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Chemical composition and larvicidal activity of essential oils from *Nepeta cadmea* Boiss. and *Pimpinella anisum* L. on the larvae of *Culex pipiens* L.

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ABSTRACT: *Culex pipiens* L. (Diptera: Culicidae) is an important vector of West Nile virus in many parts of the world. In this research, the essential oils obtained from *Nepeta cadmea* Boiss. (endemic for Turkey) and *Pimpinella anisum* L. were examined for larvicidal activity against second-third instar larvae of *Cx. pipiens*. The essential oils were distilled from aerial parts of *N. cadmea* and seeds of *P. anisum* by water distillation. The chemical composition of these oils was determined by gas chromatography-mass spectrometry (GC-MS). The lethal concentration 50 and 90 values of these essential oils were 28.7 and 49.51 ppm, and 39.82 and 74.57 ppm, respectively. As a result, both essential oils were found highly toxic to larvae of *Cx. pipiens*. Our results showed that the essential oils obtained from *N. cadmea* and *P. anisum* may be suitable for the development of potential new botanical insecticides.

KEYWORDS: Mosquito Larvae, *Nepeta cadmea*, *Pimpinella anisum*, Toxicity

1. INTRODUCTION

Mosquitoes (Diptera: Culicidae) are important vectors of many diseases such as dengue fever, malaria, yellow fever, West Nile fever and Zika [1,2]. *Anopheles*, *Aedes* and *Culex* are most abundant and important genera of mosquitoes which have over 3000 species worldwide. Mosquito fauna of Turkey consists of 54 species [3,4]. In Turkey, *Culex pipiens* L. is usually the most common mosquito in urban and suburban areas and lives in polluted water; often in septic tank systems [5]. This insect has been implicated as vector of arboviruses including St. Louis encephalitis and West Nile fever [6].

The combination of biological, cultural, mechanical-physical and chemical control methods are known as integrated pest management [7]. Using of chemicals is among the most commonly used methods to control mosquitoes. The frequent and improper usage of these pesticides can cause a variety of adverse health problems in humans and animals and also an increase in resistance. Also, some insecticides may be harmful to the non-target organisms such as fishes, predators and honey bees [8,9]. Control of insecticide resistant mosquitoes becomes more difficult day by day. Insecticides within botanical origin may serve as suitable and efficient natural products. Most botanicals are safer than synthetic insecticides and less harmful to non-target organisms [10]. Therefore, the present study aims to evaluate the larvicidal activity of essential oils obtained from *Pimpinella anisum* L. and *Nepeta cadmea* Boiss. under laboratory conditions against second-third instar larvae of *Cx. pipiens* which is an important vector of West Nile fever. The essential oil of endemic *N. cadmea* (aerial parts) has not been the subject of any research, and this study is the first report in this regard.

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2. RESULTS

The list of essential components and the percentage composition of both oils are shown in Table 1. The essential oils were extracted from the seeds of *P. anisum* and aerial parts of *N. cadmea* at yields of 2.95% and 1.86%, respectively. Eighteen components in endemic *N. cadmea* (94.48%) were identified and Caryophyllene oxide (22.96%), Viridiflorol (12.23%), *cis*-Calamenene (10.67%), *cis*-14-nor-Muurol-5-en-4-one (7.53%), *a*-Cadinol (6.92%) and Caryophylla-4(12),8(13)-dien-5-*β*-ol (6.11%) were identified as the major components. In the essential oil *P. anisum* four components were identified, which represent 100% of the total composition and the major component was anethole (94.48%).

Table 1. Essential oil compositions of *Pimpinella anisum* seeds and *Nepeta cadmea* aerial parts.

Essential Oil Composition of *Pimpinella anisum* seeds		
Retention Times (Min)	**Compounds**	**Area (%)**
36.802	*p*-Allylanisole	2.77
37.701	*γ*-Himachalene	0.41
41.364	Anethole	94.16
46.679	Anisaldehyde	2.66
	Total	**100**
Essential Oil Composition of *Nepeta cadmea* aerial parts		
Retention Times (Min)	**Compounds**	**Area (%)**
31.235	*a*-Copaene	1.15
32.66	Linalool	4.05
34.739	*β*-Caryophyllene	4.39
37.399	*γ*-Cadinene	0.89
39.485	*a*-Amorphene	2.43
41.511	*cis*-Calamenene	10.67
45.797	Caryophyllene oxide	22.96
46.74	Ledol	1.75
47.101	*cis-trans* Nepetalactone	3.09
47.306	1,10-di-epi-Cubenol	2.12
47.964	Viridiflorol	12.23
48.841	Spathulenol	2.70
49.395	Alloaromadendrene oxide	2.34
49.854	Zonarene	1.12
51.33	*a*-Cadinol	6.92
53.074	Caryophylla-4(12),8(13)-dien-5-*β*-ol	6.11
53.552	*cis*-14-nor-Muurol-5-en-4-one	7.53
54.907	13-Epimanoyl oxide	2.03
	Total	**94.48**

According to our results, both essential oils were highly toxic for larvae of *Cx. pipiens*. The essential oil obtained from *N. cadmea* showed larvicidal activity as strong as that of *P. anisum* essential oil. LC_{50} and LC_{90} values of *N. cadmea* were found 39.8 and 74.5 ppm to *Cx. pipiens* after 48 h exposure, respectively (Table 2). In general, there was a direct correlation between concentrations and mortalities. Concentrations of ≥100 ppm led to 100% mortality, while concentration of 50 ppm had moderate toxic effect to *Cx. pipiens* at 48 h (Table 3).

After 48 h exposure period LC_{50} and LC_{90} values for *P. anisum* essential oil were 28.7 ppm 49.51 ppm, respectively (Table 2). Whereas concentrations of 10 and 25 ppm had low toxicity, concentrations of ≥50 ppm caused 100% mortality after 48 h exposure (Table 3).

Table 2. LC_{50} and LC_{90} values (ppm) of tested essential oils for second-third instar larvae of *Culex pipiens* after 48 h exposure.

Test plants	LC_{50} (LL-UL)[a]	LC_{90} [LL-UL)
Pimpinella anisum	28.7 (17.25-47.75)	49.5 (23.5-104.32)
Nepeta cadmea	39.8 (21.31-67.66)	74.5 (49.69-493.48)

[a]95 % fiducial limits; LL= lower limit; UL= upper limit ppm= parts per million
LC_{50} = Lethal concentration at which 50% of the larvae showed mortality.
LC_{90} = Lethal concentration at which 90% of the larvae showed mortality.

Table 3. Larvicidal activity of *Pimpinella anisum* and *Nepeta cadmea* essential oils against second-third instar larvae of *Culex pipiens* (Percent mortalities±SE) after 48 h exposure.

Test Concentrations (ppm)	*Pimpinella anisum*	*Nepeta cadmea*
Control	0.0 ± 0.0 a[x], A[y]	0.0 ± 0.0 a, A
10	6.68 ± 6.68 ab, A	3.33 ± 3.33 a, A
25	16.67 ± 8.83 b, A	10.0 ± 5.78 a, A
50	100 c, A	66.67 ± 13.35 b, B
100	100 c, A	100 c, A
200	100 c, A	100 c, A
400	100 c, A	100 c, A

[x]: Means within a column for each concentration followed by the same letter are not significantly different (P>0.05).
[y]: Means within a line for each concentration followed by the same letter are not significantly different (P>0.05).

3. DISCUSSION

According to our literature survey, this is the first study to show toxic effect of essential oils obtained from *N. cadmea* on *Cx. pipiens* larvae, but scientists studied about the toxicity of other *Nepeta* species on important mosquito species. For example, Mahnaz et al. [11] showed that essential oil and methanol extract of the *N. menthoides* have larvicidal effect on malaria vector, *Anopheles stephensi*. The reported LC_{50} values were 69.5 and 234.3 ppm and LC_{90} values were 175.5 and 419.9 ppm for the extract and essential oil respectively. Abbas et al. [12] reported that essential oils of four *Nepeta* species showed weak larvicidal activity on *Aedes aegypti*. Sathantriphop et al. [13] reported that *N. cataria* (catnip) essential oil had very toxic effects on *A. aegypti* and *An. minimus*.

We found that there is only one research about the larvicidal activity of *P. anisum* against *Cx. pipiens.* In this research, *P. anisum* essential oil showed high larvicidal activity to *Cx. pipiens* with LC_{50} and LC_{90} values were 15.24 and 23.79 mg/L after 24 h exposure [14]. Toxic effects of *P. anisum* essential oil were tested in the different life stages of some other mosquito species. *P. anisum* essential oil showed high toxic activity against fourth instar larvae of *An. stephensi*, *A. aegypti* and *Cx. quinquefasciatus* with LD_{95} rates of 115.7, 115.7 and 149.7 µg/mL, respectively [15]. Pavela [16] examined toxic effects of *P. anisum* essential oil and its major compound (trans-anethole) on different life stages of *Cx. quinquafasciatus*. This researcher found that LC_{50} values for oil and major compound were 26-27 µL/L and 15-19 µL/L to second and fourth instar larvae, respectively. Knio et al. [17] found that *P. anisum* seed oil had toxic to larvae of *Ochlerotatus caspius* Pallas. and LC_{50} and LC_{90} values were 65 and 137 µg/mL, respectively. Erler et al. [18] tested some essential oils including the *P. anisum* for repellency on *Cx. pipiens* adults.

In addition to, there are many reports about repellent and fumigant toxicity, and also attractiveness of *P. anisum* essential oil on some other pest species. For example, Mikhaiel [19] reported that anise oil causes complete mortality to *Ephestia kuehniella* and *Tribolium castaneum* adults at 64 µl/L air concentration within 24 h. Robles-Bermúdez et al. [20] searched the effect of blue traps impregnated with anise fruit extract for capturing thrips. Cevik and Erler [21] researched fumigant activity of some essential oils and their major components (trans-anethole, eucalyptol, menthol, carvacrol and thymol, respectively) against the adult mushroom cecid flies and found that 20 and 40 µL/L air concentrations of the all essential oils and components (except for trans-anethole and menthol at an exposure period of 1 h) showed 100% mortality. Elma and Alaoglu [22] studied the toxicity of methanol extracts of eight plants that include *P. anisum*, on nymphs of *Eurygaster maura* L. They reported that young nymphs were more sensitive than olds. Nenaah and Ibrahim

[23] found that a dose of 1.50 mL/cm² of the *P. anisum* oil gave 100% and 92% mortalities on *T. castaneum* and *Trogoderma granarium* (Everts) adults after 14 days exposure period, respectively.

Erler and Cetin [24] reported that anise oil and trans-anethole produced 89.0% and 100% larval mortality on the brown-tail moth, *Euproctis chrysorrhoea* L., respectively, at 96 h at a concentration of 0.5%. Khater et al. [25] investigated toxicity of essential oils of lettuce (*Lactuca sativa*), chamomile (*Matricaria chamomilla*), anise (*P. anisum*) and rosemary (*Rosmarinus officinalis*) against the larva of *Lucilia sericata.* LC_{50} values were found as 0.57%, 0.85%, 2.74%, and 6.77% for lettuce, chamomile, anise and rosemary oils, respectively. Isıkber et al. [26] studied susceptibility of eggs of *Tribolium confusum* du Val., *Ephestia kuehniella* and *Plodia interpunctella* (Hübner) to vapors of essential oil from garlic, birch, cinnamon and anise and they reported that garlic and birch essential oils were more toxic than cinnamon and anise essential oils. Erler et al. [27] reported that *P. anisum* seed extract caused a significant decline in mushroom pest (*Megaselia halterata*) adult emerhgence rate. Tunç et al. [28] examined the fumigant toxicity of *P. anisum* essential oil against to eggs of *T. confusum* and *E. kuehniella*. The exposure to vapour of this essential oil was found highly toxic to eggs.

In previous researches anethole was recorded as major compound in *P. anisum* seed oil. The ratio of this compound may be shown variations (more than 93%) according to the collection localities, climatic conditions and distillation methods [29]. In this study the larvicidal action of anise oil has been attributed to anethole that was previously found toxic to many pest species [21, 24]. According to our results *N. cadmea* essential has many major and minor compounds. We suppose that the larvicidal activity of *N. cadmea* essential oil is attributed to the presence of the number of compounds (both minor and major), their percentage in the oil and synergistic interactions between the compounds.

As a result, both essential oils showed high larvicidal activity on *Cx. pipiens* larvae and exhibited significant concentration-dependent larvicidal activity. Based on these results, *P. anisum* and *N. cadmea* essential oils may be a candidate for the development of new botanical insecticides applied to *Cx. pipiens* larvae but further studies are needed.

4. MATERIALS AND METHODS

4.1. Mosquito culture

The mosquito, *Cx. pipiens,* used in tests was originated a pool from field areas of Antalya in Turkey and has been reared 24±2°C and 50±10% relative humidity with 10:14 dark:light photoperiod conditions more than seven years in the Vector Control and Ecology Laboratory, Department of Biology, Akdeniz University in Antalya. Second-third instar larvae of mosquito were used in the larvicidal activity tests.

4.2. Collection of plant materials

Pimpinella anisum seeds were purchased from a market in Antalya. The aerial parts of *N. cadmea* in the flowering period were collected from Antalya, Turkey (N 37º14'931"- E31º 24'420", altitude 1255) in July. The third author of this paper did taxonomic identification of plants according to Davis [30] and Güner et al. [31] and then voucher specimens (Çinbilgel 8029) were deposited in the laboratory.

4.3. Isolation of essential oils and determination of chemical compositions

Pimpinella anisum seeds and the aerial parts of *N. cadmea* were cleaned and dried in shadow for 3 weeks and powdered by a mechanical grinder. Dried plants were put in to the flask with enough water and distilated for 3 h by using a Clevenger-type apparatus. Obtained essential oils were put in glass tubes (10 ml) and stored at 4°C refrigerator until tested.

The chemical analysis was carried out on a Agilent 7890A GC/MS using a HP-5 capillary column (30 m ×0.25 mm x0.25 mm); carrier gas helium, flow rate 0,8 mL min^{-1}. The injection volume was 1 µL. The injector temperature was 250 ºC. The initial oven temperature was set at 60 °C and held for 10 min, then programmed from 60 °C to 220ºC at 4 ºC min^{-1} and then 220 ºC at 10 min. The mass spectrometer was operated in electron impact (EI) mode with the ionization energy of 70 eV. Full mass scan of 35–480 amu was used. Compounds were identified by comparing their mass spectrum to those of the database of the GC-MS (Oil Adams and Wiley), literature and retention indices.

4.4. Larvicidal activity tests

Larvicidal toxicity tests were made according to the method described by Cetin and Yanikoglu [32]. For the stock solution, 0.5 ml essential oil was dissolved in 500 ml distilled water using Tween 80 (0.3%). Six test concentrations (10, 25, 50, 100, 200, 400 ppm) of dissolved essential oils were prepared from stocks. Distilled water containing 0.3% Tween 80 was used as control group. After five minutes, ten larvae were released to plastic containers. Three replicates of each concentration were established and mortality values were recorded after 48h of exposure. Larvae were daily fed with fish food. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. All tests were conducted at 24±2 °C and 50±10% relative humidity with 10:14 dark:light photoperiod conditions.

4.5. Statistical Analysis

Lethal concentration (LC_{50} and LC_{90}) values were determined by probit analysis. All percent mortalities analyzed using Statistical Analysis System ANOVA [33]. Mean mortalities were compared with Duncan's multiple range test.

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Conflicts of Interest: The authors declare that they have no competing interests.

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