# Potential of some Essential Oils to Control *Meloidogyne Javanica* on Eggplant

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## ABSTRACT

**Nematicidal activities of eight essential oils (Castor oil, Bitter almond oil, Sweet almond oil, Watercress oil, Ginger oil, Frankincense oil, Mint oil, Camphor oil) at four different concentrations were assayed against *Meloidogyne javanica* and the Oxamyl nematicide on the egg hatching, J2 mortality percentage, disease parameters of eggplant (egg masses, numbers of nematode galls, eggs /plant and number of J2 / 250 $cm^3$ soil), plant growth parameters (shoot fresh weights and shoot dry weights and root fresh weight) as well as PPO and POD defense enzymes activities. All the essential oils at the applied concentrations suppressed egg hatching after 7 days, and J2 mortality after 72 hours' exposure. There was a progressive decrease in egg hatching with rise in oils concentration. The highest concentration that gave the highest egg hatching reduction and J2 mortality was 1000 ppm. However, Ginger oil showed the highest reduction percentage in nematode galls (88.3%), egg-masses (89.5%), eggs /plant (92.2%) and number of J2 / 250 $cm^3$ soil (90.2%), also gave the highest increased of shoot fresh weight (46.1%), shoot dry weight (62.8%), root fresh weight (42.2%), as well as POD enzyme activity (0.562) and PPO enzyme activity (0.487). This was followed by Watercress oil, then Castor oil while the treatment with Mint oil was of the least effect on reduction rate in nematode galls as compared with Oxamyl nematicide and untreated infected control.**

**In conclusion, the essential oils showed effectiveness in reducing egg hatching and J2 mortality with ginger oil being the most impactful in reducing damage and promoting plant growth.**

**Keywords: essential oils, *Meloidogyne javanica*, egg hatching, mortality.**

## INTRODUCTION

Plant-parasitic nematodes are a significant biotic factor limiting agricultural productivity, causing extensive damage to a wide variety of economically important crops. As reported by Elling (2013), global annual yield losses in major crops due to these nematodes are estimated at USD 173 billion. Among them, root-knot nematodes (*Meloidogyne* spp.), comprising over 100 species, are recognized as the most destructive group (Trinh *et al*., 2019). As of right now, the genus *Meloidogyne* has around 80 identified species (Karssen, 2002). The majority of globally significant crops are negatively impacted by nematodes of the species *Meloidogyne*, which is a disease with difficult control.

Management of root-knot nematodes is mainly based on practices of agriculture, by the use of resistant cultivars and chemical nematicides (Gohar *et al.*, 2013). Since plant parasitic nematodes are known to reside in soil, using chemical pesticides below ground has negative effects on the environment and society is much more than the aboveground applications. The use of synthetic nematicides is the primary method for controlling *Meloidogyne* species and other plant-parasitic nematodes. However, the prolonged usage of these synthetic compounds led to several problems, including soil pollution, food contamination with nematicide residues and danger to humans and non-target species. Consequently, the public awareness with these problems limited the use of many nematicides (Nyczepir and Thomas, 2009). Reducing pesticide use has become a goal shared by several countries and a major issue in public policies (Lee *et al.*, 2019) since negative impacts of pesticides on the environment and on human health have been demonstrated unambiguously. Therefore, there is an urgent need to search and develop environmentally friendly products to control plant-parasitic nematodes. Phytochemicals are among the most useful natural materials that may be employed for the nematodes control. Many plant-based products have been investigated in this respect and showed promising nematicidal activities against nematodes parasitic to plant (Faria *et al.*, 2016; Khan *et al.*, 2019 and Chen & Song, 2021).

It is commonly known that nematicidal chemicals in plants are studied. Through the using of tinctures, extracts, and the addition of organic materials, these tests have demonstrated the nematicidal capabilities of a number of medicinal plants (Pandey & Kalra, 2010; Chaudhary *et al.*, 2013 and Kokalis-Burelle *et al.*, 2013). Plants can create secondary chemicals through metabolic processes that aid in environmental adaption, attract pollinators, and offer protection. Thus, many higher plants have chemicals with nematicidal characteristics that can cause nematode death, obstruct hatching, or impair motility (Chitwood, 2002).

The plant-extracted essential oils are complex mixtures that can include anywhere from 20 to 60

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components in different concentrations. They are substances that are rich in secondary chemicals. Therefore, using these essential oils is a useful technique to investigate the nematicidal potential of plants (Andrés *et al.,* 2012). The volatile compounds called essential oils are found in aromatic plants and give them their distinct flavors and fragrances. These substances that include monoterpenes and sesquiterpenes are the most abundant constituents. The primary ingredients in essential oils, monoterpenes and sesquiterpenes, present in different subgroups, such as esters, hydrocarbons, aldehydes ethers, phenols, alcohols, oxides and ketones. Andrés *et al.* (2012) described the toxicity nature of essential oils from various plant families against the nematodes of root-knot. Djiwanti *et al.* (2024) assessed the impact of castor oils and neem on the mortality of foliar nematodes (*Aphelenchoides fragariae*) as nematicide. The findings demonstrated that, in comparison to the death rate in the water, the mortality of *A. fragariae* exposed to Castor oil and Neem oil at various concentration levels was significantly greater. The study of Zahradníková and Petříková (2012) aimed to assess the impact of watercress eluate and tomato juice plants inoculated by *Meloidogyne hapla*. It was discovered that applying solutions made from watercress to plants greatly enhanced their fruit production. When nematodes were assessed for their existence on tomato plant roots, infections were found in zero percent in treated cultivars. The nematicidal activity of essential oils of three mint species were investigated against *Meloidogyne javanica* was conducted by Caboni *et al.* (2013) and the result showed that the *Meloidogyne incognita* was significantly susceptible to the nematicidal action of the tested essential oils. The study of Sharar *et al.* (2017) on the nematicidal effectiveness of Camphor oil against *Meloidogyne javanica*, a root knot nematode, revealed that emulsion significantly reduced the mortality rate of 81% of the second-stage juveniles (J2s) of *M. incognita*. Therefore, this study aims to: (i) investigate the in vitro impact of eight essential oils on egg hatching and J2 mortality of *M. javanica*, (ii) examine the effectiveness of the essential oils and their effects on plant growth parameters of Eggplant under greenhouse conditions (*in vivo*), and (iii) determine the effect of essential oils on the defense enzyme activities in Eggplant inoculated with *M. javanica* nematode.

## MATERIALS AND METHODS

### 1. Experimental sites

Laboratory experiments (*in vitro*) and greenhouse experiments (*in vivo*) were conducted in the Department of Plant Pathology, Faculty of Agriculture, Damanhour University. The experiments were conducted in year 2023 and was repeated twice.

### 2.Culture preparation of *Meloidogyne javanica* nematode.

Under greenhouse conditions at 25–30°C, a definite population of the root-knot nematode, *Meloidogyne javanica*, was grown on Eggplants (*Solanum melongena* L. cv. Spanish patra). A single egg mass from a previously discovered female worm was used to establish the nematode culture (Taylor and Sasser, 1978) and reared on Eggplants in a greenhouse. Sodium hypochlorite solution (0.5%) was used to extract eggs from infected Eggplants as described by Hussey and Barker (1973)and kept for 48 hours at 25±2 °C for hatching. The freshly hatched J2 collected within 48 hours and eggs were used in test of oils effectiveness.

### 3. The tested essential oils

The following essential oils (Castor oil, Bitter almond oil, Sweet almond oil, Watercress oil, Ginger oil, Frankincense oil, Mint oil and Camphor oil) were obtained from the oil extraction unit, National Research Centre, Dokki, Cairo, Egypt. According to Mohamed and Abdelgaleil (2008), the essential oils were examined using gas chromatography (Hewlwett Packard 5890)/mass spectrometry (Hewlwett Packard 5989B) (GC–MS). The nematicide Oxamyl SL 24% (N,N-dimethyl-2-methylcarbamoyl-oxyimino-2-(methylthio) acetamide) containing Oxamyl (24%) produced by Dupont Company USA.

### 4. Determination of the essential oils effect on J2 of *M. javanica*

Laboratory experiment was conducted to evaluate the effect of eight essential oils on the mortality of J2 of *M. javanica*. The essential oils were tested at 4 concentrations (125, 500, 750 and 1000 ppm). The essential oil concentrations were prepared in distilled water. To guarantee total homogeneity of the necessary solutions, 0.3% of Tween 20 was added. In 10 ml glass vials, the essential oil solution (3 ml) was added. To each vial, 1 ml of J2 solution containing about 100 individuals was added. There were two control treatments set up: one with distilled water and the other with distilled water mixed with 0.3% Tween 20. Five replicates were made of each concentration. For comparison, Oxamyl was utilized as nematicide reference. All treatments were kept at 25±2 °C for 48 h in an incubator. J2 were then placed in distilled water for a whole day. Next, under a microscope (Olympus CX41RF, Olympus Optical Co., LTD), the living and deceased were noted. The J2 mortality percentages were calculated.

**1. Egg hatching experiment**

The J2 toxicity test was used to assess the impact of essential oils on *M. javanica* egg hatching. The essential oils were tested at 4 concentrations (125, 500, 750 and 1000 ppm) prepared in distilled water. To each 10 ml-glass vial, 3 ml essential oil concentration and 100 eggs were added. Each concentration was replicated five times. Similarly, control treatment was prepared without tested essential oils. For a period of seven days, every treatment was kept at room temperature (25±2 °C). Next, under a stereo microscope, the number of juveniles was noted. Using the following formula, the percentage of inhibition of egg hatching was calculated with the following equation: Inhibition (%) = (C-T)/C × 100

Where, C and T stand for the number of juveniles in control and treatment, respectively.

**2. Greenhouse experiments**

The results of J2 mortality and egg-hatching experiments using different oils at different concentrations revealed that the four essential oils (Watercress oil, Bitter almond oil, Castor oil and Ginger oil), had the highest egg hatching reduction and the J2 mortality, were selected, also the oil that gave the lowest egg-hatching reduction and J2 mortality was chosen, which is Mint oil. Therefore, these five oils were selected to assess their effectiveness for the control of *M. javanica* infected Eggplant under greenhouse condition.

In this experiment, the highest concentration that gave the highest egg hatching reduction and J2 mortality was also chosen, which is the concentration of 1000 ppm, to conduct experiments under the greenhouse. The essential oils were evaluated at 1000 ppm concentrate. Oxamyl was tested a recommended application rate (240 mg/L) for comparison.

Twenty-one-day -old Eggplant seedlings were transplanted individually in plastic pots. Twenty centimeters in diameter and fifteen centimeters in depth, each pot containing sterile soil (clay-sand; 1:3, v:v). After ten days, 5000 *M. javanica* eggs were placed into four holes at the roots of each plant, spaced three centimeters apart. Each pot received 150 milliliters of the essential oil solution supplied as a soil drench. For each treatment, ten replicates were arranged. The treatments were kept at 25-30°C in a greenhouse and arranged in a completely randomized design. All pots in all experiments were irrigated with tap water once every 2 days. Until the completion of the experiment, fertilization was carried out once a week at a rate of 2.5 g/L (200 ml/pot) using Crystalone® (N: P: K 20:20:20). After 60 days of growth in regular conditions, plants were pulled up and cleaned under running water. The fresh weight (g) of the shoot and root was determined. We counted the number of *M. javanica* galls and egg masses for each eggplant root. J2 was extracted using Coolen's technique (Coolen, 1979) from 250 g of soil in each pot, and the quantity was counted. Henderson and Tilton (1955)'s equation was used to determine the percentages of reduction in galls, egg masses, and J2. Following a 15-minute staining period in an aqueous Phloxine B dye solution (0.15 g/l water) (Holbrook *et al.,* 1983), the roots were carefully rinsed with tap water. Growth parameters of plants (shoot fresh weight, shoot dry weight and root fresh weight) were recorded.

**3.Determination the activity of the defensive enzymes**

**A. Estimation of peroxidase (POD) activity**

Hammerschmidt and Kuc (1982) peroxidase activity assay protocol was used. Using a pre-chilled pestle and mortar (4 ºC), 1 g of the fresh root Eggplant sample was homogenized in 2 ml of 0.1M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged for 15 minutes at 4 ºC at 10,000 rpm. The reaction mixture contained 1.5 ml of 0.05 M pyrogallocallol, 0.5 ml of enzyme extract, and 0.5 ml of 1% $H_2O_2$. The supernatant functioned as the enzyme supply. At 28±2 ºC, the reaction mixture was incubated. The mixture's absorbance was set to zero at 420 nm in the spectrophotometer to begin the enzyme reaction, and for three minutes, the change in absorbance was recorded at 20-second intervals. The control was a preparation of boiled enzymes. The change in the reaction mixture's absorbance $min^{-1}$ $g^{-1}$ of fresh tissue was used to express the peroxidase activity.

**B. Estimation of polyphenol oxidase (PPO) activity**

Using a pre-chilled pestle and mortar, one gram of the eggplant fresh root sample was homogenized in two milliliters of 0.1 M sodium phosphate buffer (pH 6.5). After centrifuging the homogenate for 15 minutes at 4 ºC at 10,000 rpm, the supernatant was used as an enzyme source. The method described by Mayer *et al.* (1965) was used to measure polyphenol oxidase activity. 200 µL of the enzyme extract and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) made up the reaction mixture. First, 200 µL of 0.01 M catechol was added to initiate the reaction. The absorbance at 495 nm was set to zero while the reaction mixture was incubated at room temperature. The activity was reported as change in absorbance $min^{-1}$ $g^{-1}$ of fresh tissue, and the absorbance changes were measured at 30-second intervals for two minutes.

## STATISTICAL ANALYSIS

SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to evaluate the data (treatments and timings) from *in vitro* and pot experiments. With GraphPad PRISM version 7 (GraphPad Software Inc.,

California, USA), enzyme activity data were examined. At a probability level of ≤0.05, the significant differences between means were ascertained using LSD.

## RESULTS AND DISCUSSION

Effect of essential oils on egg-hatching and J2 mortality of M. javanica in vitro.

Data in Table (1) showed the effect of the used essential oils (Castor oil, Bitter almond oil, Sweet almond oil, Watercress oil, Ginger oil, Frankincense oil, Camphor and Mint oil) at concentrations of 125, 500, 750 and 1000 ppm in addition to the Oxamyl nematicide, on the egg hatching of M. javanica was investigated. At the applied concentrations, all treatments inhibited ($P < 0.05$) hatching of the eggs at 7 days compared the untreated control and this effect increased with increasing the concentration where the highest concentration of 1000 ppm showed the highest inhibition effect. The results revealed that Watercress oil and Bitter almond oil were the highest active in decreasing eggs hatchability, with 86.7% and 83.7% after the exposure for 7 days, respectively, followed by Ginger oil with 80.1% and Castor oil with 79.3% of hatchability after a seven-day exposure.

**Table1. Effects of certain essential oils at concentrations on egg-hatching and reduction percentage of *M. javanica* (*in vitro*), 7 days after treatment**

| Treatments & Concentration (ppm) | | Hatching % | Inhibition % |
|---|---|---|---|
| **Control distilled water mixed with 0.3% Tween 20** | | **72.40$^{a}$** | **-** |
| Castor oil | 125 | 43.40d$^{ef}$ | 40.1 |
| | 250 | 23.20l$^{mno}$ | 68.0 |
| | 500 | 18.00$^{opqr}$ | 75.1 |
| | 1000 | 15.00$^{qrs}$ | 79.3 |
| Bitter almond oil | 125 | 49.40$^{cd}$ | 31.8 |
| | 250 | 46.40$^{cde}$ | 35.9 |
| | 500 | 41.40$^{ef}$ | 42.8 |
| | 1000 | 11.80$^{rs}$ | 83.7 |
| Sweet almond oil | 125 | 62.40$^{b}$ | 13.8 |
| | 250 | 37.60f$^{gh}$ | 48.1 |
| | 500 | 33.60h$^{ij}$ | 53.6 |
| | 1000 | 16.00$^{pqr}$ | 77.9 |
| Watercress oil | 125 | 42.00$^{ef}$ | 42.0 |
| | 250 | 30.80$^{ijk}$ | 57.5 |
| | 500 | 24.80$^{klmn}$ | 65.7 |
| | 1000 | 9.60$^{st}$ | 86.7 |
| Ginger oil | 125 | 42.20$^{ef}$ | 41.7 |
| | 250 | 38.00$^{fgh}$ | 47.5 |
| | 500 | 30.80$^{ijk}$ | 57.5 |
| | 1000 | 14.40$^{qrs}$ | 80.1 |
| Frankincense oil | 125 | 34.40$^{ghi}$ | 52.5 |
| | 250 | 29.40$^{ijkl}$ | 59.4 |
| | 500 | 24.60$^{klmn}$ | 66.0 |
| | 1000 | 20.60$^{nopq}$ | 71.5 |
| Mint oil | 125 | 51.60$^{c}$ | 28.7 |
| | 250 | 40.00$^{efg}$ | 44.8 |
| | 500 | 27.80$^{jklm}$ | 61.6 |
| | 1000 | 22.60$^{mno}$ | 68.8 |
| Camphor oil | 125 | 43.80$^{def}$ | 39.5 |
| | 250 | 31.80$^{hij}$ | 56.1 |
| | 500 | 24.80$^{klmn}$ | 65.7 |
| | 1000 | 21.80$^{mnop}$ | 69.9 |
| Oxamyl | | 4.40t | 93.9 |
| L.S. D | | 1.99 | - |

Values followed by different letter (s) are significantly different at 0.05 of probability.

However, treatment by Camphor oil and Mint oil had the least effect on decreasing eggs hatchability percentages with 69.90% and 68.8 %, respectively, compared to the untreated control. The results also showed that the treatment with the Oxamyl gave the highest percentage of suppression of hatchability (93.9%) following a seven-day exposure compared to the treatments with all the used oils (Table 1).

Table (2) data demonstrated that the J2 mortality percentage increased significantly ($P < 0.05$) for all treatments after 12, 24, 48, and 72h of exposure. Additionally, J2 mortality percentages increased stepwise as exposure time increased, with the highest J2 mortality percentages were at 72 hours in all oils used. Meanwhile, the highest concentration that gave the highest J2 mortality percentage in all the oils used was 1000 ppm. Treatment with Watercress oil, however, gave a J2 mortality percentages of 99.60%, followed by Bitter almond oil and Ginger oil with a J2 mortality percentages of 99.4% for each, then Castor oil, with a J2 mortality percentages of 99.20%. While Camphor oil and Mint oil had the least effect on J2 mortality percentages with 93.80 and 93.60%, respectively, compared to the control (Table 2).

**Table2. Effects of certain essential oil at different concentrations on J2 mortality of *M. javanica* after different exposure time**

| Treatment & Concentration (ppm) | | J2 Mortality% | | | |
|---|---|---|---|---|---|
| | | 12 h | 24 h | 48 h | 72 h |
| **Control** | | **5.40r** | **7.60$^{p}$** | **9.40$^{n}$** | **11.60$^{n}$** |
| Castor oil | 125 | 16.00$^{pq}$ | 28.40$^{no}$ | 75.60$^{h}$ | 91.20$^{ghi}$ |
| | 250 | 20.80$^{mno}$ | 32.60$^{mn}$ | 80.80$^{g}$ | 94.80$^{bcde}$ |
| | 500 | 24.80$^{jkl}$ | 38.00$^{iijk}$ | 89.60$^{cde}$ | 97.00$^{abc}$ |
| | 1000 | 32.60$^{fg}$ | 41.60$^{ghii}$ | 94.20$^{b}$ | 99.20$^{a}$ |
| Bitter almond oil | 125 | 16.40$^{pq}$ | 37.20$^{jkl}$ | 81.20$^{g}$ | 91.80$^{fghi}$ |
| | 250 | 21.80$^{lmn}$ | 35.60$^{jklm}$ | 84.80$^{f}$ | 95.40$^{bcd}$ |
| | 500 | 27.40$^{ij}$ | 46.00$^{g}$ | 87.40$^{def}$ | 94.20$^{cdefg}$ |
| | 1000 | 36.60$^{cde}$ | 54.40$^{ef}$ | 91.20$^{bc}$ | 99.40a |
| Sweet almond oil | 125 | 17.60$^{op}$ | 25.00$^{o}$ | 56.00$^{l}$ | 89.20$^{hij}$ |
| | 250 | 22.60$^{klm}$ | 34.40$^{klm}$ | 61.20$^{k}$ | 91.60$^{fghi}$ |
| | 500 | 34.20$^{defg}$ | 42.00$^{ghi}$ | 76.60$^{h}$ | 93.60$^{defg}$ |
| | 1000 | 37.80$^{bcd}$ | 54.00$^{ef}$ | 81.00$^{g}$ | 97.00$^{abc}$ |
| Watercress oil | 125 | 18.20$^{nop}$ | 33.40$^{lm}$ | 65.60$^{j}$ | 88.20$^{j}$ |
| | 250 | 24.80$^{jkl}$ | 39.40$^{hij}$ | 75.20 | 91.60$^{fghi}$ |
| | 500 | 30.60$^{ghi}$ | 44.00$^{g}$ | 80.80$^{g}$ | 97.00$^{abc}$ |
| | 1000 | 33.40$^{efg}$ | 53.20$^{f}$ | 88.60$^{cde}$ | 99.60$^{a}$ |
| Ginger oil | 125 | 22.20l$^{m}$ | 31.60$^{mn}$ | 66.00$^{j}$ | 88.20$^{j}$ |
| | 250 | 28.40$^{hij}$ | 51.80$^{f}$ | 76.60$^{h}$ | 94.40$^{cdef}$ |
| | 500 | 35.00$^{cdef}$ | 65.20$^{d}$ | 87.40$^{def}$ | 98.40$^{a}$ |
| | 1000 | 36.80$^{cde}$ | 69.00$^{d}$ | 90.60$^{cd}$ | 99.40$^{a}$ |
| Frankincense oil | 125 | 13.60$^{q}$ | 31.80$^{mn}$ | 61.80$^{k}$ | 75.80$^{m}$ |
| | 250 | 22.00l$^{mn}$ | 34.60$^{klm}$ | 70.20$^{i}$ | 80.80$^{l}$ |
| | 500 | 32.40$^{fg}$ | 45.00$^{g}$ | 78.20$^{gh}$ | 88.80$^{ij}$ |
| | 1000 | 40.80$^{ab}$ | 54.40$^{ef}$ | 84.80$^{f}$ | 97.60$^{ab}$ |
| Mint oil | 125 | 16.40$^{pq}$ | 31.80$^{mn}$ | 47.60$^{m}$ | 83.80$^{k}$ |
| | 250 | 23.20$^{klm}$ | 42.40$^{gh}$ | 67.00$^{j}$ | 89.20$^{hij}$ |
| | 500 | 28.40$^{hij}$ | 52.00$^{f}$ | 77.40$^{h}$ | 92.00$^{efgh}$ |
| | 1000 | 31.60$^{fgh}$ | 57.80$^{e}$ | 86.40$^{ef}$ | 93.60$^{defg}$ |
| Camphor oil | 125 | 20.60$^{mno}$ | 53.60$^{ef}$ | 80.60$^{g}$ | 91.40$^{fghi}$ |
| | 250 | 26.40$^{jk}$ | 67.00$^{d}$ | 87.80c$^{def}$ | 91.80$^{fgh}$ |
| | 500 | 34.20d$^{efg}$ | 73.00$^{c}$ | 90.00$^{cd}$ | 97.00$^{abc}$ |
| | 1000 | 42.20$^{a}$ | 78.60$^{b}$ | 90.20$^{cd}$ | 93.80$^{defg}$ |
| Oxamyl | | 38.60$^{bc}$ | 87.00$^{a}$ | 97.60$^{a}$ | 98.80$^{a}$ |
| L.D.S | | 1.22 | 1.41 | 1.08 | 0.91 |

Values followed by different letter (s) are significantly different at 0.05 of probability.

The results showed that the treatment with Watercress oil, Bitter almond oil, Ginger oil and Castor oil gave a J2 mortality percentages which were not significantly different from the Oxamyl nematicide, which exhibited J2 mortality percentages of 98.80 % (Table 2).

Effect of the tested essential oils on disease parameters of Eggplant infected with M. javanica under greenhouse.

Through the results of an egg-hatching and J2 mortality experiments using different oils at different concentrations, the oils that gave the highest egg hatching reduction and the J2 mortality were selected, which were Watercress oil, Bitter almond oil, Castor oil and Ginger oil, also, the oil that gave the lowest egg-hatching reduction and J2 mortality was chosen, which is Mint oil. The highest concentration that gave the highest egg hatching reduction and J2 mortality was also chosen, which is a concentration of 1000 ppm, to conduct experiments under the greenhouse. Results showed that all tested oils application treatments at the 1000 ppm concentration had reduced numbers of nematode galls, egg-masses, eggs /plant and number of J2 / 250 cm3 of soil compared with those obtained with the untreated infected control plants (Table 3). The highest reduction % in all nematode parameters were obtained with Oxamyl applications with (99.5%) in galls, (99.5%) in egg masses, (99.5%) in number of J2 / 250 cm3 and (99.8%) in eggs. While the results showed that Ginger oil gave the highest reduction rate in nematode galls, egg-masses, eggs /plant, number of J2 / 250 cm3 of soil with 88.3, 89.5, 92.2 and 90.2% respectively, followed by Watercress oil, then Castor oil. While the treatment with Mint oil was less effective on reduction rate in nematode galls (45.7%), in egg masses (48.9%), in number of J2 / 250 cm3 (68.8%) and in eggs (40.7%), followed by Bitter almond oil (Table 3).

Effect of the tested essential on Eggplant growth parameters infected with M. javanica under greenhouse.

The results of Table (4) showed eggplant growth parameters infected with M. javanica affected by Watercress oil, Bitter almond oil, Castor oil, Ginger and Mint oils using 1000 ppm concentration and Oxamyl pesticide. When compared to the untreated infected control plants, the data demonstrated that all treatments significantly enhanced the root fresh weights, shoot fresh weights and shoot dry weights of Eggplant infected with M. javanica.

**Table 3. Effect of some essential oils on *Meloidogyne javanica*-infected Eggplant plants' disease parameters**

| Treatment | G | R% | EM | R% | J2 | R% | Eggs | R% |
|---|---|---|---|---|---|---|---|---|
| Control | 588.6$^{a}$ | - | 518.2$^{a}$ | - | 602.0$^{a}$ | - | 139244$^{a}$ | - |
| Mint oil | 319.8$^{b}$ | 45.7 | 264.8$^{b}$ | 48.9 | 187.8$^{b}$ | 68.8 | 82505$^{b}$ | 40.7 |
| Watercress oil | 87.0$^{d}$ | 85.2 | 77.6$^{d}$ | 85.0 | 59.6$^{d}$ | 90.1 | 16189$^{d}$ | 88.4 |
| Bitter almond oil | 152.8$^{c}$ | 74.0 | 131.0$^{c}$ | 74.7 | 89.0$^{c}$ | 85.2 | 36845$^{c}$ | 73.5 |
| Castor oil | 88.2$^{d}$ | 85.0 | 73.6$^{d}$ | 85.8 | 60.2$^{d}$ | 90.0 | 16976$^{d}$ | 87.8 |
| Ginger oil | 68.8$^{d}$ | 88.3 | 54.4$^{d}$ | 89.5 | 47.2$^{d}$ | 92.2 | 13629$^{d}$ | 90.2 |
| Oxamyl | 3.20$^{e}$ | 99.5 | 2.60$^{e}$ | 99.5 | 3.20$^{e}$ | 99.5 | 331e | 99.8 |
| L.S.D | 10.64 | - | 15.52 | - | 7.17 | - | 2041 | - |

Means with the same letters (s), in each column, are not significantly different at P=0.05, Number of galls (G), egg mass (EM), number of J2/250 $cm^3$ of soil (J2), and reduction percentage (R%)

**Table 4. Effects of essential oils on Eggplant growth parameters infected with *Meloidogyne javanica* under greenhouse conditions**

| Treatment | Shoot system weight (g) | | | | Root system weight (g) | |
|---|---|---|---|---|---|---|
| | Fresh | Increase% | Dry | Increase% | Fresh | Increase% |
| Control | 31.62$^{e}$ | - | 6.40$^{e}$ | | 10.80$^{d}$ | - |
| Mint oil | 36.74$^{d}$ | 16.2 | 7.46$^{de}$ | 16.6 | 11.28$^{cd}$ | 4.4 |
| Watercress oil | 44.20$^{b}$ | 39.8 | 9.62$^{bc}$ | 50.3 | 13.66$^{b}$ | 26.5 |
| Bitter almond oil | 39.58$^{cd}$ | 25.2 | 7.98$^{d}$ | 24.7 | 12.70$^{bc}$ | 17.6 |
| Castor oil | 42.54$^{bc}$ | 34.5 | 8.82$^{cd}$ | 37.8 | 13.44$^{b}$ | 24.4 |
| Ginger oil | 46.20$^{ab}$ | 46.1 | 10.42$^{b}$ | 62.8 | 15.36$^{a}$ | 42.2 |
| Oxamyl | 49.66$^{a}$ | 57.1 | 11.88$^{a}$ | 85.6 | 17.00$^{a}$ | 57.4 |
| L.D.S | 1.27 | - | 0.47 | - | 0.57 | - |

Values with the same letters (s), in each column, are not significantly different at P=0.05.

In addition, the Oxamyl applications highest increased shoot fresh and dry weights and root fresh weight of Eggplant infected with M. javanica, while the results showed that Ginger oil gave the highest increased shoot fresh (46.1%) and shoot dry weights (62.8%) and root fresh weight (42.2%), followed by Watercress oil and Castor oil. On the contrary, the results showed that Mint oil gave the lowest increase of shoot fresh weights, shoot dry weights and root fresh weight with 16.2, 16.6 and 4.4% of Eggplant infected with M. javanica followed by Bitter almond oil compared with the untreated infected control plants.

**Effect of the tested essential oils on the defense enzyme activity of Eggplant infected with *M. javanica* under greenhouse.**

**A. Peroxidase (POD) activity**

Data in Table (5) and illustrated in Fig. (1) revealed that POD activity in eggplant roots was significantly increased compared with the control in all tested oils and all the time. Ginger oils was the most effective to enhance POD enzyme activity with overall mean of 0.562 (absorbance $min^{-1}$ $g^{-1}$ of fresh tissue) and no significant between the Ginger oils and Oxamyl pesticide, followed by Castor oil. No significant difference in POD activity was observed between Bitter almond oil and Watercress oil. While the POD activity was at its lowest was recorded in Mint oil compared with the untreated infected control. No significant in POD activity was observed between Mint oil and control. Results also showed that the POD enzyme activity in roots of Eggplant increased with increasing time after infected with *M. javanica* until POD enzyme activity reached to highest activity at 7 days after infection in all the used oils as well as the Oxamyl. Then, the activity of the peroxidase enzyme decreased at 15 days after infection compared with the untreated control plants.

**Table 5. Effects of the tested essential oils on activities of Eggplant POD enzyme in roots infected with *M. javanica* under greenhouse**

| Treatment | POD (absorbance $min^{-1}$ $g^{-1}$ of fresh tissue) | | | | Mean |
|---|---|---|---|---|---|
| | 0 day | 3 days | 7 days | 15 days | |
| Control | 0.255 | 0.428 | 0.605$^{d}$ | 0.571$^{d}$ | 0.465$^{d}$ |
| Mint oil | 0.252 | 0.433 | 0.613$^{d}$ | 0.560$^{d}$ | 0.465$^{d}$ |
| Watercress oil | 0.252 | 0.419 | 0.747$^{c}$ | 0.625$^{c}$ | 0.511$^{c}$ |
| Bitter almond oil | 0.257 | 0.424 | 0.720$^{c}$ | 0.609$^{c}$ | 0.503$^{c}$ |
| Castor oil | 0.248 | 0.433 | 0.800$^{b}$ | 0.673$^{b}$ | 0.539$^{b}$ |
| Ginger oil | 0.254 | 0.449 | 0.836$^{ab}$ | 0.707$^{a}$ | 0.562$^{a}$ |
| Oxamyl | 0.247 | 0.426 | 0.867$^{a}$ | 0.722$^{a}$ | 0.566$^{a}$ |
| L. D. S | 0.009 | 0.008 | 0.013 | 0.007 | 0. 011 |

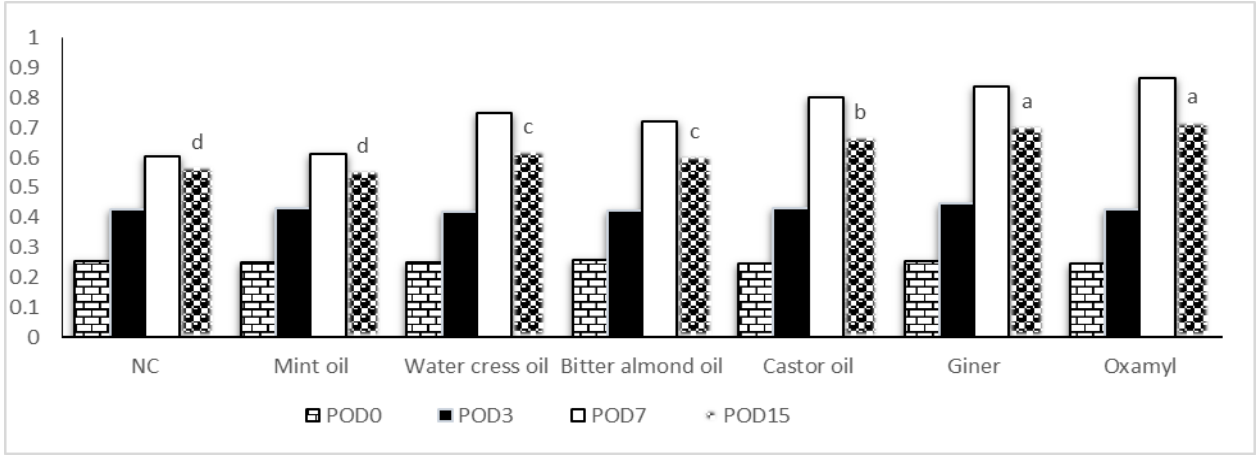


**Fig. 1. Effect of the tested essential oils on activities of Eggplant POD enzymes in roots infected with *M. javanica* under greenhouse conditions**

**Table 6. Effects of the tested essential oils on activities of Eggplant PPO enzymes infected with *Meloidogyne javanica* under greenhouse**

| Treatment | PPO | | | | Mean |
|---|---|---|---|---|---|
| | 0 day | 3 days | 7 days | 15 days | |
| NC | 0.233 | 0.390 | 0.493$^{d}$ | 0.485$^{b}$ | 0.400$^{e}$ |
| Mint oil | 0.230 | 0.398 | 0.535$^{cd}$ | 0.529$^{b}$ | 0.423$^{d}$ |
| Watercress oil | 0.221 | 0.400 | 0.615$^{bc}$ | 0.551$^{b}$ | 0.447$^{c}$ |
| Bitter almond oil | 0.237 | 0.400 | 0.595$^{bc}$ | 0.553$^{b}$ | 0.446$^{c}$ |
| Castor oil | 0.221 | 0.412 | 0.680$^{ab}$ | 0.604$^{a}$ | 0.476$^{b}$ |
| Ginger oil | 0.227 | 0.420 | 0.683$^{ab}$ | 0.617$^{a}$ | 0.487$^{a}$ |
| Oxamyl | 0.222 | 0.401 | 0.728$^{a}$ | 0.629$^{a}$ | 0.495$^{a}$ |
| L. D. S | 0.011 | 0.008 | 0.021 | 0.013 | 0.080 |

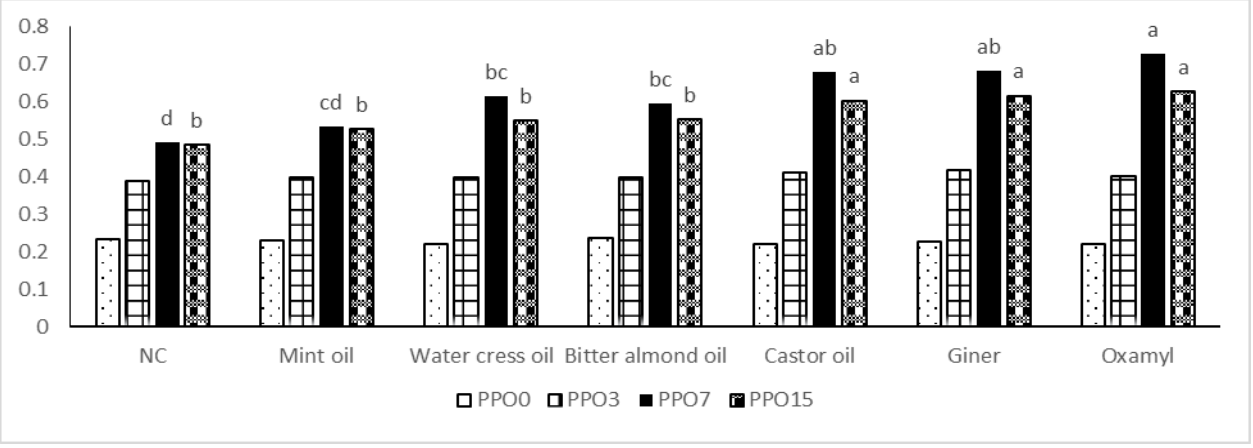


**Fig.2. Effect of the tested essential oils on activities of Eggplant PPO enzymes infected by *Meloidogyne javanica* under greenhouse**

**B. Polyphenol oxidase (PPO) activity**

Data in Table (6) and Fig. (2) revealed that PPO activity in Eggplant roots was significantly increased compared with the control in all used oils and all the time. Ginger oil was the most effective to enhance PPO enzyme activity and no significant differences between the Ginger oil and Oxamyl nematicide, followed by Castor oil. No significant in PPO activity was observed between Bitter almond oil and Watercress oil. While the POD activity was at its lowest was recorded in Mint oil as compared with the control. Results also showed that the PPO enzyme activity in roots of Eggplant increased with increasing time after infected with *M. javanica* until PPO enzyme activity reached to highest activity at 7 days after infection in all the used oils as well as the Oxamyl. Then, the activity of the PPO enzyme decreased at 15 days after infection compared with control.

**Discussion:**

Because synthetic nematicides endanger people and the environment, current European law has limited their use on agricultural crops. However, as chemical nematicides are not permitted in organic farms, other control methods must be created as organic farming gains attraction. Furthermore, most economically important crops lack resistant cultivars. Consequently, it is now crucial to identify new alternative control strategies. Furthermore, medicinal plants that act as botanical nematicides are widely available, less expensive than chemical nematicides, safe for the environment and people, and simple for farmers to make (Renco *et al.*, 2004 and Ononuju & Nzenwa, 2011).

Utilizing essential oils, especially those derived from plants, has demonstrated encouraging outcomes in controlling the nematodes of root-knot (*Meloidogyne javanica*) in crops like eggplant. *M. javanica* is a serious pest that damages plant roots, which inhibits plant growth and productivity. Research has indicated that

essential oils can function as a sustainable substitute for chemical insecticides.

When opposed to traditional pesticides, essential oils have the advantage of being less hazardous to the environment and biodegradable. According to encouraging outcomes from related crops, essential oils may be able to dramatically lower *M. javanica* infections in Eggplants (Hammad and Hasanin, 2022).

The results of the current investigation showed that all treatments with the eight essential oils (Castor oil, Bitter almond oil, Sweet almond oil, Watercress oil, Ginger oil, Frankincense oil, Camphor oil and Mint oil) with concentrations (125, 500, 750 and 1000 ppm), have a positive effect on *M. javanica* control. All treatments at the applied concentrations in vitro decreased the egg hatching, and Watercress oil and Bitter almond oil were the most highly effective in decreasing eggs hatchability followed by Ginger oil and Castor oil. These results are consistent with study by Amer-Zareen *et al.* (2003) that evaluated ginger extract against the root knot nematode *M. javanica*. *In vitro* studies revealed that higher concentrations of the extract (100% concentration) effectively inhibited root-knot nematode egg hatching and induced juvenile mortality. These findings align with those of Djiwanti *et al.* (2024), who investigated the nematicidal properties of Castor oil on the mortality of foliar nematodes (*Aphelenchoides fragariae*). Their study demonstrated a significantly higher mortality rate of *Meloidogyne javanica* exposed to varying concentrations of Castor oil compared to the control group in water. In their 2012 study, Zahradníková and Petříková also assessed the Watercress eluate's effectiveness against the root-knot nematode of tomato *Meloidogyne Hapla.* In accordance with our findings, when nematodes were assessed for their presence on tomato plant roots, all treated versions had 0% pathogen presence and a highly significant increase in fruit yield.

Meanwhile, the greenhouse investigation in the present study showed that all used oils application treatments at the 1000 ppm concentration reduced numbers of nematode galls, egg-masses, eggs /plant and number of J2 / 250 $cm^3$ of soil. The highest reduction in all nematode parameters was obtained with Ginger oil which gave the highest reduction rate in nematode galls, egg-masses, eggs /plant, number of J2 / 250 $cm^3$ of soil, followed by Watercress oil, then Castor oil. The findings align with the findings of other researchers that examined the nematicidal characteristics of castor (Abdel-Aty, 2010 and Chaudhary *et al.,* 2013). These findings agree with Amer-Zareen *et al.* (2003) study using ginger extract against root knot nematode *M. javanica*. Also, the outcomes supported the findings published by Mostafa *et al*. (2017) as oils made from Camphor, Black seed, Sesame, and Jojoba, Castor oil performed the best against *M. javanica*. Also, Zahradníková and Petříková (2012) study on Watercress oil against tomato plants root-knot nematode infection with *M. hapla*, also these finding agree with Refaat *et al.* (2020) commercial seed oils such as Castor *in vitro* tests using egg masses, free eggs, and second stage juveniles of *Meloidogyne incognita* revealed nematicidal activity at three doses. Tested oil, concentration, and exposure duration all had a significant ($P \leq 0.05$) impact on egg hatching and juvenile mortality. The tested oil's dilution had an inverse relationship with the suppression of egg hatching. Similarly, it was discovered that the tested oils were considerably ($P < 0.05$) effective against *M. javanica* second stage juveniles.

The results of the present study, also, showed that all the essential oils significantly increased shoot fresh weights, shoot dry weights and root fresh weight of Eggplant infected with *M. javanica*, while the results showed that Ginger oil gave the highest effect, followed by Watercress oil and Castor oil. Results of other studies are in agreement with this study to determine the effects of essential oils on shoot and root weight in the nematode management. Salim *et al.* (2016) which reported that Ginger oil with different concentrations obviously improved plant growth parameters (shoot length, total plant fresh and shoot dry weight) and suppressed nematode population in soil and roots. Castor oil extracts have shown significant nematicidal activity against root-knot nematodes (*Meloidogyne* spp.), leading to increased shoot and root weights in treated plants (Adomako & Kwoseh, 2013 and Salim *et al.,* 2016). Also, in potted plant studies, higher concentrations of Castor extracts resulted in improved plant growth metrics, including fresh shoot weight (Adomako and Kwoseh, 2013).

On the other side, the current study's findings showed that POD and POD activity in Eggplant roots was significantly increased compared with the control in all used oils and all the time. Ginger oil was the most effective in enzyme activity, followed by Castor oil then, Bitter almond oil and Watercress oil. Activity reached to highest level at 7 days after infection in all used oils as well as the Oxamyl. These findings in agree with many studies determination of the effect of essential oils such as Ginger oil, Watercress oil, and Castor oil on peroxidase (POD) and polyphenol oxidase (PPO) activities, particularly in relation to nematode interactions and reveal significant insights into their biochemical roles (El-Sherif *et al.,* 1980 and Sammari *et al.,* 2021). These oils may influence enzyme activities that are crucial for plant defense mechanisms against nematodes. Peroxidase and Polyphenol Oxidase: studies indicate that PPO activity is heightened in nematode-infected plants, suggesting a defensive response (El-

Sherif *et al.*, 1980). The presence of Ginger and watercress oils could potentially modulate this activity, enhancing plant resilience. Castor oil has been shown to induce oxidative stress, which may alter POD and PPO activities, potentially affecting nematode resistance mechanisms (Sammari *et al.*, 2021).

## CONCLUSION AND RECOMMENDATIONS

Through the results of the practical experiments conducted in this study, this study reveals the nematicidal activity of eight essential oils against J2 and eggs of *M. javanica*. The essential oils of Ginger followed by Watercress oil, then Castor oil Viminal's displayed the highest reduction percentage in nematode galls, egg-masses, eggs /plant and number of J2 / 250 $cm^3$ soil, also gave the highest increase of shoot fresh, shoot dry weight root fresh weight, POD and PPO enzyme activity. There was a progressive decrease in egg hatching with rise in oils concentration. The highest concentration that gave the highest egg hatching reduction and J2 mortality, which is a concentration of 1000 ppm. Therefore, it can be recommended to use these oils to control nematodes naturally and reduce the use of harmful pesticides.

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## الملخص العربي

# فعالية بعض الزيوت العطرية فى مكافحة *Meloidogyne javanica* على الباذنجان

ضياء إكرام الحبشي

تم تقييم الفاعلية كمبيد للنيماتودا لثمانية زيوت أساسية (زيت الخروع، زيت اللوز المر، زيت اللوز الحلو، زيت الجرجير، زيت الزنجبيل، زيت اللبان، زيت النعناع، زيت الكافور) بأربع تركيزات مختلفة لكل منها ضد Meloidogyne javanicaومبيد النيماتودا أوكساميل. ركزت الدراسة على تأثير هذه الزيوت على عدة معايير، منها: فقس البيض، نسبة الموت في الطور اليرقي الثاني (J2)، الصفات المرضية في نباتات الباذنجان مثل عدد كتل البيض، عدد العقد النيماتودية، عدد البيض لكل نبات، وعدد الأطوار اليرقية الثانية (J2) في ٢٥٠ سم٣ من التربة. كما تضمنت الدراسة تأثيرها على قياسات نمو النبات مثل الوزن الطازج والجاف للساق، الوزن الطازج للجذور، ونشاط الإنزيم المؤكسد للفينولات (PPO) والإنزيم المؤكسد للبيروكسيد (POD).أظهرت جميع الزيوت الأساسية المستخدمة قدرتها العالية على تثبيط فقس البيض بعد ٧ أيام وزيادة معدل الموت في الأطوار اليرقية J2 بعد ٧٢ ساعة من التعرض. كما لوحظ انخفاض تدريجي في فقس البيض مع زيادة تركيز الزيوت، حيث كان التركيز الأعلى (١٠٠٠ جزء في المليون) هو الأكثر فعالية. من بين الزيوت المختبرة، أظهر زيت الزنجبيل أعلى كفاءة، حيث قلل من عدد العقد النيماتودية بنسبة ٨٨,٣%، وكتل البيض بنسبة ٨٩,٥%، وعدد البيض لكل نبات بنسبة ٩٢,٢%، وعدد الأطوار اليرقية J2 في التربة بنسبة ٩٠,٢%. بالإضافة إلى ذلك، ساهم زيت الزنجبيل في تحسين نمو النبات، حيث زاد الوزن الطازج للساق بنسبة ٤٦,١%، الوزن الجاف للساق بنسبة ٦٢,٨%، الوزن الطازج للجذور بنسبة ٤٢,٢%، ونشاط إنزيمي POD (0.562)و .(0.487) PPOيليه زيت الجرجير، ثم زيت الخروع، بينما كان زيت النعناع أقل فعالية في تقليل عدد العقد النيماتودية مقارنة بمبيد أوكساميل والكنترول المعدى بالنيماتودا.

في الختام، أظهرت الزيوت الأساسية فعالية في تقليل الفقس ومعدل موت اليرقات، حيث كان زيت الزنجبيل الأكثر تأثيراً في تقليل الأضرار وتعزيز نمو النبات.

الكلمات المفتاحية: الزيوت العطرية، Meloidogyne javanica، فقس البيض، معدل الموت.