif, A.G., Gad, S.B., Khalil, A.M., Mohamady Rabab, H.E., 2015. Impact of Four
Organic Acids on Meloidogyne Incognita Infesting Tomato Plants under
Greenhouse Conditions. Global J. Biol. Agric. Health Sci 4 (2), 94–100.
Hayat, Q., Hayat, S., Irfan, M., Ahmad, A., 2009. Effect of exogenous salicylic acid
under changing environment: a review. Environ. Exp. Bot. 68, 14–25.
Hooper, D.J., 1986. Extraction of live-free living stages from soil. In: Southey JF (ed).
Laboratory methods for working with plant and soil nematodes. Ministry of
Agriculture Fisheries and Food, Reference Book, vol 402. HMSO, London, pp 8–10.
Kayani, M.Z., Mukhtar, T., Hussain, M.A., 2012. Evaluation of nematicidal effects of
Cannabis sativa L. and Zanthoxylum clatum Roxb, against root-knot nematodes,
Meloidogyne incognita. Crop Prot. 39, 52–56.
Kawińska, T., Mochiuzuki, A., Sato, Y., Sugeno, W., Murata, M., Seo, S., Mitsuhara, I.,
2012. Different expression profiles of jasmonic acid and salicylic acid inducible
genes in the tomato plant against herbivores with various feeding modes.
Arthropod-Plant Interactions 6, 221–230.
Khan, A., Khan, M.Z., Hussain, F., Akhter, M., 2013. Effect of humic acid on the
growth, yield, nutrient composition, photosynthetic pigment and total sugar
contents of peas (Pisum sativum). J. Chem. Soc. Pak. 35, 206–211.
Khattab, M., Ayman, M.E., Arafà, H., 2012. Effect of humic acid and amino acids on
pomegranate trees under deficit irrigation. I. Growth, Flowering and Fruit.
J. Hort. Sci. Ornamental. Plant 4, 253–259.
Khoshfarman-Borji, H., Yali, M., Pahlavan, Bozorg-Amirkalea, M., 2020. Induction
of resistance against Brevicoryne brassicae by Pseudomonas putida and salicylic
acid in canola. Bull. Entomol. Res. 1-14, https://doi.org/10.1017/S0007485320000097.
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.
Refaat, Manar, M., Mostafa, E., Mahmoud, Ramadan, M., El-Ashyry, Amr, M.,
ElMarzoky, 2020. Nematicidal properties of commercial seed oils of certain
medicinal plants on egg hatching and juvenile mortality of the root-knot
nematode, Meloidogyne incognita in vitro. Zagazig J. Agric. Res., 47 (2) : 143–156.
Seenivasan, N., Senthilnathan, 2017. Effect of humic acid on Meloidogyne incognita
(Rhofoud & White) Chitwood infecting banana (Musa spp.). International journal
of pest management.
Seo, Y.H., Kim, Y.H., 2014. Control of Meloidogyne incognita using mixtures of organic
acids. Plant Pathol. J. 30, 450–455.
Tabarant, P., Villeneuve, C., Ruseve, J.M., Roger-Estrade, J., Theriès, L., Dorel, M., 2011.
Effects of four organic amendments on banana parasitic nematodes and soil
nematode communities. Appl. Soil Ecol. 49, 59–67.
Upadhyay, R.S., Rai, B., 1988. Biocontrol agents of plant pathogens: their use and
practical constraints. In: Mukerji, K.G., Garg, K.L. (Eds.), Biocontrol of Plant
Diseases Using Biotic and Abiotic Inducers Under Field Conditions. Res. J. Agric. and Biol. Sci. 3 (6), 767–776.

Zhao, L.Y., Chen, J.L., Cheng, D.F., Sun, J.R., Liu, Y., Tian, Z., 2009. Biochemical and molecular characteristics of Stiboniov annae-induced wheat defense responses. Crop Prot. 28, 413–422.

Zinovieva, S.V., Vasyukova, N.I., Udalova, Z.Z., Gerasimova, N.G., Ozertsekovskaya, O. L., 2011. Involvement of Salicylic Acid in Induction of Nematode Resistance in Plants. Biol. Bull. 38 (5), 453–458.

Martínez-Medina, A., Fernández, I., Lak, G.B., Pozo, M.J., Pieterse, C.M., Van Wees, S. C., 2017. Shifting from priming of salicylic acid-to-jasmonic acid-regulated defences by Trichoderma protects tomato against the root knot nematode Meloidogyne incognita. New Phytol. 213 (3), 1363–1377.

Nagachandrabose, S., Baiddoo, R., 2021. Humic acid—a potential biosource for nematode control. Nematol. 1 (1), 1–10.

Singh, S., Singh, B., Singh, A.P., 2015. Nematodes: a threat to sustainability of agriculture. Procedia Environ. Sci. 29, 215–216. https://doi.org/10.1016/j.pesr.2015.07.270.

Zhong, S., Gan, Y., Ji, W., Xu, B., Hou, B., Liu, J., 2017. Mechanisms and characterization of Trichoderma longibrachiatum T6 in suppressing nematodes (Heterodera avenae) in wheat. Front. Plant Sci. 8, 1491. https://doi.org/10.3389/fpls.2017.01491.

Rehan, B., Mohd, A.G., Kavita, P., Mansoor, A.S., Usman, A., 2012. Management of Root Knot Nematode, Meloidogyne incognita Affecting Chickpea, Cicer arietinum for Sustainable Production. Biosci. Int. 1 (1), 01–05.

Horváth et al., 2007. Induction of abiotic stress tolerance by salicylic acid signaling. J Exp. Bot. 58, 351–361.

El-Deeb, A.M., El-Ashry, R.M., El-Marzoky, A.M., 2020. Nematicidal activities of Meloidogyne spp. The resistance genes in pepper (Capsicum annuum L.) are Meloidogyne incognita and races. In: Nickle, W.R. (Ed.), Manual of Agricultural Nematology. Marcell Dekker, New York, pp. 55–88.

Katoori, N., Moghadam, E.M., Taheri, A., Nasrollahnejad, S., 2010. Management of root-knot nematode (Meloidogyne incognita) on cucumber with the extract and oil of nematicidal plants. Int. J. Agric. Res. 5, 582–586.

Bradshaw, C.J., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., 2016. Massive yet grossly underestimated global costs of invasive insects. Nat. Commun. 7, 1–8. https://doi.org/10.1038/ncomms12586.

Djyan-Caporalino, C., Fazari, A., Arguel, M.J., 2007. Root-knot nematode (Meloidogyne spp.) resistance mechanisms in pepper (Capsicum annum L.) are clustered on the P9 chromosome. Theor. Appl. Genet. 114, 473–486. https://doi.org/10.1007/s00122-006-0447-3.

Wosielowska, A., Jadczyk, D., Grzeszczuk, M., 2011. Chemical composition of the pepper fruit extracts of hot cultivars Capsicum annum L. Acta Sci. Pol. Hortorum. Cultus 10, 171–184.

Bae, H., Jayaprakasha, G.K., Jinon, J., Patil, B.S., 2012. Variation of antioxidant activity and the levels of bioactive compounds in lipophilic and hydrophilic extracts from hot pepper (Capsicum spp.) cultivars. Food Chem. 134, 1912–1918.

Ha, S.H., Kim, J.B., Park, J.S., Lee, S.W., Cho, K.J., 2007. A comparison of the carotenoid accumulation in Capsicum varieties that show different ripening colours: Deletion of the capsanthin–capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. J. Exp. Botany 58, 3135–3144.

García-Closas, R., Berenguer, A., José Tormo, M., José Sánchez, M., Quirós, J.R., 2004. In: Garcia-Closas, R., Berenguer, A., José Tormo, M., José Sánchez, M., Quirós, J.R. (Eds.), Dietary strategies to prevent breast cancer in Spain. Breast Cancer Res. 6, 5949–5960.

Taylor, A.L., Sasser, J.N., 1978. Influence of environmental factors on the hatch and mobility of the root-knot nematode Meloidogyne incognita in columns filled with glass beads, sand or and soil. Appl. Soil Ecol. 43, 200–205.

Chini, R., Hamada, E., Bettiol, W., 2008. Climate change and plant diseases. Scientia Agricola 65 (SPE), 98–107.

Goodell, P.B., Ferris, H., 1989. Influence of environmental factors on the hatch and survival of Meloidogyne incognita. J. Nematol. 21 (3), 328.

Hunt, D.J., Handoo, Z.A., 2009. Taxonomy, identification, and principal species. In: Perry, R.N., Moens, M., Stari, J.L. (Eds.), Root-Knot Nematodes. CAB International, USA, pp. 55–88.

Kayani, M.Z., Mukhtar, T., Hussain, M.A., 2017. Effects of southern root knot nematode population densities and plant age on growth and yield parameters of cucumber. Crop Prot. 92, 207–212.

Nicol, J.M., Turner, S.J., Coyne, D.L., Den Nijs, L., Hockland, S., Maafi, Z.T., 2011. Current nematode threats to world agriculture. In: Genomics and molecular genetics of plant-nematode interactions. Springer, Netherlands, pp. 21–43.

Ozdemir, E., Gaoz, U., 2017. Efficiency of some plant essential oils on root-knot nematode Meloidogyne incognita. J. Agric. Sci. Technol. A 7 (2017), 178–183. https://doi.org/10.17265/2161-6256/2017.03.005.

Ralini, N.H.A.A., Khandaker, M.M., Mat, N., 2016. Occurrence and control of root knot nematode in crops: a review. Aust. J. Crop Sci. 11, 1649–1654.

Shah, S.J., Anjam, M.S., Mendy, B., Anwer, M.A., Habash, S.S., Lozano-Torres, J.L., Grundler, F.M.W., Siddique, S., 2017. Damage-associated responses of the host contribute to defence against cyst nematodes but not root-knot nematodes. J. Exp. Bot. 68, 5949–5960.

Taylor, A.L., Sasser, J.N., 1978. Biology, identification, and control of root-knot nematodes (Meloidogyne species). North Carolina State University, USA, Dept. of Plant Pathology.

Taylor, A.L., Sasser, J.N., Nelson, I.A., 1982. Relationship of climate and soil characteristics to geographical distribution of Meloidogyne species in agricultural soils. Cooperative Publication, Department of Plant Pathology, North Carolina State University and US Agency for International Development, Raleigh, North Carolina.

Vrain, T.C., Barker, K.R., Holtzman, G.I., 1978. Influence of low temperature on rate of development of Meloidogyne incognita and M. hapla larvae. J. Nematol. 10 (2), 166.

Wheeler, T.A., Starr, J.L., 1987. Incidence and economic importance of plant parasitic nematodes on peanut in Texas. Peanut Sci. 14, 94–96.

Yadav, U., 2017. Recent trends in nematology management practices: the Indian context. Int. Res. J. Eng. Tech. 12, 482–489.

Refaat, M.M., Mahrous, M.E., El-Ashry, R.M., El-Marzoky, A.M., 2020. Nematicidal proprieties of commercial seed oils of certain medicinal plants on egg hatching and juvenile mortality of the root-knot nematode, Meloidogyne incognita In Vitro. Zagazig J. Agric. Res. 47 (2), 487–497.