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Efficiency of Some Plant Essential Oils on Root-Knot Nematode *Meloidogyne incognita*

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Abstract: During the last few decades, researchers have been in the search for environmental friendly ways to cope with agricultural pests, instead of using chemical pesticides. The use of essential oils has a high potential to become an alternative control strategy against plant parasitic nematodes. This study was conducted to determine the toxicity of 10 essential oils (*Artemisia absinthium*, *Citrus bergamia*, *Eucalyptus citriodora*, *Hypericum perforatum*, *Lavandula officinalis*, *Mentha arvensis*, *Ocimum basilicum*, *Piper nigrum*, *Thymus serpyllum* and *Zingiber officinale*) against the second stage juveniles of the root-knot nematode *Meloidogyne incognita.* The aqueous solutions of these essential oils have been applied to the second stage juveniles in three different concentrations (1%, 3% and 5%) at four different application time intervals (12, 24, 48 and 72 h). The interactions between the variables have been examined with repeated measure analysis of variance (ANOVA). This showed that the interactions of essential oil-time and essential oil-concentration were statistically significant, and it is concluded that *L. officinalis*, *A. absinthium*, *P. nigrum*, *C. bergamia* and *M. arvensis* have the most toxic effect in all concentrations and times, respectively.

Key words: Root-knot nematode, *Meloidogyne incognita*, essential oils, environmental friendly pest management.

1. Introduction

In agricultural pest management, the unconscious use of chemical pesticides, incorrect application methods and the effort to eradicate the pests have disrubted the ecological balance and caused the appearance of new pests in the fields. At the same time, the intensive use of chemicals caused the disappearance of many beneficial organisms, also it became a big threat for the protection of clean water supplies. The chemical pesticides also damage the balance of beneficial soil microorganisms. The pesticide residuals on the foods have dangerous risks for human health.

Plant parasitic nematodes are a major worldwide threat for agricultural production. The root knot nematodes *Meloidogyne* sp. attack over 2,000 different plant species and they cause approximately 5% of global crop loss [1-3]. The root knot nematode species, *M. incognita* is the most widespread and probably the most serious plant parasitic nematode pest of tropical and subtropical regions throughout the world [4]. The control of root-knot nematodes is mainly based on cultural practices, resistant cultivars and chemical nematicides. However, the intensive use of chemical nematicides has led to environmental and health concerns. Because of these concerns, and also the difficulties in the utilization and high costs of the cultural practices and the limited number of resistant varieties, more ecologically rational alternative systems, such as biological control and natural plant products, are gaining more importance [3]. Many of the chemical nematicides have been banned (91/414/EEC) or are under evaluation (2009/1107/EU) by strict legislations in European Union countries [5]. It has now become more essential to search for alternative environmental friendly ways for the management of nematodes in various agricultural crops.

Plants have been serving as effective pesticidal compounds and considered as an unlimited source of

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harmless pesticides, which have low or no toxicity against plant and vertebrates, such as fishes, birds and mammals [6]. They are not only easily biodegradable, but also get easily catabolized in the environment [7, 8]. In this context, the effects of plant essential oils against root knot nematodes, especially on their egg hatching, mortality, the immobility of the second stage juveniles and root galling, etc., have become worth to study.

The objective of this study was carried out to determine the toxicity of 10 plant essential oils at three different concentrations and at four different application time intervals on the second stage juveniles of root-knot nematode *Meloidogyne incognita*.

2. Materials and Methods

The nematodes, *M. incognita*, used in this study, have been obtained from a single egg mass and reproduced on Troy tomato cultivar which is highly susceptible to *M. incognita*. For this purpose, 3-week-old tomato seedlings were transferred into the pots containing 2 kg of sterilized sandy clay soil (80% sand and 20% clay), and one week after transplantation, 1,000 freshly hatched second stage juveniles (J2s) of *M. incognita* were inoculated in each pot with 20 mL of tap water. The inoculated plants have been placed in a climate chamber at 26 ± 2 °C and exposed to 16 h light, 8 h darkness and watered regularly.

The infected roots were removed from the pots, washed under a slow stream of tap water until the roots were purified from soil and debris. Then the roots were cut into 2-3 cm small pieces, and the root segments placed in a one liter capacity jar with of 0.25% sodium hypochlorite (NaOCl) to cover the roots and stirred vigorously for about 2-3 min. The suspension poured through 80 mesh, 200 mesh and 500 mesh sieves from top to bottom, respectively. The eggs on the 500 mesh were gently washed with a slow stream of tap water to rinse off residual NaOCl. Afterwards, the eggs were collected from the 500 mesh sieve into a beaker [9]. The egg suspensions were concentrated in a volume of 2 mL to determine the number of eggs per mL.

The essential oils tested in this study were *Artemisia absinthium*, *Citrus bergamia*, *Eucalyptus citriodora*, *Hypericum perforatum*, *Lavandula officinalis*, *Mentha arvensis*, *Ocimum basilicum*, *Piper nigrum*, *Thymus serpyllum* and *Zingiber officinale*. The source of plants, plant parts and the obtaining methods are shown in Table 1.

The experiment was 11 × 3 × 4 randomized complete block design with three repetitions. The trial was conducted twice. Factors and the levels of each factors consisted of 10 essential oils with a control group, three different aqueous concentrations (1%, 3% and 5%) of each essential oils and four application times (12, 24, 48 and 72 h). 20 mL of each concentration of each essential oils were poured into the 12 pitted plates and 100 freshly hatched J2s have been placed into each pit. The mortality of J2s was counted in time periods of 12, 24, 48 and 72 h under a light microscope by using a fine needle.

A repeated measures analysis of variance (ANOVA) was used to examine the effects of each essential oil concentration on mortality over time. In the analyses, two between-subject factors: (1) essential oils and (2) concentrations (1%, 3% and 5%) were evaluated together with the within-subject factor: time (12, 24, 48 and 72 h). The alpha level was set at 0.05. The mortality of J2s for each essential oil treatments in each concentration and time interval were summarized as means ± standard error (SE). ANOVA was performed using SPSS software (version 20, SPSS, Inc, Chicago, IL, USA) and means were compared using the Duncan's multiple range test.

3. Results and Discussion

The results of variance analysis, which has been used to indicate if the essential oil treatments, application times and application doses make difference on J2s, have been shown in Table 2.

Table 1 Essential oils used in the study.

No.	Common name	Scientific name	Plant parts	Extraction method
1	Wormwood	*Artemisia absinthium*	Leaves	Steam distillation
2	Bergamot	*Citrus bergamia*	Bark	Cold press
3	Lemon-scented gum	*Eucalyptus citriodora*	Leaves	Steam distillation
4	St. John's wort	*Hypericum perforatum*	Flowers	Cold press
5	Lavander	*Lavandula officinalis*	Leaves	Steam distillation
6	Field mint	*Mentha arvensis*	Leaves	Steam distillation
7	Basil	*Ocimum basilicum*	Leaves	Steam distillation
8	Black pepper	*Piper nigrum*	Seeds	Steam distillation
9	Wild thyme	*Thymus serpyllum*	Leaves	Steam distillation
10	Ginger	*Zingiber officinale*	Rhizomes	Steam distillation

ANOVA test indicated the absence of a triple interaction between essential oil treatments, dose and time ($P > 0.05$). However, dual interactions of essential oil treatments with the application dose and essential oil treatment with the time were found ($P < 0.01$). The presence of these interactions suggests that essential oil treatment-time interactions have occurred at all concentrations, and essential oil treatment-dose interactions have occurred for all of the time points. No interaction of dose with time was found ($P > 0.05$), which means dose-time interaction did not occur in any essential oil treatments (Table 2).

One of the main purposes of this study is to understand the toxic effects of each essential oil applied to the J2s in different application doses at the same time intervals and also in different time intervals at the same application doses. Thus, the repeated measure ANOVA has been used.

The effect of essential oil treatments and application time interactions on J2s mortality rates is shown in Table 3. The essential oil applications of *A. absinthium*, *C. bergamia*, *H. perforatum*, *L. officinalis*, *M. arvensis*, *P. nigrum* and *Z. officinale* in the different application times showed no statistically significant difference on the mortality rates of J2s. While the statistically significant differences were found at the end of 24 h for *E. citriodora*, 48 h for *O. basilicum* and 12 h for *T. serpyllum* ($P < 0.01$).

As the analysis result, *L. officinalis*, *P. nigrum*, *A. absinthium* and *C. bergamia* essential oils have the most toxic effects on J2s, followed by *M. arvensis*, *T. serpyllum* and *E. citriodora* essential oils, respectively, with a less mortal effect. Some of these potential botanical pesticides are used within this study, and an important amount of J2 mortality was achieved by *A. absinthium*, *C. bergamia*, *L. officinalis*, *M. arvensis* and *P. nigrum* essential oils in all concentrations and time intervals.

The effect of essential oil treatments and application dose interactions on J2s mortality rates is shown in Table 4. There were statistically significant differences found among all application doses of *E. citriodora*, and between 1% and 5% application doses of *T. serpyllum* ($P < 0.01$). However, there were no statistically significant differences between the doses of other essential oil treatments ($P > 0.05$).

In consequence, when considered independent application times, the most effective essential oil treatments were *L. officinalis*, *A. absinthium*, *P. nigrum* and *C. bergamia*, followed by *M. arvensis*, *E. citriodora* and *T. serpyllum* with a less mortality effect.

These results are substantially similar to previous essential oil efficacy studies which were conducted on different organisms [8-15].

Owing to the fact that the efficacy of essential oils among organisms is affected by a wide range of variables, such as plant's ripeness period, cultivation area, essential oil obtaining technology, etc., different results can be observed. Although most of the essential

Table 2 Analysis of variance to compare the average nematode mortality according to essential oil, application dose and time.

Variance	df	MS	F
Essential oil	9	4,107,292.1	540.764^{**}
Dose	2	706,320.1	92.994^{**}
Time	3	67,595.8	8.900^{**}
Essential oil × dose	18	55,506.1	7.308^{**}
Essential oil × time	27	14,428.9	1.900^{**}
Dose × time	6	3,612.9	0.476
Essential oil × dose × time	54	5,658.4	0.745

df: degree of freedom; MS: mean square; ** significant different at $P < 0.01$ level.

Table 3 Average mortality rate of J2s of *Meloidogyne incognita* at different application times.

Times	*A. absinthium*	*C. bergamia*	*E. citriodora*	*H. perforatum*	*L. officinalis*	*M. arvensis*	*O. basilicum*	*P. nigrum*	*T. serpyllum*	*Z. officinale*
12 h	88.68 ± 1.95^{aA}	81.94 ± 1.15^{aAB}	43.24 ± 7.04^{Bcd}	23.43 ± 2.8^{aE}	90.76 ± 1.10^{aA}	77.02 ± 1.20^{aB}	35.87 ± 4.20^{aD}	82.43 ± 1.16^{bAB}	53.03 ± 3.25^{aC}	16.04 ± 1.03^{aE}
24 h	91.98 ± 1.05^{aA}	82.65 ± 1.81^{aAB}	46.59 ± 6.05^{bC}	24.64 ± 2.79^{aDE}	91.27 ± 1.15^{aA}	78.58 ± 2.07^{aB}	32.35 ± 3.17^{abD}	85.35 ± 1.46^{abAB}	55.60 ± 2.77^{bC}	20.19 ± 1.90^{aE}
48 h	92.56 ± 1.25^{aA}	83.86 ± 2.22^{aA}	58.40 ± 6.25^{aB}	27.20 ± 2.50^{aCD}	92.38 ± 1.14^{aA}	81.78 ± 1.85^{aA}	36.01 ± 4.95^{aC}	87.40 ± 1.71^{abA}	58.39 ± 3.61^{bB}	21.68 ± 2.03^{aD}
72 h	90.12 ± 1.89^{aA}	87.60 ± 1.98^{aA}	61.59 ± 5.80^{aC}	27.83 ± 2.63^{aD}	93.78 ± 0.94^{aA}	76.01 ± 8.42^{aB}	26.62 ± 4.39^{bD}	90.54 ± 1.47^{aA}	61.12 ± 4.61^{bC}	22.16 ± 1.49^{aD}

Means followed by the different small letters within a column and the different capital letters within a row are significantly different according to Duncan multiple range test at $P <$ 0.01 level.

Table 4 Average mortality rate of J2s of *Meloidogyne incognita* at different application doses.

Doses	*A. absinthium*	*C. bergamia*	*E. citriodora*	*H. perforatum*	*L. officinalis*	*M. arvensis*	*O. basilicum*	*P. nigrum*	*T. serpyllum*	*Z. officinale*
1%	88.59 ± 0.92^{aA}	78.36 ± 1.01^{aA}	34.01 ± 4.31^{cBC}	18.68 ± 1.43^{aCD}	88.31 ± 0.64^{aA}	77.78 ± 1.43^{aA}	26.93 ± 4.23^{aCD}	82.20 ± 1.19^{aA}	49.07 ± 2.26^{bB}	16.10 ± 0.85^{aD}
3%	89.82 ± 1.69^{aA}	87.59 ± 0.95^{aA}	50.22 ± 3.04^{bB}	24.45 ± 1.02^{aC}	92.66 ± 0.41^{aA}	78.61 ± 1.45^{aA}	28.37 ± 1.96^{aC}	86.61 ± 1.21^{aA}	54.59 ± 2.19^{abB}	18.86 ± 0.86^{aC}
5%	94.08 ± 0.93^{aA}	86.09 ± 1.50^{Aab}	73.14 ± 2.20^{aBC}	34.20 ± 1.60^{aDE}	95.16 ± 0.40^{aA}	78.65 ± 6.39^{aABC}	42.84 ± 2.68^{aD}	90.48 ± 1.03^{aA}	67.45 ± 2.24^{aC}	25.09 ± 1.50^{aE}

Means followed by the different small letters within a column and the different capital letters within a row are significantly different according to Duncan multiple range test at $P <$ 0.01 level.

oils which were used in this study had been tested on different organisms, such as insects, mites, funguses and bacteria, they have not been tried on *M. incognita* before. Thus, most of the results of this study can be seen as the first record essence. Similar efficacies as in the other organisms have been examined on J2s of *M. incognita* as well.

As stated before, exposure of three essential oils (*A. absinthium*, *L. officinalis* and *P. nigrum*) to *M. incognita* produced more than 90% mortality. All of the plant species from which the essential oils produced are angiosperms and they belong to seven different families. Lamiaceae is the most common one with four members (*L. officinalis*, *M. arvensis*, *O. basilicum* and *Thymus serpyllum*). But at this point, it is important to emphasize that it is not the family which determines the mortality degree, because while *L. officinalis* is one of the most effective, *O. basilicum* is one of the least effective essential oils. In order to understand the logic behind this mechanism, it is necessary to distill the secondary metabolites of these essential oils, and afterwards the effective compounds should be reapplied to *M. incognita*. At the same time, these results must be supported by field experiments.

4. Conclusions

This study is an evaluation of 10 plant essential oils against to *M. incognita* under laboratory conditions. This study is the first report of nematicidal activity against *M. incognita* of these essential oils in laboratory conditions.

The findings showed that the essential oils *L. officinalis*, *A. absinthium*, *P. nigrum*, *C. bergamia* and *M. arvensis* have the most toxic effect in all time intervals and application doses on *M. incognita*.

It has a great importance to find and use alternative control strategies against the root-knot nematode *M. incognita* as in all agricultural pest species. Based on the positive results of this study, which is limited to 10 essential oils, new studies can be conducted by using different doses and time intervals of the highly effective essential oils.

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