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RESEARCH ARTICLE

Larvicidal effect of *Cedrus libani* seed oils on mosquito *Culex pipiens*

Huseyin Cetin, Yusuf Kurt, Kani Isik, and Atila Yanikoglu

Biology Department, Faculty of Arts and Science, Akdeniz University, Antalya, Turkey

Abstract

The aim of this research was to determine the larvicidal activity of essential oils obtained from the seeds of six different *Cedrus libani* A. Rich. (Pinaceae) populations on *Culex pipiens* L. (Diptera: Culicidae). The essential oil was obtained from seeds by hydrodistillation. Third and fourth instar mortalities from six concentrations (10, 25, 50, 100, 200, and 400 ppm) of oil from each *Cedrus* population were compared with those of a standard larvicide, temephos. Seed oils from Pozantı, Degirmenlik, Aslankoy, Avlankuzu, Camkuyusu, and Sogutozu populations in Turkey showed high larvicidal activities, with LC_{50} values of 47.8, 59.0, 66.6, 105.5, 109.3, and 116.0 ppm, respectively. The data showed that there were significant differences among the oils from different *C. libani* populations in terms of larvicidal activity. Our overall results suggest that essential oil of *C. libani* has potential to be used in the search for chemical components as new larvicides.

Keywords: *Mosquito control; essential oil; insecticidal activity; natural insecticide*

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Introduction

Several tree species in the world are commonly called "cedar", but only four of them are true cedars: *Cedrus atlantica* (Endl.) Manetti, *Cedrus deodara* (Roxb.) G. Don, *Cedrus brevifolia* Henry, and *Cedrus libani* A. Rich (Pinaceae). The second is native to the mountains of western Himalaya, occurring at altitudes of 1500–3200 m, and the others to the Mediterranean region, at 1000–2200 m above sea level. Many of the species belonging to genus *Cedrus* have been previously reported for their various biological activities (Singh & Agarwal, 1988; Shinde & Phadke, 1999). The hexane-soluble extract of the wood of *C. deodara* (deodar cedar) was found to possess significant antiallergic activity (Gupta et al., 1997). Extracts of different parts of some cedar species, such as *C. libani* and *C. deodara*, have been used as traditional medicines. The essential oils of these two cedar species, obtained from roots and wood, are also used as perfume fixatives in the soap and cosmetic industries (Usher, 1974; Hafızoğlu, 1987; Yesilada et al., 1993; Chevallier, 2001; Kurt et al, 2008a). On the other hand, oil obtained from cedar wood was one of six mosquito repellent substances tested by Curtis et al. (1987) against four *Anopheles* species. Of these, *A. stephansi* was found to be the most susceptible. Naples et al. (1992) reported that cedrol, in particular, a principal component of cedar wood oil, seems to have a high toxicity to cercariae (*Schistosoma mansoni*), a parasite for humans. Chromatographic fractions of Himalayan cedar wood oil (*C. deodara*) showed insecticidal activity against *Callosobruchus analis* F. and *Musca domestica* L. (Singh & Agarwal, 1988).

The widest distribution of *C. libani* occurs in Turkey, on the Taurus Mountains in the Mediterranean region of the country. In Lebanon, from where the name derives, there is only a small stand of cedar forest (Boydak, 2003; Khuri et al., 2000). It is a tree 20–40 m high and up to 3 m in diameter, with glaucous blue-green leaves, 8– 25 mm. It can live as long as 1500–2000 years. Timber of this species has been used in shipbuilding and 'katran' (wood extracts) production since ancient times (Kurt et al., 2008a). The species is economically important

Address for Correspondence: H. Cetin, Biology Department, Faculty of Arts and Science, Akdeniz University, 07058 Antalya, Turkey. Tel: 00902423102393. Fax: 00902422278911. E-mail: hcetin@akdeniz.edu.tr, hcetin4477@yahoo.com

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in Turkey: essential oils from the wood are used in perfumery and also the leaves and the wood are antiseptic and expectorant (Usher, 1974; Hafızoğlu, 1987; Chevallier, 2001; Kurt et al., 2008a). Antimicrobial activity of *C. libani* was reported by Digrak et al. (1999) and Kizil et al. (2002).

Culex pipiens L. (Diptera: Culicidae) is one of the most widely distributed mosquitoes in the world. The species, commonly referred to as "house mosquito", can be found in urban and suburban areas and lives near people, but feeds primarily on birds (Bernard et al., 2001). This mosquito can transmit many arbovirus encephalitides and lymphatic filariases. More than 120 million people worldwide are infected with the *Wuchereria bancrofti* form of lymphatic filariasis (Cao et al., 1997; Turell et al., 2000). Several commercially available insecticides (e.g. temephos, chlorpyrifos-methyl, diflubenzuron) can be effective to control the species at immature stages (Cetin et al., 2006a, 2006b). However, many of these chemical insecticides are expensive and harmful to the environment as well as to humans.

Many biological larvicides are effective and less toxic than many chemical adulticides used at present. Botanical larvicides appear to be widely applicable over the coming years and some of them will probably prove to be effective. Efforts to discover and develop new botanical insecticides have increased dramatically in recent years (Cetin et al., 2004; Habluetze et al., 2007). Hence, many researchers have focused on developing new, natural, and more effective phytochemical compounds against mosquitoes. In this study, we therefore aimed at determining the larvicidal activity of essential oils from the seeds of six different *C. libani* populations against *C. pipiens* under laboratory conditions.

Materials and methods

C. libani *seed samples*

Seeds of different *C. libani* populations collected in 2003 and 2004 from six different locations in Turkey in 2003 and 2004 were used in this study (Table 1). Taxonomic authentication of seeds was performed by the second and third authors of this article (Kurt et al, 2008b). The seed samples were deposited at about 4°C in the Pesticide Test Laboratory of the Department of Biology, Akdeniz University, Antalya.

Hydrodistillation of the essential oils

The seeds were separated from the "wings" before the hydrodistillation process. The volatile oils were obtained from the seeds (100 g) by hydrodistillation for 2 h using a Clevenger-type apparatus, as described by Cetin and Yanikoglu (2006). Oil samples were stored at 4°C until needed.

Table 1. Locations of *C. libani* populations in Turkey and contents of essential oil in their seeds.

Population	Altitude (m)	Latitude N	Longitude E	Mean essential oil (%)
Avlankuzu Mountain, Elmalı, Antalya	1100	36°34′48″	29°57′57″	0.80
Camkuyusu, Elmalı, Antalya	1800	36°35′86″	30°01′55″	1.25
Degirmenlik, Mut, Mersin	1800	36°41′20″	33°03′34″	2.50
Sogutozu, Mut, Mersin	1650	36°46′30″	33°32′20″	2.75
Aslankoy, Mersin	1800	37°00′00″	34°14′00″	1.75
Pozantı, Adana	1320	37°30′32″	34°57′38″	1.25

Mosquito culture

C. pipiens used in the assays originated from Varsak, Antalya (southwestern Turkey) and were collected from a septic tank in September 2005. The larvae were reared at 26 ± 2°C, 60 ± 10% relative humidity (RH), and a 12:12 (light/dark) photoperiod in an insectarium in the Biology Department, Akdeniz University, Antalya. Third–fourth instar larvae were used in the bioassays.

Larvicidal assays

Larvicidal activity against *C. pipiens* was assessed using the World Health Organization (WHO) standard method (WHO, 1996). For the assays, 1 mL of essential oil from each *C. libani* population was dissolved in 100 mL distilled water + Tween 80 (0.3%). Series concentrations ranging from 10 to 400 ppm of dissolved oil were prepared. The mixture of oil + 0.3% Tween 80 + water was stirred for 30 s with a glass rod. After about 5 min, 25 larvae taken on a strainer with fine mesh were transferred gently to the test medium by tapping. Four replicates of each concentration were run at a time. Two control groups were set up, including negative (distilled water containing 0.3% Tween 80) and positive (1 ppm temephos (Temephos M-Toks® 50 EC)) controls. Mortality was recorded after an exposure of 24 h. During the exposure period, no food was given to the larvae. All experiments were conducted at 24 ± 2°C and 50 ± 10% RH with a 14:10 (light/dark) photoperiod. Larvae were considered dead when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time, 15 min) or of showing the

characteristic diving reaction of live larvae when the water was disturbed. Larvae were also observed for discoloration, unnatural position, and incoordination, to verify mortality.

Data analysis

All data were analyzed using Statistical Analysis System analysis of variance (ANOVA) (SPSS, 1999). Means were compared with Duncan's multiple range test, and the data were also subjected to Probit analysis in order to estimate the LC_{50} values (EPA, 2007).

Results and discussion

The mean essential oil contents of different cedar populations varied from 0.8% (Avlankuzu population) to 2.75% (Sogutozu population) (Table 1).

All the essential oils tested demonstrated significant larvicidal activity on *C. pipiens*, with LC_{50} values ranging from 47.8 to 116.0 ppm. Oils from Pozantı, Degirmenlik, Aslankoy, Avlankuzu, Camkuyusu, and Sogutozu populations showed high larvicidal activities, with LC_{50} values of 47.8, 59.0, 66.6, 105.5, 109.3, and 116.0 ppm, respectively (Table 2). The Degirmenlik population was found to be interesting. It had relatively high essential oil content (2.5%), and also had a relatively high larvicidal activity (LC_{50} = 59 ppm). We found that there was no statistically significant relation between the geographical origins of seed sources (i.e. altitude, latitude, and longitude) of seed sources and essential oil contents. As expected, the percent larval mortality increased with increasing concentrations of the oil. A concentration of 400 ppm of the oil was found to be 100% larvicidal. However, at a concentration of 100 ppm the oil killed more than 45% of third and fourth instars.

At concentrations of 100 and 400 ppm, there were no significant differences among the oils from different cedar populations in terms of larvicidal activity (Table 3). At the high concentration (400 ppm), mortality was not significantly different from the reference, temephos (1 ppm). When the larvicidal activity of *C. libani* seed oils is compared to that of temephos, all oils from the six cedar populations were much less toxic against *C. pipiens.* However, many mosquito species are known to have developed resistance to temephos in many parts of the world (Braga et al., 2004; Cui et al., 2006).

Many researchers have examined the larvicidal activity of various plant seed oils and extracts against mosquitoes (Hellpap & Zebitz, 1986; Sagar & Sehgal, 1996). Elbanna (2006) reported that the extract from *Eucalyptus* seeds contained toxic compounds against *C. pipiens* larvae. Also, Knio et al. (2008) found that anis and parsley seed oils had strong larvicidal activity against *Ochlerotatus caspius* (Pallas), with LC_{50} values of <100 ppm. No reports are available on the larvicidal property of *C. libani* seed oil against *C. pipiens*. However, some researchers have shown that essential oils obtained from different plant parts of *Cedrus* sp. are highly repellent of adult mosquitoes (Curtis et al., 1987; Makhaik et al., 2005).

This study showed that *C. libani* seed oil has the potency to control mosquito larvae. The study also

Table 2. LC_{50} values [ppm (95% confidence limits)] at 24 h of essential oil application of six *C. libani* populations against third–fourth instar *C. pipiens.*

Avlankuzu	Camkuyusu	Degirmenlik	Sogutozu	Aslankoy	Pozantı
105.5	109.3	59.0	116.0	66.6	47.8
(91.7–118.5)	(68.5–147.6)	(22.3–119.2)	(101.2–130.0)	(10.0–210.0)	(4.2–150.4)

Table 3. Mean percent mortality ± SE of seed essential oils from six *C. libani* populations at six concentrations when compared with temephos (TMP) and a distilled water (DW) control.

Dose (ppm)	Avlankuzu	Camkuyusu	Degirmenlik	Sogutozu	Aslankoy	Pozantı
10	$14.8 \pm 2.8^{b,A}$	$16.7 \pm 1.0^{b,A}$	$20.0 \pm 1.0^{b,A}$	$15.9 \pm 1.0^{b,A}$	$26.5 \pm 1.0^{b,AB}$	$32.8 \pm 1.0^{b,B}$
25	$16.9 \pm 2.8^{b,A}$	$21.3 \pm 1.8^{b,AB}$	$32.1 \pm 1.0^{bc,ABC}$	$22.7 \pm 0.0^{b,AB}$	$28.0 \pm 1.8^{b,C}$	$39.0 \pm 2.0^{bc,BC}$
50	$22.7 \pm 1.0^{b,A}$	$22.0 \pm 1.0^{b,A}$	$47.0 \pm 1.0^{d,B}$	$20.3 \pm 1.0^{b,A}$	$39.0 \pm 1.8^{b,A}$	$46.9 \pm 2.0^{bc,B}$
100	$48.2 \pm 2.8^{c,A}$	$53.4 \pm 1.8^{c,A}$	$51.0 \pm 1.0^{cd,A}$	$46.0 \pm 2.8^{c,A}$	$45.6 \pm 2.8^{b,A}$	$54.5 \pm 2.8^{c,A}$
200	$88.8 \pm 3.7^{d,C}$	$78.7 \pm 2.0^{d,AB}$	$73.5 \pm 1.0^{e,A}$	$87.2 \pm 2.8^{d,BC}$	$71.8 \pm 1.8^{c,A}$	$67.7 \pm 2.8^{d,BC}$
400	$100.0 \pm 0.0^{e,A}$	$100.0 \pm 0.0^{e,A}$	$100.0 \pm 1.0^{f,A}$	$100.0 \pm 0.0^{e,A}$	$100.0 \pm 0.0^{d,A}$	$100.0 \pm 0.0^{d,A}$
TMP	$100.0 \pm 0.0^{e,A}$	$100.0 \pm 0.0^{e,A}$	$100.0 \pm 1.0^{f,A}$	$100.0 \pm 0.0^{e,A}$	$100.0 \pm 0.0^{d,A}$	$100.0 \pm 0.0^{d,A}$
DW	$0.0 \pm 0.0^{a,A}$	$0.0 \pm 0.0^{a,A}$	$0.0 \pm 0.0^{a,A}$	$0.0 \pm 0.0^{a,A}$	$0.0 \pm 0.0^{a,A}$	$0.0 \pm 0.0^{a,C}$

Means followed by the same lower case letter within a given population (columns) are not significantly different (Duncan's multiple range test, $p < 0.05$); means followed by the same upper case letter at a given dose (row) are not significantly different (Duncan's multiple range test, $p < 0.05$).

demonstrated that essential oils of the same plant species from different localities could have different larvicidal activities. Further studies are needed to determine and isolate major oil components that are most effective for larvicidal or other insecticidal activities.

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