Larvicidal and ovicidal effects of selected chemicals found in plant-derived essential oils for mosquito control

Sivrisineklerle mücadelede bitkisel kökenli esansiyel yağlarda bulunan çeşitli kimyasalların larvisidal ve ovisidal etkileri

Fatma BURSALI[1] (ID)

ABSTRACT

Objective: *Aedes aegypti* (L., 1762) (Diptera: Culicidae) is a vector for numerous viral diseases including dengue fever, Chikungunya, Zika fever, and yellow fever in densely populated urban areas. Mosquito control programs rely heavily on applying larvicides in breeding areas and applying insecticides targeting adult mosquitoes in the *Ae. aegypti* populations. Essential oils are primarily composed of terpenoids and phenylpropanoids and have a range of beneficial properties, including insecticidal activities.

Methods: This study primarily investigated the ovicidal and larvicidal activity of selected chemicals (verbenone, propionic acid, lactic acid, 1-butanol, 2-butanol and citronellol) identified in essential oils of plants against *Ae. aegypti* in wells of 24-well plates.

Results: Verbenone, propionic acid and lactic acid were observed to present a dose dependent effect on mosquito larvae. Verbenone emerged as the most effective against larvae with an LC_{50} value

ÖZET

Amaç: *Aedes aegypti* (L., 1762) (Diptera: Culicidae), dünya nüfusunun büyük bir kısmını etkileyen dang humması, Chikungunya, Zika humması ve sarıhumma gibi çok sayıda viral hastalığın vektörüdür. Sivrisinek kontrol programları büyük ölçüde üreme alanlarında larvasitlerin uygulanmasına ve *Ae. aegypti* ergin popülasyonlarını hedef alan böcek ilaçlarının uygulanmasına dayanmaktadır. Esansiyel yağlar temel olarak terpenoidler ve fenilpropanoidlerden oluşmaktadır ve böcek öldürücü aktiviteleri de dahil olmak üzere çeşitli faydalı özelliklere sahiptir.

Yöntem: Bu çalışmada çeşitli bitkilerin uçucu yağlarından tanımlanmış bazı kimyasal maddelerin (verbenon, propiyonik asit, laktik asit, 1-butanol, 2-butanol ve sitronellol) *Ae. aegypti* larva ve yumurtalarına karşı ovisidal ve larvisidal aktiviteleri 24 gözenekli plakada araştırılmıştır.

Bulgular: Verbenon, propiyonik asit ve laktik asidin sivrisinek larvaları üzerinde doza bağlı etki gösterdiği gözlenmiştir. Verbenon, 29.369 ppm LC_{50} değeri ile larvalara karşı en etkili madde olarak belirlenmiştir.

[1]Aydın Adnan Menderes University, Faculty of Science, Department of Biology, Aydın, Türkiye

İletişim / Corresponding Author : Fatma BURSALI
Aydın Adnan Menderes Üniversitesi Fen Fakültesi Biyoloji Bölümü Aydın - Türkiye
E-posta / E-mail : fatma.gunerkan@adu.edu.tr

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of 29.369 ppm. Propionic acid and lactic acid were highly effective with LC_{50} values ranging between 40.45-47.63 ppm. 1-butanol, 2-butanol and citronellol did not cause any mortality. This study is among the few studies that have assessed the ovicidal effects of essential oils on mosquito eggs. After 120 hours of exposure, verbenone, propionic acid and 1-butanol were observed to present a dose dependent effect on mosquito eggs. Verbenone also emerged as the most effective against eggs with an LC_{50} value of 11.877 ppm. At 200 and 100 ppm <25% hatched but afterwards a drastic increase in egg hatching was observed with decreased with concentration.

Conclusion: EOs have promising potential as a safe and effective larvicidal and ovicidal agent for controlling *Aedes* mosquito populations. Such studies pave the way for developing new mosquito control formulations utilizing these effective essential oils.

Key Words: *Aedes*, verbenone, citronellol, essential oil, vector control

Propiyonik asit ve laktik asit, 40.45-47.63 ppm arasında değişen LC_{50} değerleri ile oldukça etkili olmuştur. 1-bütanol, 2-bütanol ve sitronellol herhangi bir etki göstermemiştir. Bu çalışmada ayrıca uçucu yağların sivrisinek yumurtaları üzerindeki ovisidal etkileri de değerlendirilmiştir. Verbenon, propiyonik asit ve 1-bütanolün 120 saatlik değerlendirme süreci sonunda sivrisinek yumurtaları üzerinde doza bağlı bir etki gösterdiği gözlemlenmiştir. Verbenon 11.877 ppm LC_{50} değeri ile yumurtalara karşı en etkili madde olarak ortaya çıkmıştır. 200 ve 100 ppm'de <%25 yumurtadan çıkma oranı gözlemlenmiş ancak daha düşük konsantrasyonlarda yumurta açılma oranında artış ortaya çıkmıştır.

Sonuç: Esansiyel yağlarda bulunan bazı etken maddeler *Aedes* sivrisinek popülasyonlarının kontrolünde larvisidal ve ovisidal olarak güvenli ve etkili bir mücadele yöntemi olma potansiyeline sahiptir. Bu tür çalışmalar sivrisinek mücadelesinde esansiyel yağlar kullanılarak yeni formülasyonların geliştirilmesi yolunu açacaktır.

Anahtar Kelimeler: *Aedes*, verbenon, sitronellol, esansiyel yağ, vektör mücadelesi

INTRODUCTION

Aedes aegypti (L., 1762) (Diptera: Culicidae), commonly known as the yellow fever mosquito, poses a significant threat to public health due to its role as a vector for numerous viral diseases including dengue fever, Chikungunya, Zika fever, and yellow fever. This mosquito thrives in densely populated urban areas, where it preferentially feeds on humans, even when other mammals are available (1,2). *Ae. aegypti* exhibits primarily diurnal biting behavior that is often inconspicuous with peak activity occurring around sunrise and sunset. However, they can also bite at night in well-lit areas (3,4). This mosquito exhibits a cosmo-tropical distribution in tropical regions year-round, its reach. Additionally, its geographic range extends beyond its African origins and now encompass more temperate areas during the summer months (5). Human activities, particularly international travel and trade have significantly facilitated this global spread. Historically, maritime transportation played a major role in establishing *Ae. aegypti* populations across continents (6–8).

Mosquito control programs rely heavily on monitoring *Ae. aegypti* populations to assess their prevalence, dispersal patterns, and abundance. This involves inspecting potential breeding sites, employing traps for both adult mosquitoes and eggs (ovitraps), and eliminating potential breeding sites or applying larvicides (chemicals that kill mosquito larvae) in these areas and applying insecticides targeting adult mosquitoes (adulticides) can be used (9,10). Common chemical adulticides including organophosphates (like malathion) and pyrethroids (like permethrin) can be applied through various methods, such as indoor residual spraying, bed net impregnation, or aerial spraying (11–13). The effectiveness of chemical insecticides is often short-lived, as mosquitoes can develop resistance with continuous use, and many are associated with potential health and environmental concerns (14). Biocontrol agents like bacteria (*Bacillus thuringiensis israelensis*) or Insect growth regulators (IGRs) which kill or disrupt mosquito's development cycle can also be used to control mosquitoes as they pose minimal risk to humans, and other beneficial organisms (15,16) .

Essential oils (EOs) are a class of concentrated, aromatic, and volatile liquids extracted from various plant parts (flowers, leaves, stems, bark, and fruits) using established techniques (hydro distillation, steam distillation, dry distillation) or more ecologically friendly methods (supercritical fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction) (17,18). These EOs are primarily composed of terpenoids and phenylpropanoids and have a range of beneficial properties, including antioxidant, antibacterial, and insecticidal activities (19). Consequently, EOs have potential applications in various fields, including pharmaceuticals, food science, and agriculture. Notably, their insecticidal properties have been explored against a diverse array of pests (17,20,21).

This study primarily investigated the ovicidal and larvicidal activity of some chemicals identified in essential oils of plants against *Aedes aegypti*.

MATERIAL and METHOD

Aedes aegypti mosquitoes were reared in cages within an insectary maintained at a temperature of 28-30°C and 70-80% relative humidity, with a 12-hour photoperiod in Vector Control Laboratory at Aydın Adnan Menderes University, Türkiye. To facilitate blood feeding, females were presented with defibrinated sheep blood via an artificial feeder (4). Eggs were hatched in plastic containers, and the larvae were subsequently fed crushed fish scale (Tetramin®) for larvicidal experiments. Eggs were air dried for 2 days before use in ovicidal experiments.

Commercially available chemicals (purity > 85%) found in plant essential oils were obtained from Sigma Aldrich (Table 1, Figure 1). Stock solutions of 200, 100, 50, and 25 ppm (µg/mL) were prepared by dissolving these chemicals in distilled water for use in the bioassays.

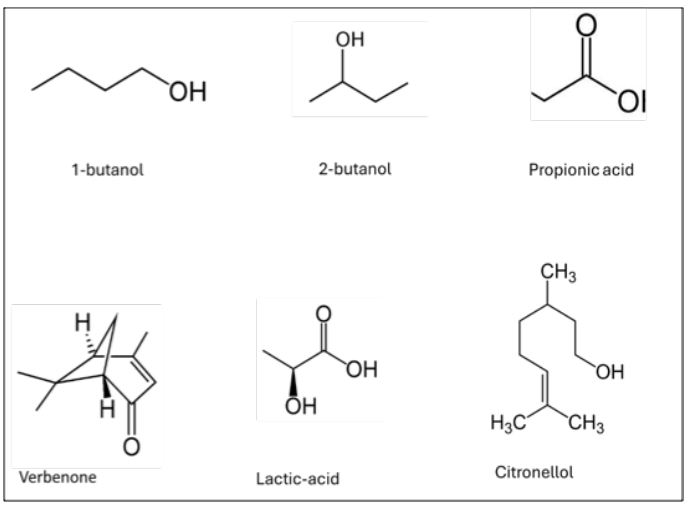

Figure 1. Chemical structures of verbenone, propionic acid, lactic acid, 1-butanol, 2-butanol and citronellol

Table 1. Information and lethal values of the selected chemicals from plant-derived essential oils

Chemicals	Plant source	Compound type	Company (purity)	Larvicidal		Ovicidal	
				LC_{50} (µg/mL) (95% CL)	Chi-square X^2	LC_{50} (µg/mL) (95% CL)	Chi-square X^2
Citronellol	Rose, geranium and lemongrass (22,23)	terpenoid	≥95% (Sigma)	ND	ND	138.037 (101.190-243.685)	2.462
Verbenone	Rosemary (41)	terpene	≥93% (Sigma)	29.369 (27.718-31.662)	0.769	11.877 (0.911-24.481)	2.194
Propionic acid	Rhynhosia bedd-domei (42)	carboxylic acid	≥99.5% (Sigma)	40.454 (22.800-55.619)	15.330	30.576 (15.294-43.212)	3.924
1-butanol	Jack fruit, Japanese ginger, maize, musk melon (24,25)	alcohol	≥99.9% (Sigma)	ND	ND	80.634 (25.312-56.627)	12.666
2-butanol	Truffle (43)	alcohol	≥99.8% (Sigma)	ND	ND	85.535 (7.246-45.751)	12.894
Lactic acid	Sugar plant wastes (44)	carboxylic acid	≥85% (Sigma)	47.631 (41.648-53.451)	4.627	99. 416 (68.171-190.042)	0.314
Mortality (%) ±SD	Negative control	0.0 ±0.0	0.0 ±0.0	-		-	
	Positive control	100±0.0	100±0.0	-		-	

LC values are expressed in ppm (µg/mL) and they are considered significantly different when 95% CL fail to overlap. Values are means ± S.D. Negative control: distilled water. Positive control: Vectobac® 12AS 0.19 ml/L for larvicidal. ND: Not determined.

Larvicidal bioassay

Ten third instar mosquito larvae were dispensed into wells of 24-well plates (Sigma, Corning Costar Multiple Well Plates, CLS3524) and exposed to varying concentrations of chemicals (200, 100, 75, 50, 25 ppm), while a negative control group received only distilled water and a positive control the commercial *Bti* (0.19 ml/l), (*Bacillus thuringiensis var. israelensis*), (VectoBac 12AS, Valent Biosciences, USA). Each well contained a total volume of 1 ml and 10 larvae per well. Required concentrations of the compounds were dissolved in distilled water. The plates were incubated at 24°C, and larval mortality was assessed after 48 hours. Larvicidal activity was determined by calculating the LC_{50} which represents the concentration (in µg/ml) that induced 50% mortality within 48 hours. Larvae were considered dead if they remained immobile after probing. Each treatment had six replicates and the entire experiment was repeated three times (26). Experiments were done under laboratory conditions.

Ovicidal bioassay

Ten intact and healthy mosquito eggs were dispensed into wells of 24-well plates (Sigma, Corning Costar Multiple Well Plates, CLS3524) using a fine brush and exposed to varying concentrations of chemicals (200, 100, 75, 50, 25 ppm), while a negative control group received only distilled water. Required concentrations of the compounds were dissolved in distilled water. Each well contained a total volume of 1 ml and 10 eggs per well. The plates were incubated at 24°C, and egg hatching rates was assessed after 120 hours. Ovicidal activity was determined by calculating the LC_{50} which represents the concentration (in µg/ml) that induced 50% mortality. Eggs were considered dead if there is no eggshell fracture with no rising of the egg buster and there was no L1 stage in the well. Each treatment was replicated six times, and the entire experiment was repeated three times (26). Experiments were done under laboratory conditions.

Statistical analyses

LC values and their 95% confidence interval of the compounds against eggs (ovicidal) and larvae (larvicidal) was evaluated using probit analysis. Data from control groups with less than 5% mortality were excluded from the analysis. To compare differences on the effects of the compound, a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test ($p < 0.05$) was employed. Prior to ANOVA, the data underwent arcsine transformation. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Larvicidal bioassay

The larvicidal effects of the chemicals found in various plant derived EOs against *Ae. aegypti* larval mortality is given in Figure 1. After 48 hours of exposure, verbenone, propionic acid and lactic acid were observed to present a dose dependent effect on mosquito larvae. At 200, 100 and ppm these compounds caused 100% mortality but afterwards a drastic drop in efficacy was observed with decreased with concentration. The LC_{50} value of Verbenone, propionic acid and lactic acid against *Ae. aegypti*were determined as Table 1. 1-butanol, 2-butanol and citronellol did not cause any mortality. Analysis of variance results showed that there were significant differences between the chemical compounds ($F_{(5, 150)} = 366$; $p<0.0001$), tested concentrations ($F_{(4, 150)} = 593.3$; $p<0.0001$) and their interaction ($F_{(20, 150)} = 142.4$; $p<0.0001$) (Figure 2).

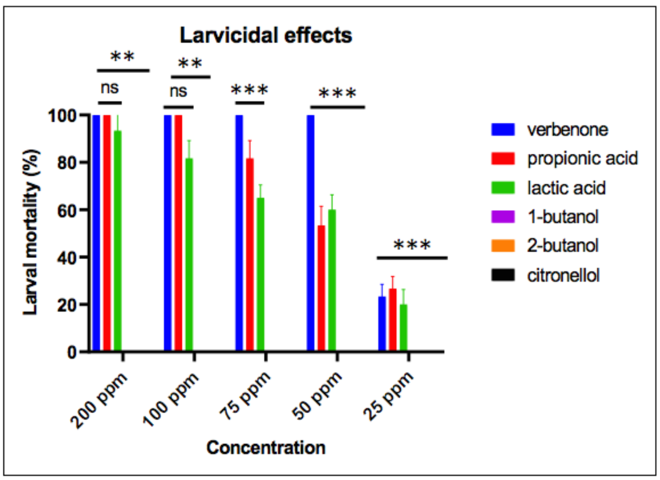

Figure 2. Larvicidal effects of different doses of verbenone, propionic acid, lactic acid, 1-butanol, 2-butanol and citronellol against 3rd instar *Aedes aegypti* larvae

Ovicidal bioassay

The ovicidal effects of the chemicals found in various plant derived EOs against *Ae. aegypti* larval mortality is given in Figure 3. After 120 hours of exposure, verbenone, propionic acid and 1- butanol were observed to present a dose dependent effect on mosquito eggs. At 200 and 100 ppm <25% hatched but afterwards a drastic increase in egg hatching was observed with decreased with concentration.

The LC_{50} value of the chemicals against *Ae. aegypti* eggs were determined as Table 1. Analysis of variance results showed that there were significant differences between the chemical compounds (F (6, 175) = 25.56; p<0.0001), tested concentrations (F (4, 175) = 35.01; p<0.0001) and their interaction (F (24, 175) = 2.369; p=0.0007) (Figure 3).

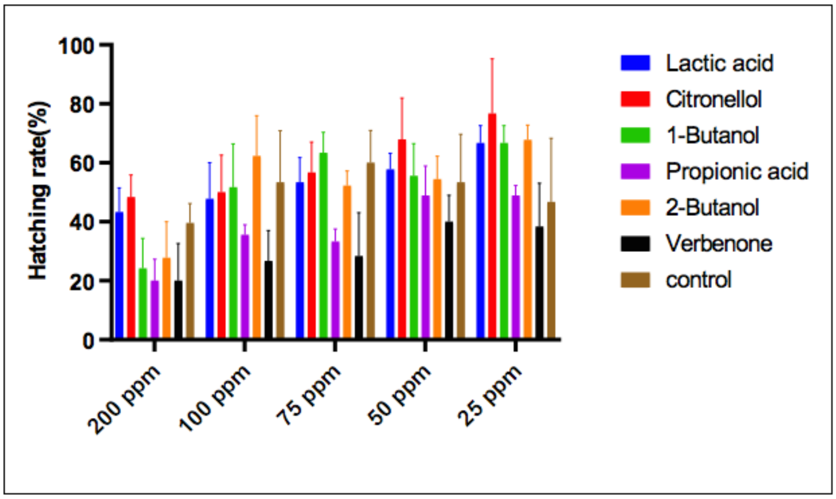

Figure 3. The hatching rate of the mosquitoes after treatment with chemicals

DISCUSSION

Essential oils (EOs) possess complex chemical compositions and are characterized by a diversity of distinct compounds (phytochemical diversity) with different biological properties. Some EOs and their components can kill insects or disrupt their growth at various stages of their life cycle (17,27). This study assessed the effects of six chemicals found in various plant derived EOs against *Ae. aegypti* eggs hatching and larval survival. Results showed that the chemicals tested showed varying effects. After 24 hours of exposure, verbenone, propionic acid and lactic acid were observed to present a dose dependent effect on mosquito larvae. Verbenone emerged as the most effective against larvae with an LC_{50} value of 29.369 ppm. Propionic acid and lactic acid were highly effective with LC_{50} values ranging between 40.45-47.63 ppm. 1-butanol, 2-butanol and citronellol did not cause any mortality.

Numerous studies have explored the potential of essential oils and their components for mosquito larvae control, these studies have shown that commercially available oils like turmeric (*Curcuma longa*), sweet orange (*Citrus sinensis*), lemongrass oil, cinnamon bark oil and lemon (*Citrus limon*) are particularly effective against different mosquito species belonging to the *Culex*, *Aedes*, and *Anopheles* genera (28-33). For instance, (34) investigated the potential of four essential oils (lemon, lavender, peppermint, and neem) as larvicides against *Ae. aegypti* and revealed high larvicidal activity for lemon, peppermint, and lavender oils, with lemon oil exhibiting the strongest effect (LC_{50} = 10.676 ppm). Conversely, neem oil displayed the lowest larvicidal potency (LC_{50} = 38.058 ppm). Manimaran et al. (28) evaluated the larvicidal and knockdown effects of 25 essential oils against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. Eight oils (calamus, cinnamon, citronella, clove, eucalyptus, lemon, mentha, and orange) demonstrated complete larvicidal activity at a concentration of 1000 ppm and complete knockdown effect at 10% concentration. Such studies pave the way for developing new mosquito control formulations utilizing these effective essential oils. Selvi et al. (45) evaluated the activities of some naturally growing medicinal plants (*Salvia verticillata, Leucanthemum vulgare, Inula vulgaris* and *Matricaria chamomilla*) in Türkiye. Plant extracts had high larvicidal activity on *Ae. albopictus* larvae. Similarly, Usta et al. (46) evaluated oviposition deterrent, ovicidal and skin repellent activities of *Salvia verticillate* and *Matricaria chamomilla* against *Aedes albopictus* and stated that these plants have potential in this sense.

While research on larvicidal effects of EOs is extensive, ovicidal effects remain less explored. This study demonstrated that after 120 hours of exposure, verbenone, propionic acid and 1- butanol were observed to present a dose dependent effect on mosquito eggs. At 200 and 100 ppm <25% hatched but afterwards a drastic increase in egg hatching was observed with decreased with concentration. Few studies have assessed the ovicidal effects of essential oils on mosquito eggs. Muturi et al. (35) investigated the chemical composition and ovicidal activity of essential oils from garlic and asafoetida on *Cx. pipiens* and *Cx. restuans*. They identified ten and twelve distinct compounds in garlic and asafoetida essential oils, respectively with Allyl disulfide found as the most prevalent compound in garlic oil. These EOs exhibited significant impact on egg hatching as majority of the *Culex* egg rafts exposed to either garlic or asafoetida oil failed to hatch. In another study (36), *Cinnamomum verum* EO and its main compound, trans-cinnamaldehyde, showed strong ovicidal activity against mosquito eggs at high concentrations (30,000 ppm). EOs have promising potential as a safe and effective ovicidal agent for controlling *Aedes* mosquito populations. Research shows promise for using extracts from various plants to kill mosquito eggs (37). Studies have demonstrated the ovicidal activity of crude extracts from plants such as *Andrographis paniculata* (Lamiales: Acanthaceae), *Cassia accidentalis* (Fabales: Fabaceae), *Euphorbia hirta* (Malpighiales: Euphorbiaceae), *Eclipta alba* (Asterales: Asteraceae), *Cardiospermum halicacabum* (Sapindales: Sapindaceae), *Aegle marmelos* (Sapindales: Rutaceae), *Andrographis lineata* (Lamiales: Acanthaceae), *Cocculus hirsutus* (Ranunculales: Menispermaceae), *Tagetes erecta* (Asterales: Asteraceae), *Melothria maderaspatana* (Cucurbitales: Cucurbitaceae) and *Acalypha alnifolia* against various mosquito species, including *An. stephensi*, *An. subpictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* (38–40). Further research is needed to optimize their use and develop practical control strategies.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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