Effects of Essential Oils Distilled from Some Medicinal and Aromatic Plants against Root Knot Nematode (*Meloidogyne hapla*)

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**ABSTRACT:** Essential oils of medicinal and aromatic plants are important and promising to manage the nematological problems in agriculture. In this study, five of the plants including *Oreganum onites*, *Salvia officinalis*, *Lippia citriodora*, *Mentha spicata* and *Mentha longifolia* for egg hatching inhibition and four of the plants including *Mentha piperita*, *Foeniculum vulgare*, *Coriandrum sativum* and *Ocimum basilicum* for juvenile mortality were tested on *Meloidogyne hapla* under laboratory conditions. The oils were achieved by using water distillation method with a Clevenger apparatus. As the results of egg hatching trial, the highest egg hatching inhibition rate was found as 54% for *O. onites*. In addition, the other inhibition rates varied as 31.4%, 21.6%, 23.8%, 25.7% for the other plants, *S. officinalis*, *M. longifolia*, *M. spicata* and *L. citriodora*, respectively. Essential oil of each plant components were determined by gas chromatography (GC). Carvacrol was found as the main component (68.8%) of *O. onites* followed by Thujone 27.7% for *S. officinalis*, l-Menthone 76.9% for *M. longifolia*, Carvone 27.1% for *M. spicata* and Citral 19.3% for *L. citriodora*. For the juvenile mortality, *Mentha piperita* showed the highest mortality rate as 93.2% and was followed by *F. vulgare* 72.9%, *C. sativum* 69.3% and *O. basilicum* 64.9%. The main component of the used plants were Carvone 39.3%, Anethole 40.2%, Linalool 81.3% and Linalool 54.6%, respectively.

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The root-knot nematodes, *Meloidogyne* spp, are one of the most economically damaging genera of plant parasitic nematodes on horticultural and field crops (Andrès et al., 2012) and synthetic nematicides were used to manage nematode yield losses problem until realizing some side effects of them. Especially, the increasing recognition that nematicidal residues became a health problem promoted much more research for safe and cheap alternative methods of nematode control. Natural products provide such an alternative model for plant-parasitic nematodes and essential oils of the plants are one of the resources to be mentioned for future alternative use for nematode management. The nematicidal activity of some plant essential oils has been demonstrated against *Meloidogyne* spp. (Oka, 2000; Chitwood, 2002; Zasada et al., 2006). Some investigations so far were carried out to prove the effectiveness of the oils on *Meloidogyne* species. Abd-Elgawad and Omer (1995) pointed out that essential oils of *Mentha spicata* and *Mentha longifolia* inhibited the egg hatching of *Meloidogyne incognita* 92.2 % and 82.6%, respectively. Chatterjee et al., (1982) and Walker et al. (1996) reported that essential oils of *O. basilicum*, *M. piperita*, and *M. spicata* have nematicidal activity. Oka (2001) tested the inhibition effects of ten components of the oils on *Meloidogyne javanica* egg hatching and found that four of the components are promising to inhibit egg hatching. Ibrahim et al., (2006) examined the nematicidal activity of 18 plants and the components of the oils belonging to the plants. Among the components, the hatching of *M. incognita* eggs was completely inhibited at low concentrations (2, 4 mg liter⁻¹) of carvacrol, thymol, and linalool. Joen et al. (2016) showed that *Alpinia galanga* significantly reduced hatching of *Meloidogyne hapla* eggs at 7, 14, and 21 days after treatment and even at the end of 21 th day, the mean number of hatched eggs was below 10 individuals. Some effects of the oils or components were also observed on juveniles of *Meloidogyne* species. Accordingly, the oils of *Foeniculum vulgare*, *Oreganum syriacum* and Mentha microphylla caused 86%, 65% and 56% juvenile mortality (J2) of *M. incognita* at 100 mg/L concentration, respectively (Ibrahim et al., 2006). In another experiment, the essential oils of *F. vulgare*, *Mentha rotundifolia* and *Mentha spicata* contributed the immobilization of *M. javanica* juveniles (J2) more than 98% at 800 μl/liter after 48 hours (Oka et al., 2000). Similarly, the plants, *F. vulgare* and *Mentha pulegium* were the plants causing 100 % mortality on the *M. hapla* juveniles.

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after the exposure with the 1000 μg/mL dose for 24 hours (Joent et al., 2016).

As seen on the previous investigations, the oils are effective on egg inhibition of major root knot nematodes by their different concentrations, constitutes and exposure times. In this context, different medicinal and aromatic plants may be the substitution according to their different constitutes which are the component of their essential oils. *Meloidogyne hapla* population extracted from infected kiwifruit roots was multiplied on tomato and used in this study. This nematode species is known to have a wide host range affecting more than 550 crop and weed species (Jepson, 1987). It is found to be a common parasite of kiwifruit in different countries in the world (Sale, 1985). It has been reported in Chile, New Zealand, United States, Iran, China, Korea and Turkey (Akyazi et al., 2017; Haygood et al., 1990; Philippi et al., 1996; Ma et al., 2007; Watson et al. 1992).

The aim of this investigation is to determine the nematicidal activity of some medicinal and aromatic plants on *M. hapla* egg-hatching inhibition and juvenile mortality. By obtaining positive results, that will be possible to give recommendations to kiwifruit growing farmers and to contribute the literature.

**MATERIAL AND METHODS**

**Plant materials:** All medicinal and aromatic plants used in this study were obtained from the experimental greenhouse of the Ordu university. The nine plant species and part of the plant used for oil extraction are presented in Table 1.

| Plant species          | Common name     | Plant parts          |
|------------------------|-----------------|----------------------|
| *Oregano nitus*        | Oregano         | Flowers, stems, leaves |
| *Salvia officinalis*   | Sage            | Leaves               |
| *Lippia citriodora*    | Lemon verbena   | Leaves, seeds        |
| *Mentha piperita*      | Spearmint       | Leaves               |
| *Mentha longifolia*    | Mint            | Leaves               |
| *Mentha piperita*      | Peppermint      | Leaves               |
| *Coriandrum sativum*   | Fennel          | Seeds                |
| *Ocimum basilicum*     | Coriander       | Seeds                |
| **Axis Detector**      | **Basil**       | **Leaves**           |

**Distillation of the oils from the plants:** Different part of the medicinal and aromatic plants were used for essential oil extraction. The essential oils from parst of the plants were extracted using water distillation method with a Clevenger system. Fifty grams of each plant were submitted to hydrodistillation with a Clevenger-type apparatus according to the European Pharmacopoeia and extracted with 500 ml of distilled water for 120 min. Then the oil per plant was collected, stored at 4 °C until used.

**Preperation of nematode inoculum:** The inoculum of *Meloidogyne hapla* obtained from infected kiwifruit roots (*Actinidia delicosa A. Chev.*) and replicated on Rutgers tomato (*Lycopersicon lycopersicum*) as pure culture in pot cultures was used for the trials. Egg masses were handpicked from tomato roots and sterilized by sodium hypochlorite solution (NaOCl) (2.5 %) for 4 min. with hand-shake (Hutangura et al., 1998). The egg suspension was washed through sieve of 500 meshes and eggs retained on the sieve were poured into beher glass containing distilled water in order to condense the sterilized eggs. The eggs suspension was adjusted to a final concentration of 100 egg/ml distilled water and used for hatching assay. To mortality assay, the eggs were transferred to a hatching chamber and incubated for 24 hours in the dark at room temperature 26±2°C. As last, the fresh hatched juveniles were adjusted as 40 J2s/ml distilled water as inoculum.

**Chemical analysis of essential oils:** The essential oils of plants were analyzed with an Agilent Technology 7890A GC system coupled to a 5975C inert MSD with Triple-Axis Detector (Agilent Technologies) on a capillary column [Agilent Technologies HP-5ms (30 m x 0.25 mm I.D. x 0.25 μm film thickness)]. GC temperature program was as follow: Initial temperature was 60°C and increased to 240°C at a rate of 4°C/min. Inlet temperature was 250°C. Spectra were obtained for the range of 50–550 m/z. The GC temperature program was run with helium as carrier gas, at a flow rate of 1 mL/min and injections in split mode (1:200). The mass-spectrometer interface temperature was set to 250°C. The temperature of the ion source was 230°C, electron energy 70 eV and quadruple temperature 150°C. The injection volume was 1 μL.

**Trial and Treatment:** The treatments were arranged in a completely randomised designed with three replicates. For the homogenisation process of the oils, 2% gum arabic solution was used and 4 μl oils was added into 1 ml filtered gum arabic solution to prepare the last stock solution. Five ml of stock solution was added into petri dishes (35 mm diam.) and 1 ml of M.
the consideration component among longifolia, M. spicata 23.8%, 25.6% the other inhibition rates varied activity belongs to the solution of ranging five Egg hatching was recorded after 7 days exposure to oils. At the end of the trial, cumulative hatched and unhatched eggs were counted under Zeiss inverted light microscope at 40X magnification. In addition, after 24 hours, the juveniles were washed under tap water on a 500 mesh sieve and left in the new petri dishes with water during another 24 hours and then observed under Leica (S8APO) stereo microscope as dead and alive juveniles by touching. As last, Abbott’s formula was used to calculate egg hatching inhibition and juvenile mortality rates. The trial was carried out once.

Statistics: Before ANOVA, the assumptions which are data normality and homogeneity of variance were tested. If the assumptions fitted, then, the variables were analysed by one-way ANOVA. The mean results of ANOVA were compared in letters by Tukey’s post-hoc test. All calculations were performed with Minitab 17 statistical software. The alpha level was preferred as 5%.

RESULTS AND DISCUSSION
The nematicidal activity of essential oils from medicinal and aromatic plants against M. hapla second-stage juveniles (J2) and eggs was evaluated under laboratory conditions. The results of the treatments revealed that there is a significant difference between the groups for the trial of egg hatching inhibition, but not for those of juvenile mortality. The highest success for egg hatching inhibition was valid for O. onites and for juvenile mortality was for M. piperita with the probable success contributed by the highest constitutes as carvacrol and carvone, respectively. The other rates was also mentioned below as sperate headlines for hathing inhibition and juvenile mortality.

Egg hatching inhibition: The essential oils of the tested five plants revealed significant inhibition activity ranging between 21.6 - 54 %. The highest inhibition activity belongs to the solution of O. onites 54.0% and the other inhibition rates varied as 31.4%, 21.6%, 23.8%, 25.6 % for the other plants, S. officinalis, M. longifolia, M. spicata and L. citriodora, respectively. The level of the constituents per plant was given in table 4. Carvacrol was detected as the highest (68.8 %) among the constituents of O. onites and the most effective component on egg-hatching inhibition of M. hapla. By consideration the min. and max. effects of this plant oil, the lowest inhibition rate is beginning with 51.4 % (Table 2). This is the evidence to show the relatively high potential of the plant for nematode management. Even the 1-Menthone is the highest level (76.9%) among the constituents for M. longifolia, the effectiveness of the plant was lowest in comparison to the others. The other constitute rates were close to each other as Thujone 27.8% for S. officinalis, Carvone 27.1 % for M. spicata, Citral 19.3% for L. citriodora.

Table 2. Tested plant, inhibition rates and highest constitutes of the oils

| Plants     | Mean inhibition rates (%) and SE | Minimum- Maximum (%) |
|------------|----------------------------------|----------------------|
| O. onites  | 31.4 ± 5.1 b                     | 21.3 - 36.7          |
| S. officinalis | 25.6 ± 1.5 b                   | 23.1 - 28.7          |
| M. spicata | 23.8 ± 4.5 b                     | 14.9 - 28.9          |
| M. longifolia | 21.6 ± 3.4 b                   | 14.8 - 25.1          |

p-value 0.000

*Different letters next to the values in the column indicate significant differences at p<0.05

Fig 1. Appearance of dead second stage juvenile of M. Hapla

Juvenile mortality: Four essential oils of medicinal and aromatic plants were tested for juvenile mortality (Table 3). There was no significant differences among the essential oils of the tested plants, but high mean mortality rates which are more than 60 % for each one. The mean mortality rates ranged between 64.9 - 932 %. Mentha piperita showed the highest death rate as 93.2% and was followed by Foeniculum vulgare 72.9%, Coriandrum sativum 69.3 % and Ocimum basilicum 64.9 %. These results are also consistent with min-max rates which are waved as similar like means. When considered the constitutes, the main component of the used plants were Carvone 39.3%, Anethole 40.2%, Linalool 81.3 % and Linalool 54.6 %, respectively (Table 4). Even the rates of Linalool were different for Coriandrum sativum and Ocimum basilicum, the mortality rates are almost similar for both mean and min-max parameters. As result, the impacts are promising for the management of M. hapla.

Table 3. Tested plants for juvenile mortality and obtained results

| Plants     | Mean mortality rates (%) and SE | Minimum- Maximum % |
|------------|---------------------------------|-------------------|
| M. piperita | 93.2 ± 0.7 a                    | 91.7-93.9         |
| F. vulgare | 72.9 ± 11.1 a                   | 51.5-88.5         |
| C. sativum | 69.3 ± 18.5 a                   | 34.1-96.8         |
| O. basilicum | 64.9 ± 15.4 a                   | 35.6-88.1         |

p-value 0.48

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Effects of Essential Oils Distilled from Some Medicinal species. The further vision must be based on trying the pure main constitute of the plants if there is similar affect on nematode. This will also be beneficial to confirm if the impact is coming directly from the main constitute or not. When the dead nematodes were observed under the microscope, it’s appearance was straight or straight with very few bent. The intestinal system of the larvae was damaged and small bubbles were observed in the form of swellings (Fig 1).

In the present study, the examined essential oil solutions were the subjects of high nematotoxic effect on egg hatching inhibition and juvenile mortality on M. hapla. Even there were by now high numbers of researches on essential oils with root knot nematodes, the focus has happened mainly on other Meloidogyne species beyond M. hapla. Therefore, the results of present study were discussed by comparing M. hapla with other Meloidogyne species. Firstly, the most effective plant in the present study was O. onites on the highest egg inhibition rate and this effect probably was the result of the major constitute, carvacrol. The previous researches also proved the promising effects of carvacrol. Oka (2001) considered the nematotoxic activity of carvacrol as intermediate level and it effectively reduced the root galling caused on plant by M. javanica in case of applied at 30 mg/kg soil. In addition, Carvacrol at 100 mg/kg increased the plant fresh shoot weight into greatest level among the all applications. Ibrahim et al. (2006) used again pure Carvacrol on the eggs of M. incognita and observed almost completely inhibited hatching. They reported that hatching did not exceed 6% with carvacrol at 2 mg L\(^{-1}\) and carvacrol at 4 mg L\(^{-1}\) showed the inhibition effect mainly in the first week. The other plants of the present study were S. officinalis, L. citriodora, M. spicata, M. longifolia to observe the effectiveness against M. hapla eggs. Essential oil of this plant was

### Table 4. Chemical constitutes of essential oils distilled from nine medicinal and aromatic plant

| Plants                  | Highest Constitutes (%)                                      |
|-------------------------|-------------------------------------------------------------|
| *Origanum onites*       | Carvacrol (68.8%), Thymol (14.4%), α-Cymene (5.7%), γ-Terpine (4.2%) |
| *Salvia officinalis*    | Thujone (27.7%), Camphor (27.2%), Eucalyptol (8.6%), Camphene (5.6%) Thujone (5.6%), γ-Gurjunene (3.7%), Humulene (3.1%) |
| *Mentha longifolia*     | L-Menthone (76.9%), Isomenthone (5.4%), Isopulegone (3.1%), Eucalyptol (2.9%), β-Terpiny acetate (2.1%) |
| *Mentha spicata*        | Carvone (27.1%), Menthol (26.4 %), Isomenthone (14.4%), Eucalyptol (7.1%), Limonene (5.3%) |
| *Lippia citriodora*     | Citral (19.3%), Limonene (16.1%), Citral (15.6%), Eucalyptol (8.6%), α-Curcumene (6.5%), Caryophyllene oxide (5.1%), Spathulenol (5.0%), Caryophyllene (2.9%) |
| *Mentha piperita*       | Carvone (39.3%), Menthol (17.6 %), Isomenthone (11.0%), Eucalyptol (7.4%) , Limonene (7.2%), Menthone (3.3%) |
| *Foeniculum vulgare*    | Anethole (40.2%), Estragole (31.4%), Fenchone (19.7%), Anisaldehyde (4.7%) |
| *Coriandrum sativum*    | Linalool (81.5%), Camphor (4.4%), γ-Terpine (3.5%), α-Pinen (3.4%), Geranyl acetate (1.8%) |
| *Ocimum basilicum*      | Linalool (54.6%), Estragole (21.9%), Eucalyptol (3.5%), Citral (3.1%), tau-Cadinol (2.5%) |

**Fig 2**: Chemical structures of the major constituents in essential oils.
previously reported against Bursaphelenchus xylophilus with very low (1.9 %) efficacy (Kim et al., 2011), but in our study that was considerably high, at 25.6 %, against M. hapla eggs. Another plant at past, M. spicata was used against M. incognita and the oil of the plant suppressed the M. incognita eggs at 94 % after 5 days (Andress et al., 2012) or at the end of 16 days, hatching of the nematode eggs was also inhibited as 92.2 % (Abd- Elgawad, 1995), but in our investigation, the suppression rate was 23.8 % for M. hapla eggs after seven days. As the last plant, oil of M. longifolia inhibited the egg hatching of M. incognita as 82.6 % at the end of 16 days (Abd- Elgawad, 1995), but our result showed the rate as 21.6 % for M. hapla eggs after seven days. On the other hand, the juvenile (J2) mortality/immobilisation was the other part of the our research and the promising results were achieved according to the four plants, M. piperita, F. vulgare, C. sativum, O. basilicum. The peppermint, M. piperita was the most successful plant by creating the highest mortality rate, 93.2 % after 24 hours against M. hapla and also evaluated by now in different studies on the other root knot nematodes. For example, M. javanica juveniles were immobilised at 71.8 % after 2 days by the effect of M. piperita essential oil (1,000 µl/liter) (Oka et al., 2000). As a different species, M. incognita juveniles died at rate, 35 % after 24 hours at 1000 ppm concentration (Phadey et al., 2000). The oil of second plant, F. vulgare caused 67.4 % mortality on the M. hapla juveniles after the exposure with the 1000 µg/mL dose for 24 hours (Joen et al., 2016). The mortality of the same plant for M. incognita juveniles after 24 h exposure of the oil was 86 % at 100 mg l¹ (Ibrahim et al., 2006). Our mortality result for F. vulgare was 72.9 % after 24 hours on M. hapla juveniles. The last two plants of our study were C. sativum and O. basilicum which shared the same highest constitute, Linalool, and close mortality rates, 69.3 % and 64.9, respectively. C. sativum was applied on B. xylophilus and caused mortality as 100 % on the nematode after 24 h with the dose 2 mg/mL. (Kim et al., 2008). In case of the effectiveness for O. basilicum oil, the different concentrations 0.5, 0.1, 0.02 % showed a mean 91.4 % mortality on the juveniles of M. incognita (Gill, et al., 2001). As seen from the published studies, the number of publications are limited for M. hapla, but the effectiveness of plants used in our study is consistent with the previous investigations conducted other nematode species. Therefore, it is possible to emphasize that the egg hatching inhibition or juvenile mortality results of the plants based on essential oils are promising to modify for nematode management.

In conclusion, our results suggest that essential oils showed nematicidal potential for the management of M. hapla in present study. Among the tested plants O. onites and M. piperita were found the most effective for egg hatching inhibition and juvenile mortality, respectively. A mortality rate of more than 50% was observed. However, further experiments are needed to evaluate nematicidal activity under field conditions with nematode species.

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