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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Mosquito Oviposition Deterring and Larvicidal Effect of Cinnamaldehyde and Eugenol

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Abstract: *Aedes* spp. females feed from various hosts including humans and can vector arboviruses of public health importance like dengue, Zika and chikungunya viruses. These infections affect millions of people so insect populations need to be monitored and effectively controlled. Cinnamaldehyde and eugenol are naturally occurring compounds found in essential oils of various spices like cinnamon, cloves, cassia and have different biological activities. I investigated the larvicidal and oviposition deterrence effects of cinnamaldehyde and eugenol against *Ae. aegypti* and *Ae. albopictus.* Both chemicals effectively killed mosquito larvae and deterred female oviposition for both mosquito species at 50-200 µg/mL concentration. Larvicidal and oviposition-deterrent compounds like cinnamaldehyde and eugenol are useful in biological control of mosquitoes. They offer the potential to reduce population numbers by killing immature stages and repelling mosquitoes away from human settlements.

Keywords: *Aedes*, cinnamaldehyde, eugenol, larvicidal, oviposition-deterrence.

Sinnamaldehit ve Eugenolün Sivrisinek Yumurtlamasını Engelleme ve Larvisidal Etkisi

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Öz: Dişi *Aedes* spp. sivrisinek türleri insanlar da dahil olmak üzere çeşitli konakçılardan beslenirler ve Dang humması, Zika ve Chikungunya virüsleri gibi halk sağlığı açısından önem taşıyan arbovirüslerin vektörlüğünü yapabilmektedir. Bu enfeksiyonlar milyonlarca insanı etkilediğinden dolayı böcek popülasyonlarının izlenmesi ve etkili bir şekilde kontrol edilmesi gerekmektedir. Sinnamaldehit ve eugenol, tarçın, karanfil gibi çeşitli baharatların esansiyel yağlarında bulunan ve farklı biyolojik aktivitelere sahip doğal bileşiklerdir. Cinnamaldehit ve eugenolün *Ae. aegypti* ve *Ae. albopictus*'a karşı larva öldürücü ve yumurtlamayı önleyici etkilerini araştırdım. Her iki kimyasal da sivrisinek larvalarını etkili bir şekilde öldürdü ve her iki sivrisinek türü için 50-200 µg/mL konsantrasyonda dişi yumurtlamasını engelledi. Sinnamaldehit ve eugenol gibi larva öldürücü ve yumurtlamayı önleyici bileşikler, sivrisineklerle biyolojik mücadelede oldukça faydalıdır. Larva evreleri öldürerek ve sivrisinekleri insan yerleşimlerinden uzaklaştırarak nüfus sayılarını azaltma potansiyeli sunarlar.

Anahtar kelimeler: *Aedes*, sinnamaldehit, eugenol, larvasidal, yumurtlama önleyici.

INTRODUCTION

Container-breeding mosquitoes *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) are adaptive species that live close to urban areas in tropical and tropical regions. Females feed from various mammals and birds during the day and can vector arboviruses of public health importance like yellow fever, dengue, Zika and chikungunya viruses. These infections affect millions of people esp. children and pregnant women. Both species are emerging in various locations in temperate regions in the world through been transported into new areas during international shipping and climate influences (Kraemer et al., 2015; Lwande et al., 2020). This spread outside their native range around the world is a serious global health threat, so populations need to be monitored and effectively controlled. Control strategies involve spraying insecticides, use of transgenic adults, reducing potential breeding sites, or use of larvicides. Environmental pollution and health risks associated with insecticides, and development of insecticide resistance have spur research on the search for safer and effective substitute strategies (Chadee, 2004; Patil et al., 2018; Rodriguez et al., 2007).

Another possible point of target for mosquito control is to treat breeding sites of mosquitoes with biolarvicides such as *Lysinibacillus sphaericus*, methoprene, temephos, *Bacillus thuringiensis israelensis*. These locally accessible biolarvicides are highly effective against larvae and are also safe to non-target organisms (Mittal, 2003). Besides scientist are researching for new larvicidal compounds or products from plants, bacteria and fungi to complement these products. Cinnamaldehyde and eugenol are naturally occurring compounds found in essential oils of various spices like cinnamon, cloves, cassia (Kowalska et al., 2021). Cinnamaldehyde is a yellowish aldehyde with a characteristic cinnamony aroma, whereas eugenol is a colorless liquid with a clove odor. They have different biological activities that include antibacterial, antifungal, antioxidant etc. (Kowalska et al., 2021; Suppakul, 2016).

I investigated the larvicidal and oviposition deterrence effects of cinnamaldehyde and eugenol against *Ae. aegypti* and *Ae. albopictus.*

MATERIAL AND METHOD

Insects: *Aedes albopictus* and *Ae. aegypti* were reared in cages kept in an insect room at 28-30 °C and 70-80% relative humidity, 12 h photoperiod. Females were fed defibrinated sheep blood using an artificial blood feeder. Eggs were hatched in plastic containers and larvae were fed crushed fish scales (Shah et al., 2021).

Chemicals: Eugenol (>98% purity) and cinnamaldehyde (>95% purity) were purchased from Sigma Aldrich. They were dissolved in distilled water to concentrations of 200, 100, 50, and 25 ppm and used in the bioassays.

Larvicidal assay: This study was conducted using 3rd stage mosquito larvae dispensed in wells of 24-well plates. In each well, 10 larvae were treated with different concentrations of eugenol and cinnamaldehyde. The control was prepared only with distilled water. The total volume in each well was 1 ml. The setup was incubated at 24 °C and larval mortality was recorded after 24 h. Larvicidal activity was reported as LC_{50}, representing the concentration in micrograms per milliliter that caused 50 % larval mortality in 24 h. Immobile larvae after probing were considered dead. Each treatment had 6 replicates and the study was repeated thrice (n = 18).

Oviposition deterrence assay: First blood fed females were transferred from stock cultures to new cages (10♀/cage). Cages were placed in an insect room at 28-30 °C and 70-80% relative humidity, 12 h photoperiod for 4 days. Ten % sugary water was presented at all times. Two plastic containers were added to the cages. One had 50 ml water treated with different concentrations of eugenol or cinnamaldehyde (200,100, 50 and 25 ppm) and the other had distilled water. The edges of the containers had strips of filter paper as oviposition substrates. After 3 more days of incubation the eggs laid in each container were recorded and the oviposition deterrence effects were calculated. The containers were 40 cm apart and positions were changed between replicates. Each treatment had 6 replicates and the study was repeated thrice (n = 18). Effects were determined using the formula below (Rajkumar and Jebanesan, 2009).

$$\text{Effective Repellency \%} = \frac{no.of\ eggs\ in\ control - no.of\ eggs\ in\ treated\ cups}{no.of\ eggs\ in\ control} \times 100$$

***Statistical analysis**:* Data was analyzed in SPSS 23 software. One-way ANOVA followed by Tukey's test (p<0. 05) was used to compare the larvicidal and oviposition deterrence effects of eugenol and cinnamaldehyde against mosquito larvae.

RESULTS

***Larvicidal assay*:** The larvicidal activity of cinnamaldehyde and eugenol against *Ae. aegypti* is presented in Figure 1. Results showed that cinnamaldehyde and eugenol induced >90% larval mortality of *Ae. aegypti* after 24 h with a dosage of 200 µg/mL (Fig. 1). At 100 µg/mL concentration cinnamaldehyde caused 66% mortality whereas eugenol showed 78%. The concentration of the compounds had a significant effect on larvicidal activity against *Ae. aegypti* (F=135.129; df=8,162; P<0.0001) (Fig. 1). The LC_{50} value of cinnamaldehyde and eugenol against *Ae. aegypti* were determined as 55 and 69 µg/mL, respectively.

In the experiment with *Ae. albopictus*, the larvicidal activity of cinnamaldehyde and eugenol is presented in Figure 2. Cinnamaldehyde and eugenol showed 100% larval mortality at 200 µg/mL concentration. There was a statistically significant difference in larvicidal activity of cinnamaldehyde and eugenol against *Ae. albopictus* depending on the dosage (F=390.390; df=8,162; P<0.0001) (Fig. 2). Eugenol showed >95% larvicidal activity at 100 µg/mL concentration and there was no significant difference between 200 µg/mL concentration. The LC_{50} value of cinnamaldehyde and eugenol against *Ae. albopictus* were determined as 65 and 55 µg/mL, respectively.

No larval mortality was observed in control groups.

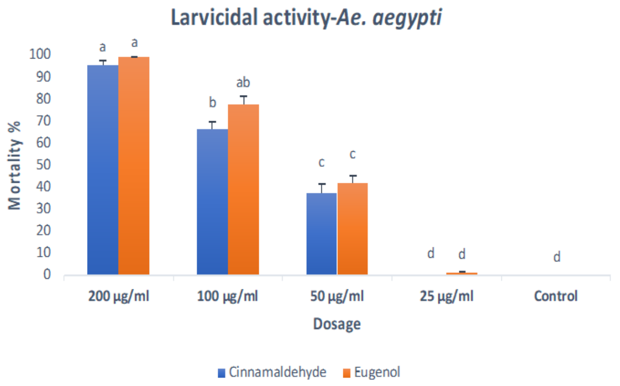

Figure 1. Larvicidal effects of different doses of cinnamaldehyde and eugenol against 3rd instar *Aedes aegypti* larvae.

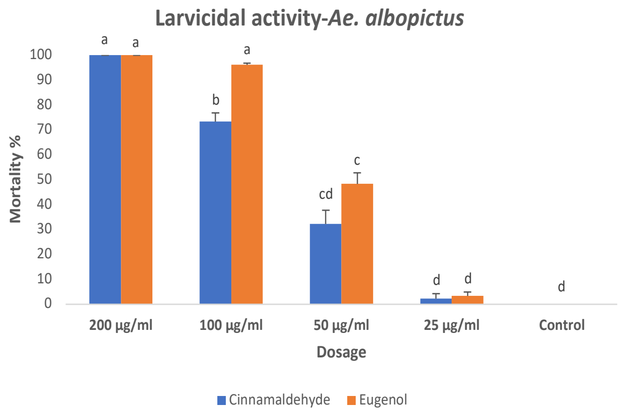

Figure 2. Larvicidal effects of different doses of cinnamaldehyde and eugenol against 3rd instar *Aedes albopictus* larvae.

Oviposition deterrence assay: The oviposition deterrence effect of cinnamaldehyde and eugenol against *Ae. aegypti* and *Ae. albopictus* presented in Figure 3 and Figure 4, respectively. Both chemicals effectively deterred oviposition for both mosquito species (Fig. 3 and Fig. 4). 200 and 100 µg/mL concentration were the most effective (Fig. 5), followed by 50 µg/mL. There was a statistically difference for cinnamaldehyde and eugenol (*Ae. aegypti*: F=918.180; df=7,144; P<0.0001) (*Ae. albopictus*: F=790.311; df=7,144; P<0.0001) (Fig. 3 and Fig. 4).

DISCUSSION

Cinnamaldehyde and eugenol have been investigated for use as bio-insecticide to control *Ae. aegypti* and *Ae. albopictus*. This study has revealed that cinnamaldehyde and eugenol showed larvicidal activities with <70 µg/mL LC_{50} value after 24 h, towards the two mosquito species *Ae. aegypti* and *Ae. albopictus*. The present LC50 values differ slightly from other studies, which probably reflects differences in methodologies and analyses (Cheng et al., 2004). Cheng et al., (2004) reported that cinnamon leaf essential oils showed larvicidal activity against fourth-instar *A. aegypti*. among the 11 compounds tested for 24 h, cinnamaldehyde, cinnamyl acetate, eugenol, and anethole exhibited the strongest activities (LC50 < 50 µg/mL). Cinnamaldehyde and eugenol can be compared with other essential oils from plants. Araujo et al., (2003) demonstrated that 1,8-cineole caused a high mortality rate of *A. aegypti* larvae after 1 day of test at a dosage as low as 100 mg/L. Ramsewak et al., (2001) found that both linoleic acid and oleic acid isolated from the hexane of *Dirca palustris* seeds had an LC_{50} value of 100 µg/mL when tested against fourth-instar *A. aegypti* larvae at 24 h. In another investigation, Rahuman et al., (2000) also found that n-hexadecanoic acid in *Feronia limonia* dried leaves was effective against fourth-instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi*, and *A. aegypti* with LC_{50} values of 129.24, 79.58, and 57.23 ppm, respectively.

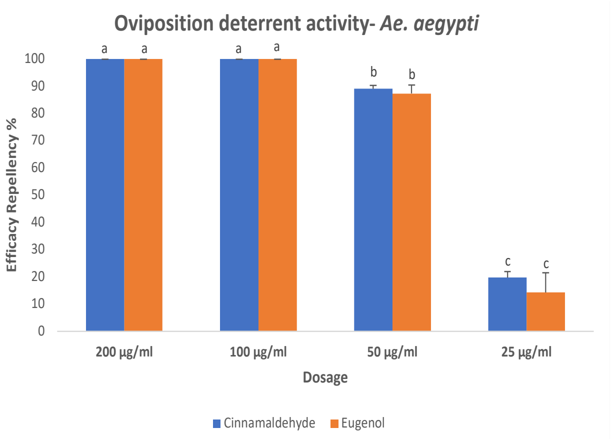

Figure 3. The ovipositional deterrence effects of cinnamaldehyde and eugenol on *Aedes aegypti*.

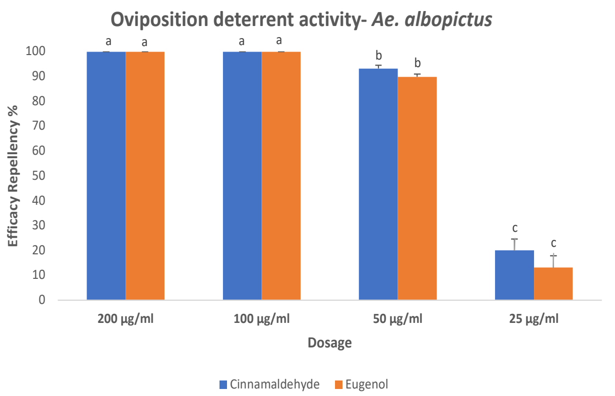

Figure 4. The ovipositional deterrence effects of cinnamaldehyde and eugenol on *Aedes albopictus*

Cinnamaldehyde and eugenol also effectively deterred *Ae. aegypti* and *Ae. albopictus* oviposition in a concentration-dependent manner. Oviposition is a vital part in the mosquito life cycle of mosquitoes. Females are known to prefer certain oviposition sites that they find safer for their offspring over others. They can sense the chemical signals using sensory receptors on the antenna (Davis and Bowen, 1994). Oviposition-deterrent compounds like cinnamaldehyde and eugenol are useful in biological control of mosquitoes. They offer the potential to repel mosquitoes away from human settlements by preventing or reducing female oviposition as they seek out suitable aquatic habitats for their offspring (Bentley and Day, 1989; Clements, 1999; Xue et al., 2003).

Figure 5. The ovipositional deterrence effects of cinnamaldehyde with 100 µg/mL concentration on *Aedes albopictus*. The black dots on the left in the control group with only water in it are mosquito eggs.

In conclusion, cinnamaldehyde and eugenol have potential for the development of new and safe control product for mosquito vectors. Thus, effective oviposition-deterrent and larvicidal could be useful and developed further in the integrated approach to mosquito control programmes against container-inhabiting mosquitoes. These results could encourage the search for new active natural compounds offering an alternative to synthetic larvicidal and oviposition-deterrent from other plants especially indigenous ones.

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