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Araştırma Makalesi

Screening of the Nematicidal Potential of Some Essential Oils against the Columbia Root-Knot Nematode, *Meloidogyne chitwoodi*

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ABSTRACT

In this study, the nematicidal effects of four essential oils, *Rosmarinus officinalis* L. (Rosemary), *Thymus vulgaris* L. (Thyme), *Sesamum indicum* (Sesame), *Lavandula* spp. (Lavender), on *Meloidogyne chitwoodi* Golden et al., 1980 (Tylenchida: Meloidogynidae) were investigated by petri experiments. In the study, 125 ppm, 250 ppm, 500 ppm, 10000 ppm, 20000 ppm, 30000 ppm concentrations of essential oil were used to prevent egg hatching and to evaluate the lethal effect on second stage juveniles. The results showed that each essential oil inhibited hatching at 24 h, 48 h, 72 h and 7 days at varying levels. Sesame and lavender oils also had the highest rate of preventing egg hatching. Lavender, thyme, and rosemary oils exhibited higher juvenile mortality than sesame in all concentrations. More than 92.2% mortality of *M. chitwoodi* juveniles occurred after a 24 hour exposure to a 10000 ppm oil solution of lavender, thyme, rosemary. All exposure times and concentrations resulted in a 100% mortality rate of *M. chitwoodi* in thyme. The most effective result within 24 hours was observed in thyme oil. The results suggest that it should be set up pot experiments for in vitro results.

Keywords: Control, Columbia root knot nematodes, mortality, inhibition, non-chemical methods.

Kolombiya Kök-ur nematodu, *Meloidogyne chitwoodi*'ye karşı Nematisit Potensiyeli Olan Bazı Esansiyel Yağların İncelenmesi

ÖZ

Bu çalışmada dört uçucu yağ *Rosmarinus officinalis* L. (Biberiye), *Thymus vulgaris* L. (Kekik), *Sesamum indicum* (Susam), *Lavandula* spp. (Lavanta)'nın *Meloidogyne chitwoodi* Golden ve ark.,1980 (Tylenchida: Meloidogynidae) üzerindeki nematisit etkileri petri deneylerinde araştırılmıştır. Çalışmada uçucu yağın 125 ppm, 250 ppm, 500 ppm, 10000 ppm, 20000 ppm, 30000 ppm konsantrasyonları kullanılarak yumurtadan çıkışın engellenmesi ve ikinci dönem larva üzerindeki öldürücü etkisi değerlendirilmiştir. Sonuçlar, her bir esansiyel yağın, 24 saat, 48 saat, 72 saat, 7. günde farklı seviyelerde yumurtadan çıkmayı engellediğini göstermiştir. Susam ve lavanta yağları yumurtadan larva çıkışının önlemesinde en yüksek orana sahip olmuştur. Lavanta, kekik ve biberiye yağlarının tüm konsantrasyonlarında susamdan daha yüksek larva ölümü gözlenmiştir. *M. chitwoodi* %92,2'sinden fazla larva ölümü, 10000 ppm'lik lavanta, kekik ve biberiye yağ çözeltisine 24 saat maruz kaldıktan sonra meydana gelmiştir. Kekikte tüm maruz kalma süreleri ve konsantrasyonlarda, *M. chitwoodi* larvalarının %100 ölüm oranı ile sonuçlanmıştır. 24 saat içerisinde en etkili sonuç kekik yağında gözlenmiştir. Buna göre, in vitro'daki sonuçlarla saksı deneyleri yapılması gerektiğini göstermektedir.

Anahtar Kelimeler: Kontrol, Kolumbia kök ur nematodu, ölüm oranı, engelleme, kimyasal olmayan yöntemler.

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Introduction

Plant-parasitic nematodes are microscopic organisms that can wreak havoc on agricultural crops causing significant economic losses worldwide (Blew et al., 2007). *Meloidogyne* (root knot nematode) is comprised of over ninety species (Moens et al., 2009) and it is commonly observed in a wide range of climates, from tropical to subtropical regions to mild temperate zones (Sasser and Carter, 1985). The Columbia root-knot nematode also known as *Meloidogyne chitwoodi* is a significant threat to various crops such as potatoes, carrots, and other vegetables. In our country, *M. chitwoodi* is commonly found in potato growing areas (Evlice and Bayram, 2016; Evlice et al., 2021). Chemical nematicides have been traditionally used to control these plant parasitic nematodes but concerns about their environmental impact and potential harm to human health have led to the search for alternative sustainable solutions (Catani et al., 2023). In recent years, phytochemicals, and essential oils derived from plants such as sesame, rosemary, lavender, and thyme have shown promise as natural nematicides (Chitwood, 2002).

Sesame oil (*Sesamum indicum*) has garnered attention for its nematicidal properties in last studies. These have indicated that sesame oil possesses biostimulator properties, interfering with the nematode's ability to infect plant roots and reproduce (Laquale et al., 2018). Researchers believe that the presence of natural compounds like sesame oil plays a role in disrupting the nematode's life cycle (D'Addabbo et al., 2011; D'Addabbo et al., 2019). These compounds have been shown to inhibit egg hatching and reduce the motility of nematode juveniles (J2) ultimately leading to decreased nematode populations in the soil. Rosemary oil (*Rosmarinus officinalis*) is known for its aromatic qualities and has demonstrated potential as a natural nematicide (Erdoğuş, 2002). Additionally, rosemary oil has the potential to encourage plant defences against nematode infestations which makes it a promising tool for integrated pest management strategies. Lavender oil (*Lavandula* spp.) is celebrated for its pleasing fragrance, but it also possesses nematicidal properties. The primary nematicidal compound in lavender oil is linalool which interferes with nematode mobility and causes paralysis (D'Addabbo et al., 2021). Lavender oil has demonstrated its ability to reduce nematode populations in agricultural soils. offering a natural and eco-friendly alternative to synthetic nematicides. Lastly, thyme oil (*Thymus vulgaris*) is well-known for its culinary applications, but it also has powerful nematicidal properties (Abd-Elgawad and Omer, 1995). Thymol, a major constituent of thyme oil has been shown to exhibit strong nematicidal activity against various nematode species, including *Meloidogyne chitwoodi*. Thyme oil is particularly effective in reducing nematode populations in the soil when used in appropriate concentrations.

Essential oils can be applied as soil drenches, and fumigants or incorporated into organic amendments. However, their effectiveness may vary depending on factors such as nematode species, soil conditions, and climate. It is crucial to conduct thorough research and consider local conditions before implementing essential oils as part of a nematode management strategy. The nematicidal effect of these essential oils on *Meloidogyne chitwoodi* is examined in this work.

Material and Method

The pure culture of *M. chitwoodi* in Çukurova University Plant Protection Department nematology laboratory and propagated in Cüsseli tomato cultivar was used in the experiments. Egg masses were taken out of the infected tomato roots. After extracting that, these eggs were used for both egg hatching and mortality juvenile experiments (Hussey and Barker, 1973). The 125 ppm, 250 ppm, 500 ppm, 10000 ppm, 20000 ppm, 30000 ppm concentrations were prepared by diluting essential oil solutions (10 % ethanol, vol/vol) with water containing 0.3 % (Tween 20, vol/vol). The concentration of ethanol at the end was less than 1 % (Oka et al., 2000). 100 second stage juveniles were given to each petri experiments. The egg hatching experiments in 5 egg masses for each petri were established, and a 10000 ppm dose was tried at 24 h, 48 h, 72 h and 7 d. The Petri dishes were maintained at a temperature of room temperature (24 ± 3°C) at 24 h, 48 h, 72 h.

Nematodes in distilled water, Tween 20, and Tervigo solutions served as negative and positive checks. If they didn't move with a fine needle, the J2 was considered dead (Cayrol et al. 1989). The assays were conducted completely randomized experimental design with 4 replicates in two times. The results necessary were transformed to the base on Arcsin and Ln (x+1) for the homogenized error variances. SPSS 22 ANOVA program was used, and statistical analysis was performed according to the Tukey separation test.

Results and Discussion

This study evaluated the nematicidal effect of rosemary, lavender, sesame, and thyme essential oils against *Meloidogyne chitwoodi*. The effect of 4 essential oils was evaluated at 125 ppm, 250 ppm, 500 ppm, and 10000 ppm, 20000 ppm, 30.000 ppm (Table 1 and 2). Thyme essential oil significantly increased the mortality rate of nematodes by 100 % after 24 hours in all concentration. Increases in mortality of nematodes were found when the effects of essential oils after concentration of 10.000 ppm were shown significant. Thyme oil was found to cause an almost complete mortality of root-knot nematode (J2) and eggs in vitro studies. The best strong nematicidal activity of thyme was observed on J2 at 125 ppm in 24 h which indicates that it has potential effect. In this study, it was observed nematicidal activity in rosemary, lavender, and thyme oils. Sesame oil was demonstrated for a suppressive activity on root-knot nematode populations. The mortality rate of larvae in sesame was not significant, but it was observed that egg hatching of *M. chitwoodi* was prevented. Biostimulants (Sesame oil) resulted in a significant reduction of nematode eggs and galls on tomato roots since it was significantly more suppressive on tomato plants (D'Addabbo et al., 2019). In our results, it was observed on suppression of egg hatching experiments. According to Oka et al. (2000), there is a significant connection between nematicidal and insecticidal activity and it has been suggested that essential oil components may impact the nervous system of nematodes. The aromatic plants of Lavandula, Rosmarinus, and Thymus have been widely studied (Oka et al., 2000). Oka et al. (2000) tested *T. vulgaris* oil against root-knot nematodes, but it only had a moderate effect on J2 immobilization and egg hatching inhibition of *M. javanica* at 1.000 l/l. However, our results showed that thyme is more effective on mortality rate of (J2) than other essential oils. These results overlap with the results of experiments conducted on other nematodes (Andres et al., 2012). *T. vulgaris* is grown in the Mediterranean region as native plants, so it can be intraspecific variability. Rosemary and Lavandula were found moderate effects on nematodes similarly.

All the results vary according to the type and concentration of the essential oil. The increase in oils concentration and exposure period led to an increase in mortality percentage. This increase was observed as a general trend for other essential oil.

Table 1. % Mortality of *Meloidogyne chitwoodi* second-stage juveniles in direct contact with 4 essential oils at a concentration of 125 ppm, 250 ppm and 500 ppm

Concentration of essential oil		Sesame (Mean±SE)	Rosemary (Mean±SE)	Lavender (Mean±SE)	Thyme (Mean±SE)
125 ppm	24 h	0.6 ± 0.6 e	2.0 ± 1.1 d	2.4 ± 0.6 g	100.0 ± 0.0 a
	48 h	1.3 ± 0.8 e	2.0 ± 1.1 d	4.9 ± 1.3 efg	100.0 ± 0.0 a
	72 h	2.7 ± 0.5 de	9.3 ± 1.0 bcd	12.2 ± 1.1 bcd	100.0 ± 0.0 a
250 ppm	24 h	0.9 ± 0.4 e	7.1 ± 2.0 cd	4.0 ± 1.4 fg	100.0 ± 0.0 a
	48 h	4.0 ± 0.6 cde	8.3 ± 1.9 cd	4.0 ± 1.4 fg	100.0 ± 0.0 a
	72 h	4.0 ± 0.6 cde	10.0 ± 1.7 bcd	13.6 ± 0.4 bc	100.0 ± 0.0 a
500 ppm	24 h	6.1 ± 0.6 bcde	8.4 ± 0.7 cd	6.7 ± 1.5 cdefg	100.0 ± 0.0 a
	48 h	6.3 ± 0.6 bcde	9.3 ± 1.3 cd	7.1 ± 1.6 cdefg	100.0 ± 0.0 a
	72 h	7.0 ± 0.4 bcd	17.2 ± 2.9 b	14.1 ± 0.9 b	100.0 ± 0.0 a
Control (Water)	24 h	2.9 ± 1.4 de	2.9 ± 1.4 d	2.9 ± 1.4 fg	2.9 ± 1.4 d
	48 h	5.4 ± 1.4 cde	5.4 ± 1.4 cd	5.4 ± 1.4 defg	5.4 ± 1.4 cd
	72 h	7.3 ± 0.9 bcd	7.3 ± 0.9 cd	7.3 ± 0.9 bcdefg	7.3 ± 0.9 bcd
Control (Tween 20)	24 h	8.2 ± 1.4 bcd	8.2 ± 8.2 cd	8.2 ± 1.4 bcdefg	8.2 ± 0.0 bcd
	48 h	9.4 ± 1.7 bc	9.4 ± 9.4 bcd	9.4 ± 1.7 bcdef	9.4 ± 0.0 bc
	72 h	11.9 ± 3.3 b	11.9 ± 11.9 bc	11.9 ± 3.3 bcde	11.9 ± 0.0 b
Control (Tervigo)	24 h	100.0 ± 0.0 a	100.0 ± 100.0 a	100.0 ± 0.0 a	100.0 ± 0.0 d
	48 h	100.0 ± 0.0 a	100.0 ± 100.0 a	100.0 ± 0.0 a	100.0 ± 0.0 cd
	72 h	100.0 ± 0.0 a	100.0 ± 100.0 a	100.0 ± 0.0 a	100.0 ± 0.0 bcd

In a column, numbers followed by the same letter are not significantly different (P <- 0.05) according to the Tukey multiple-range test.

Table 2. % Juvenile mortality of *Meloidogyne chitwoodi* in direct contact with 4 essential oils at a concentration of 10 000 ppm. 20 000 ppm and 30 000 ppm

Concentration of essential oil		Sesame (Mean±SE)	Rosemary (Mean±SE)	Lavender (Mean±SE)	Thyme (Mean±SE)
10 000 ppm	24 h	12.4 ± 1.9 def	93.9 ± 1.6 a	92.2 ± 0.8 a	100.0 ± 0.0 a
	48 h	16.7 ± 2.2 cdef	94.5 ± 1.1 a	92.2 ± 0.8 a	100.0 ± 0.0 a
	72 h	18.2 ± 2.3 cdef	95.8 ± 1.8 a	92.2 ± 0.8 a	100.0 ± 0.0 a
20 000 ppm	24 h	27.5 ± 1.8 bcde	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	48 h	28.9 ± 1.1 bcd	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	72 h	32.4 ± 2.0 bcd	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
30 000 ppm	24 h	31.2 ± 4.0 bcd	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	48 h	32.7 ± 3.3 bc	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	72 h	38.2 ± 3.4 b	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Control (Water)	24 h	2.6 ± 0.7 f	2.6 ± 0.7 b	2.6 ± 0.7 b	2.6 ± 0.7 b
	48 h	15.1 ± 9.3 cdef	15.1 ± 9.3 b	15.1 ± 9.3 b	15.1 ± 9.3 b
	72 h	18.7 ± 10.5 bcdef	18.7 ± 10.5 b	18.7 ± 10.5 b	18.7 ± 10.5 b
Control (Tween 20)	24 h	3.0 ± 1.1 f	3.0 ± 1.1 b	3.0 ± 1.1 b	3.0 ± 1.1 b
	48 h	7.7 ± 1.7 ef	7.7 ± 1.7 b	7.7 ± 1.7 b	7.7 ± 1.7 b
	72 h	15.5 ± 2.9 cdef	15.5 ± 2.9 b	15.5 ± 2.9 b	15.5 ± 2.9 b
Control (Tervigo)	24 h	100 ± 0 a	100 ± 0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	48 h	100 ± 0 a	100 ± 0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	72 h	100 ± 0 a	100 ± 0 a	100.0 ± 0.0 a	100.0 ± 0.0 a

In a column, numbers followed by the same letter are not significantly different (P <0.05) according to the Tukey multiple-range test.

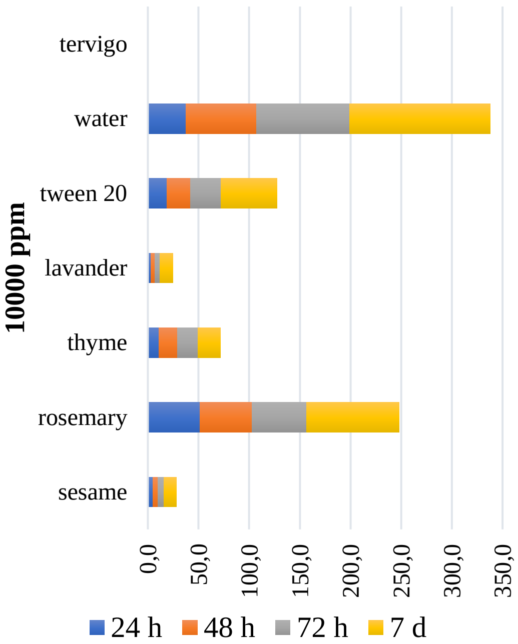

Figure 1. Effect of essential oils on mobility of *Meloidogyne chitwoodi* hatching of eggs

The effectiveness of essential oils is variable. Their chemical composition may vary considerably between aromatic plant species and varieties, and within the same variety from different geographic areas. Moreover, the composition of plants can be drastically influenced by plant maturity at the time of oil extraction and phenotypic differences (Lahlou and Berrada, 2003). While there are studies on other plant parasitic nematodes (Barbosa et al., 2010), there are not enough studies on the control of *Meloidogyne chitwoodi* with essential oils. This study draws attention to the deficiency in this regard.

Conclusion

For the past few decades, researchers have been searching for more sustainable alternatives that have the same effectiveness but have a limited impact on the environment and ecosystems. Bioactive compounds are found in essential oils. The nematicidal properties of essential oils derived from sesame, rosemary, lavender, and thyme offer promising alternatives to chemical nematicides for controlling *Meloidogyne chitwoodi* and other plant-parasitic nematodes. These natural solutions not only show effectiveness in reducing nematode populations but also come with the added benefits of being environmentally friendly and safe for human health. However, further research is needed to optimize their application methods and determine their efficacy under various conditions. As sustainable agriculture practices gain importance. essential oils may play a significant role in managing nematode infestations while promoting the health of both crops and the environment.

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