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Research Article

Larvicidal Effect of Essential Oils

Larvicidal Effect of Some Essential Oils Against Larvae of the European Pine Sawfly [*Neodiprion Sertifer* (Geoff.)]

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Abstract: Many of the species that cause damage to pine trees by feeding on needle leaves, and trees without leaves may be vulnerable to secondary pests as well as increment loss. One of the important leaf pests that damage Turkish red pine (*Pinus brutia*) and the Anatolian Black pine (*Pinus nigra* A.Rich) in Turkey is the pine sawfly *Neodiprion sertifer* (Geoff.). Only mechanical and chemical control methods are carried out with this pest. Due to the negative effects of chemical control methods on the ecosystem and nature, alternative methods have come to the fore front in recent years. One of them is the use of essential oils against harmful insects. The aim of the study is determine to larvicidal effects of some essential oils against larvae of *N. sertifer*. To achieve of this aim our objective is applied three different dosages (0.1%, 0.5% and 1.0%) of essential oils obtained from *Eucalyptus camaldulensis*, *Melissa officinalis* and *Origanum onites* plants to the 4th and 5th instar larvae of pine sawfly. Trials were carried out in the laboratory under controlled conditions (25±5°C temperature and 65±5% proportional humidity). Dead larvae in each box were counted 12, 24, 36 and 48 hours after application. According to the application dosages, the highest deaths of *N. sertifer* larvae occurred at 1% doses of all three essential oils. It was observed that the larval mortality rates increased as the dosages increased. As a result, essential oils of all three plant species were found to be hopeful in the controlling of *N. sertifer*, of which essential oil of *O.onites* was found more effective on the 4th and 5th instar larvae than the essential oils of the other two plant species. They have shown that essential oils can be a potential alternative to synthetic insecticides in the control of pine sawfly.

Keywords: Pine Sawfly, Essential Oil, Eucalyptus camaldulensis, Melissa officinalis, Origanum onites

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1. INTRODUCTION

Pine sawfly (*Neodiprion sertifer*) causes significant damage in all age classes of forest trees, especially the Turkish red pine (*Pinus brutia* Ten.) and the Anatolian Black pine (*P. nigra* A.Rich), which are pine species that make up the majority of forest areas in Turkey. *Neodiprion sertifer* prefers trees between 10-15 years of age in unsuitable soil conditions and is a primary character species. The larvae causes the main damage by eating the needle leaves of the trees, leaving the stand in a leafless state, reducing its resistance to other biotic and abiotic factors and negatively affecting the diameter development of the trees. [1-5] Biological, mechanical and chemical control methods are used against pine sawfly. Mechanical control methods are not sufficient in the areas where the damage is intense. As a result of the negative effects of insecticides used in chemical control on human and environmental health, the use of alternative applications becomes inevitable. Essential oils have an important place among these alternative applications. Essential oils are rich in components such as flavonoids, terpenoids and alkaloids, that can be used to control pests and serve as an alternative to insecticides. Also, plant essential oils have biological activities against plant pests and may use fumigants, contact insecticides, repellents, and antifeedants, or they can affect the growth rate, reproduction, and behavior of insect pests[6,7.] One third of about 300 plant families that spread in nature contain essential oil and grow in hot climates. Therefore, the Mediterranean Region is one of the richest areas within flora in Turkey. Essential oils obtained from plants are preferred in the controlling of pests because they are present in nature, do not form long-term residues, do not create additional toxic substances, do not cause water and soil pollution. As a result of the previous studies, it has been revealed that essential oil components have effects such as insecticide, antifeedant, attractant, egg killer (ovicide), repellant, growth and reproduction inhibitor in the fight against pests[8-17.] Although there are many studies on the insecticidal effect of essential oils against different pests in forest areas, there is no study on the lethal effect against pine sawfly species. In this study, the lethal effect of essential oils of three plant species *Origanum onites* L., *Eucalyptus camaldulensis* Dehn. and *Melissa officinalis* L. on *Neodiprion sertifer* larvae was investigated.

2. MATERIALS AND METHODS

The study was carried out in the laboratory under controlled conditions (25±5°C temperature and 65±5% proportional humidity). *Eucalyptus camaldulensis* which is actually exotic and used in plantations in the Mediterranean Region in Turkey for many years, as well as *O. onites* and *M. officinalis* grown in natural areas. The larvicidal effects of essential oils obtained from these plant species against the 4th and 5th instar larvae of *N. sertifer* were investigated.

2.1 Collecting of plant materials

During the vegetation periods of 2020-2021, leaf and flower samples of *E. camaldulensis*, *M. officinalis* and *O. onites* taxa were collected from the Antalya region of Turkey. Specimens were collected from at least three flowering plants from each plant for identification. The collected plant samples were dried and pressed according to the herbarium technique methods and the information about the samples was recorded. Identification of the plant materials was made in the Laboratory of Bursa Technical University Faculty of Forestry, Department of Forest Botany and it was kept under protection. At least 1 kg of leaf samples were collected from each plant to be used in essential oil analysis. The collected plants were placed in bags and the bags were coded and labeled, and information such as collection time, location and altitude were recorded on the label. These plants were then dried at room temperature in a semi-shaded, airy place to be used in essential oil analysis. Leaf and flower specimens of selected plants *Eucalyptus camaldulensis*, *Melissa officinalis* and *Origanum onites* were authenticated by corresponding author Assoc.Prof.Dr. Ayse Gul Sarikaya. Voucher specimens of the plants have been deposited in the herbarium of Department of Forest Botany, Faculty of Forestry in Bursa Technical University.

2.2 Hydrodistillation

The collected plant materials were weighed after drying at room temperature (25°C), and then distilled for about three hours in a hydrodistillation device connected to the Clevenger apparatus, and the essential oil yields were determined as volume/weight (v/w) and the volatile components obtained were determined at +4°C. It was stored until analysis.

2.3 Gas chromatography-mass spectrometry

Qualitative and quantitative analysis of volatile compounds obtained from leaf samples of the collected plants were determined by GC/MS (QP 5050 gas chromatography/mass spectrometry) device. The GC/MS analysis conditions; CP-Wax 52 CB (50 m x 0.32 mm; film thickness = 0.25 μ m) was used as the column. Oven temperature program was increased from 60°C to 220°C by increasing 10°C per minute and kept at 220°C for 10 minutes. Injection block temperature was 240°C, detector temperature was 250°C, detector energy flow was 70 eV, ionization type: EI, carrier gas: helium (20 ml/min) and flow rate was 10 psi. Wiley, NIST and Tutor mass spectrometry libraries were used to identify injected volatile compounds. This process was performed in 3 replications and the arithmetic average of the ratios of the determined compounds was taken.

2.4 Trials on Neodiprion sertifer larvae

Neodiprion sertifer larvae were collected from same population in young the Turkish red pine (*Pinus brutia*) forests in Bursa-Orhaneli region, Kınık locality (39°42'52" N; 29°2'19" E) in May and June. Essential oils were dissolved in ethanol at a ratio of 1:2, set against larvae of the same age, with 3 replications for different doses of each essential oil type (0.1%, 0.5% and 1.0%). The study was carried out in plastic containers measuring 10x10 cm. Blotting papers moistened with distilled water were placed in these containers and sufficient amounts of pine needles were placed to feed the larvae. Ten larvae were placed in each plastic container. The larvae used in the experiment were selected from a single colony. Different doses of essential oils used in the study (0.1%, 0.5 and 1.0) were prepared using distilled water and 0.3% tween 80 (Emulsifier). The prepared dosages were sprayed in a way that 1 ml came into the plastic containers in which the larvae were placed. Only pure water was sprayed into the control boxes. Dead larvae in each box were counted 12, 24, 36 and 48 hours after application. In order to determine whether there is a statistically significant difference between the obtained data, two-way analysis of variance (ANOVA) was applied using the SPSS 23.0 software package and the differences between the means were tested with the Duncan's test.

3. RESULTS

3.1 Chemical composition of plant oils

Qualitative and quantitative analysis of volatiles was determined by GCMS (QP 5050 gas chromatography/mass spectrometry). Thirty four different components were determined in *E. camaldulensis*, of them the main components were 1,8-cineole (RT (Retention Time): 26.000), α--pinene (RT: 8.239), o-cymene (RT: 28.131) (Figure 1); 52 different components were determined in *M. officinalis* and the main components were geranial (RT: 28.131), neral (RT: 26.000) and citronellal (RT: 8.184) (Figure 2), and also 61 different components were determined for *O. onites*, the main components were determined as carvacrol (RT:15.156), thymol (RT: 28.495) and linalool (RT:12.372) (Figure 3).

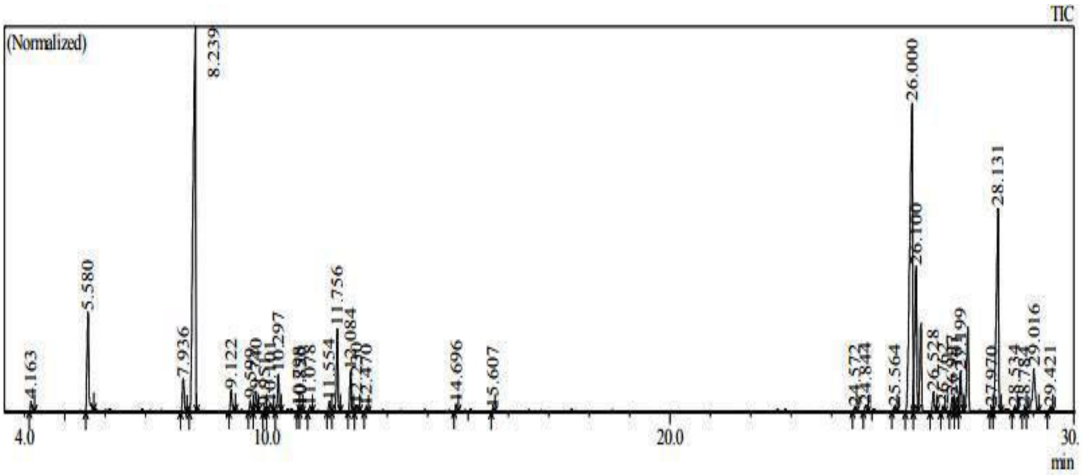

Fig 1. GC-MS analysis chromatogram of *Eucalyptus camaldulensis*

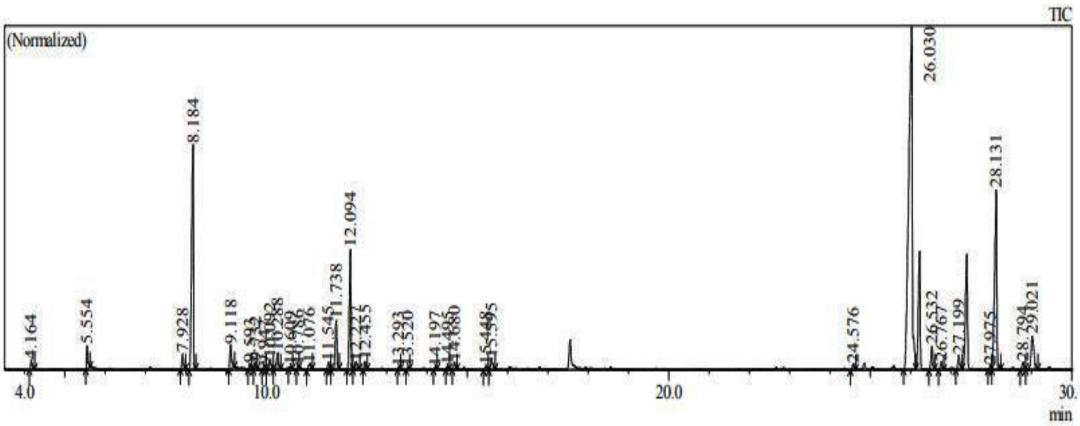

Fig 2. GC-MS analysis chromatogram of *Melissa officinalis*

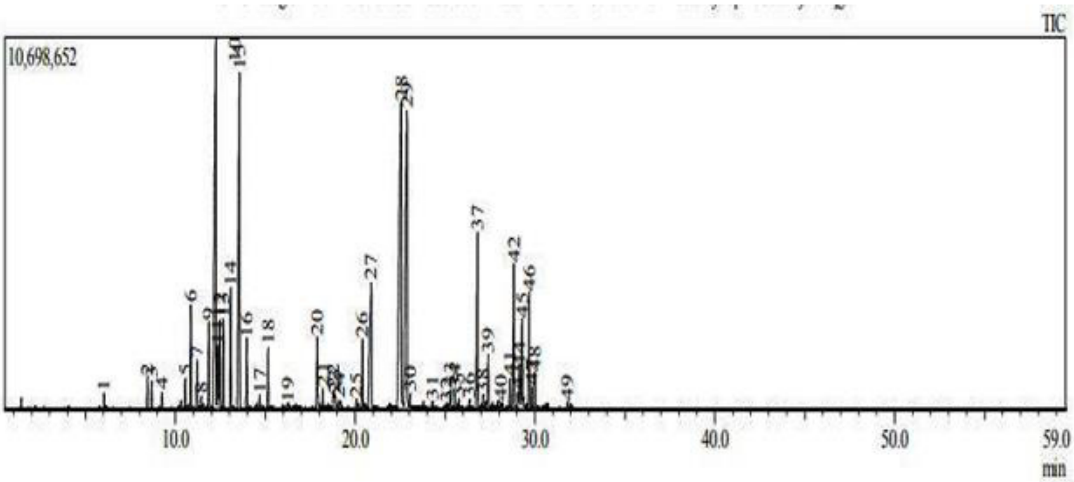

Fig 3. GC-MS analysis chromatogram of *Origanum onites*

3.2 Phytotoxicity of essential oils on Neodiprion sertifer larvae

As a result of the tests, it was observed that the applications at the dosages of 0.1%, 0.5% and 1% of the essential oils caused different rates of death on the fourth and fifth stages of the pine leaf bee when compared with the control groups. According to the application dosages, the highest deaths of *N. sertifer* larvae occurred at 1% doses of all three essential oils. It was observed that the larval mortality rates increased as the dosages increased. When the dosages of all oils were evaluated within themselves, the upper doses were statistically different from the other dosages (Table 1).

Table 1. Mean (%) mortality of 4th and 5th instars of *Neodiprion sertifer* treated with essential oil of *O. onites*, *E. camaldulensis* and *M. officinalis*.

Plant Species		12h	Sd	24h	Sd	36h	Sd	48h	Sd
	CON	0,00dA	0,00	0,00cA	0,00	6,67cA	1,15	20,00bA	1,73
O. onites	D 0.1	20,00cC	1,00	50,00bB	1,00	73,33bA	0,58	90,00aA	1,00
(4. instar)	D 0.5	40,00bC	1,00	60,00bBC	1,00	76,67bAB	1,53	96,67aA	0,58
	D 1.0	83,33aB	0,58	96,67aA	0,58	100,00aA	0,00	100,00aA	0,00
	CON	0,00cA	0,00	0,00dA	0,00	6,67cA	1,15	20,00bA	1,73
O. onites	D 0.1	6,67cD	1,15	26,67cC	1,15	56,67bB	1,15	86,67aA	0,58
(5. instar)	D 0.5	33,33bD	1,15	50,00bC	1,00	66,67bB	0,58	96,67aA	0,58
	D 1.0	73,33aC	0,58	86,67aB	0,58	90,00aAB	1,00	100,00aA	0,00
	CON	0,00cA	0,00	0,00cA	0,00	6,67cA	1,15	20,00bA	1,73
E. camaldulensis	D 0.1	10,00cC	1,00	36,67bBC	1,53	60,00bAB	1,00	83,33aA	2,08
(4. instar)	D 0.5	30,00bC	0,00	43,33bC	0,58	63,33abB	1,53	83,33aA	0,58
	D 1.0	60,00aC	1,00	73,33aBC	0,58	83,33aAB	0,58	90,00aA	1,00
	CON	0,00bA	0,00	0,00cA	0,00	6,67cA	1,15	20,00cA	1,73
E. camaldulensis	D 0.1	13,33bB	1,15	26,67bAB	1,73	46,67bAB	2,08	56,67bA	2,52
(5. instar)	D 0.5	13,33bB	0,58	30,00bB	0,58	46,67bA	1,53	60,00bA	1,00
	D 1.0	60,00aB	1,00	76,67aA	0,58	83,33aA	0,58	93,33aA	1,15
	CON	0,00cA	0,00	0,00cA	0,00	6,67dA	1,15	20,00bA	1,73
M. officinalis	D 0.1	3,33cC	0,58	13,33cC	0,58	43,33cB	0,58	80,00aA	1,00
(4. instar)	D 0.5	30,00bC	1,00	40,00bBC	2,00	60,00bAB	1,00	80,00aA	1,00
	D 1.0	60,00aC	1,00	76,67aB	0,58	90,00aA	0,00	96,67aA	0,58
	CON	0,00cA	0,00	0,00cA	0,00	6,67cA	1,15	20,00bA	1,73
M. officinalis	D 0.1	10,00bcC	1,00	6,67cC	0,58	33,33bB	1,15	70,00aA	1,00
(5. instar)	D 0.5	23,33bB	0,58	43,33bAB	2,31	60,00aA	1,73	76,67aA	2,08
	D 1.0	46,67aC	1,15	73,33aB	0,58	80,00aAB	1,00	93,33aA	0,58

a-d (↓) Mean% mortality for different concentrations within the same instar and time followed by the same small letter are not significantly different (P>0.05) by Duncan's test. A-C (→) Mean% mortality for different times within the same instar and concentrations followed by the same capital letter are not significantly different (P>0.05) by Duncan's test. Sd: standard deviation

It is noteworthy that in dose applications, *O. onites* is more effective on the 4th and 5th instar larvae than the essential oils of the other two plant species. In the applications of essential oil of *O. onites*, it was determined that the mortality rate reached 100% at the end of 48 hours. In all treatments, it was observed that the mortality rate on the 4th instar larvae was high. Another important result is that the lowest effect on the larvae was seen in the application of *M. officinalis* on the fourth instar larvae. The least deaths were detected at the dosage of 0.1% for the 4th and 5th larval stages of the essential oil of *M. officinalis*.

4. DISCUSSION

No studies were found on the larvicidal effects of plant essential oils on *N. sertifer* larvae. On the other hand, there were studies in which different trials were conducted against some other forest pest species. Of these, Faria [18] determined that essential oil components of *O. onites* and *Thymus vulgaris* showed the highest effect in the trials she conducted against pine processionary moth (PPM) species *Thaumetopoea pityocampa* and *T. wilkinsoni*. In another study, chemical composition of *Vitex agnus-castus* L. (Verbenaceae) essential oil tested against pine processionary moth, β -caryophyllene, germacrene D, 1,8-cineole and ζ -gurjunene were recorded as the main components of this essential oil. At the 96th hour of the study, essential oil caused deaths between 13.30% and 96.6%.[19] Bozhüyük [20] tested *V. agnus-castus* on pine processionary moth and determined that it caused deaths between 20-100% at the 96th hour of application. Kesdek et al.[21] applied the essential oil obtained from *Achillea biebersteinii* Afan (Asteraceae) at different doses and times against *T. pityocampa* larvae. As a result of the study, it was determined that the mortality of larvae varied between 3.33% and 100%. Also, Yigit et al.[22] applied three different dosages (0.1%; 0.5% and 1.0%) of essential oils obtained from *Rosmarinus officinalis*, *Teocrium polium*, and *Sideritis libanotica* plants against PPM larvae. They determined that the essential oil of *S. libanotica* can be used as a promising method in the fight. In another study, Yigit et al. [23] found that thyme oil, poppy oil, sage oil and garlic oil from these essential oils were effective against PPM larvae by using 8 different essential oils against pine processionary moths. The study of Kaya et al. [24] showed that some plant essential oils can be used in studies investigating new environmentally friendly active substances in the control of stored product pests such as *Callosobruchus maculatus* adults. Bozhüyük et al. [25] determined that the essential oils of *Artemisia santanicum* and

Artemisia absinthium have larvicidal effects in all larval stages of PPM. Potential uses of *Rosmarinus* and *Lavandula* essential oils as bioinsecticides on *Orgyia trigotephras* (Lepidoptera, Lymantriidae) and can be considered as an alternative to the use of synthetic products [26]. Kesdek et al. [27] tested the essential oils obtained from eight different plant species against the 2nd, 3rd and 4th larval stages of PPM and reported that the mortality rate reached 100%. Varçin and Kesdek[19] analyzed the chemical composition of the essential oil obtained from chaste tree (*Vitex agnus-castus* and they determined its larvicidal effectiveness at 250, 500 and 1000 µL /L, and 24, 48, 72 and 96 h against the five instars of the pine processionary moth (*Thaumetopoea pityocampa*). In this study, β--caryophyllene (16.8%), germacrene D (14.7%), 1,8-cineole (14.5%) and ζ -gurjunene (11.8%) were identified as main compounds of the essential oil. Ninety-six hour after treatment, the essential oil caused between 13.3 and 96.6% mortality. The highest mortality at 250, 500 and 1000 µL /L doses were 70.0% for L1, 80.0% for L3 and 96.6% for L1, L3 and L4 instars after 96 hours, respectively. Karamaouna et al.[6] tested the insecticidal activity of essential oils from peppermint (*Mentha piperita*), thyme-leaved savory (*Satureja thymbra)*, lavender (*Lavandula angustifolia*) and basil (*Ocimum basilicum*) against *Planococcus ficus*. The essential oils from citrus, peppermint and thyme leaved savory were more or equally toxic compared to the reference product, whereas the lavender and basil essential oils were less toxic than the paraffin oil. Mansour et al.[10] analyzed essential oils from 14 species of Labiatae and bean leaf discs freshly sprayed with different concentrations of the acetonic solutions of the oils caused mortality and induced repellency in adult females of the carmine spider mite (*Tetranychus cinnabarinus*) and egg-laying was reduced. According to their results on the basis of EC-50s, the most effective oils were: *Lavandula angustifolia* x *L. latifolia* (0.09%); *L. angustifolia* (0.1%); *Melissa officinalis* (0.12%); *Mentha piperata* (1.3%); *Salvia ruticosa* (1.4%); *Ocimum basilicum* (1.4%); and *Rosmarinus officinalis* (2.2%). Yıldırım et al. [28] applied *Hypericum hyssopifolium*, *Pistacia lentiscus* and *P. terebinthus* essential oils to *Tribolium confusum* Du Val and *Sitophilus granarius* adults at the maximum dose (40 µl) for 96 hours and the mortality rates were 50%, 67% and 40% for *T. confusum*, respectively, and listed 33%, 43% and 43% for *S. granarius*. Kanat and Alma [29] applied essential oils obtained by steam distillation from nine plant species against *T. pityocampa* larvae. As a result of the study, the most effective essential oil was found to be steam distilled wood turpentine, followed by thyme oil, juniper berry oil, bay leaf oil, lavender oil, respectively. flower oil, eucalyptus leaf oil, lavender leaf oil and cypress berry oil followed.

5. CONCLUSION

Considered together, this is the first study on the insecticidal activity of essential oils on *Neodiprion sertifer*. More detailed studies should be conducted to demonstrate the effectiveness of essential oils in open field conditions. However, based on the results of this study, the data obtained showed that essential oils isolated from *Eucalyptus camaldulensis*, *Melissa officinalis* and *Origanum onites* plants can be used against the larvae of *Neodiprion sertifer*, one of the most important pests that damages Turkish pine forests. *Origanum onites* is more effective on the 4th and 5th instar larvae than the essential oils of the other two plant species. The mortality rate reached 100% at the end of 48 hours for this plant. Considering the studies, they have shown that essential oils can be a potential alternative to synthetic insecticides in the control of pine sawfly.

6. AUTHORS CONTRIBUTION STATEMENT

Dr.Oguzhan Sarikaya and Tutku Gencal designed the study and Dr.Ayse Gul Sarikaya curated the data and prepared the original draft. Dr.Oguzhan Sarikaya, Dr.Ismail Sen and Dr.Ayse Gul Sarikaya discussed the methodology and analyzed the data and also provided valuable inputs towards designing of the manuscript. All authors read and approve the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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