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Larvicidal activity of *Zataria multiflora*, *Eucalyptus caesia Benth* and *Mentha piperita* against *Culex* mosquito

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Abstract

Background and Objective: Mosquito is a vector of several life threatening diseases affecting humans. The use of synthetic insecticides in the vector control is not advisable due to concern about environmental sustainability, harmful effect on human health and increasing insecticide resistance. So, the objective of this study was to assess larvicidal activity of essential oils (EOs) of *Zataria multiflora*, *Eucalyptus caesia Benth*, and *Mentha piperita* against the *Culex* mosquito.

Materials and Methods: The larvicidal activity of the essential oils were tested according to the WHO procedure. The larvae exposed to three-fold serial dilution of oils (2.5-400 ppm) using a dipping method for 24 h and then simultaneously each replicate was incubated in separate petri dishes at 27°C and 80-90% relative humidity. Mortality rate was recorded after an exposure of 24 h. LC_{50} and LC_{90} were calculated using Probit analysis and all data were analyzed using ANOVA and post hoc Tukey test.

Results: In the comparative analysis of the essential oils, LC_{50} & LC_{90} of *Z. multiflora*, *E. caesia Benth* and *M. piperita* were 5.09429 and 26.9919, 3.66376 and 35.3173, and 8.3115 and 218.888 ppm, respectively. Also, as the concentration of essential oil increased, mortality rate of larvae increased too.

Conclusion: This study concluded that the essential oils of *Z. multiflora*, *E. caesia Benth* and *M. piperita* have appropriate larvicidal activity against *Culex*, therefore, they can be used as good alternative to the *Culex* biological control.

Keywords: *Culex*, *Eucalyptus caesia Benth*, larvicidal activity, *Mentha piperita*, *Zataria multiflora*

1. Introduction

Mosquitoes are urban insects that proliferate near human housings. In addition to their biting disturbances, they are potential vectors of infectious diseases such as Dengue, West Nile virus, encephalitis, Rift Valley Fever, Zika virus infection, yellow fever, Malaria and so on (1-3). The *Culex* that is known as ordinary mosquito, classified as arthropods of the Diptera order and the Culicidae family. The *Culex* transmits biological diseases between vertebrate hosts by feeding human and animal blood and carries a number of arboviruses (arthropod born viruses) and parasites (4). The *Culex* induces angioedema (a cutaneous allergy in humans), and is vector of various infectious diseases such as: lymphatic filariasis, Japanese encephalitis, equine encephalitis and West Nill Virus (5,6). Mosquito larvae are relatively immobile and often easily accessible. By targeting the larval stages, the insects killed before spreading to human habitat. Unlike adults, mosquito larvae cannot change their habitat, so control strategies are more successful (7). Nowadays, synthetic insecticides cause several serious problems such as long survival in the environment, entering the food chain, expensiveness and development of mosquito-resistant populations (8).

Using the herbal insecticides (medicinal plants extracts and essential oils) is a good alternative, that is

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generally considered safe for the environment and health. Several essential oils can be used as herbal larvicides against important vectors (9). *Z. multiflora* (ZM) is belonging to the *Lamiaceae* family that is grown widely in Iran, Pakistan, and Afghanistan (10). This plant has several traditional uses such as antiseptic, anesthetic and antispasmodic. Many pharmacological studies showed that ZM possesses wide biological properties including its antioxidant, antimicrobial, antinociceptive and anti-inflammatory activities (11). Phytochemical studies showed that the essential oil of ZM contains significant amounts of thymol and carvacrol (37.59% and 33.65%, respectively), which are well-known anti-microbial and anti-fungal agents. p-Cymene is the other main component in the ZM oil. Other significant components of this plant contain linalool, caryophyllene, γ-terpinene and borneol (12). *Eucalyptus caesia Benth*, a species of the *Myrtaceae* family, has traditionally been used to treat various infectious diseases, especially respiratory disorders such as sinusitis, pharyngitis and bronchitis. *Eucalyptus* essential oil also has several pharmacologic effects such as antiseptic, disinfectant, antibacterial, and anti-inflammatory effects (13,14). Major constituents of *E. caesia Benth* are 1,8-cineole (40.2%), p-Cymene (14.1%), γ-terpinene (12.4%), α-pinene (7.7%), and terpinen-4-ol (5.6%) (15). *Mentha piperita* belongs to the *Lamiaceae* family, phytochemistry of the plant demonstrated that the major components of *M. piperita* essential oil are menthol (30.35 %), menthone (21.12%), and trans-carane (10.99%) (16). *M. piperita* in the Iranian traditional medicine is used as a carminative, stimulant, tonic, anti-viral and anti-fungal agent. Pharmacological studies have indicated that *M. piperita* essential oil has antimicrobial, antifungal, anti-inflammatory and anti-nociceptive effects, also larvicidal, bio-pesticidal, neuroprotective, hepatoprotective and antioxidant properties (17). The purpose of this study was to investigate the larvicidal activity of the essential oils of *Zataria multiflora*, *Eucalyptus caesia Benth*, and *Mentha piperita* against *Culex* mosquito.

2. Materials and Methods

2.1. Mosquito rearing

The *Culex* larvae were collected from Golpayegan (a city located in northwest of Isfahan province) at the end of spring and summer. The larvae transferred to the parasitology laboratory, faculty of veterinary medicine, Shahrekord University. Identification of the larvae was done and confirmed. The *Culex* larvae maintained at 27 ±2°C with a photoperiod of 14 hours light and 10 hours dark in 80-90% relative humidity. A dried yeast powder was used as food source.

2.2. Plant material

Z. multiflora, *E. caesia Benth* and *M. piperita* essential oils were purchased from Barij Essence Co., Kashan, Iran. According to the instructions, plant oils were obtained by hydro-distillation of sterilized plant materials (leaves) using a Clevenger-type apparatus as follows: The plant leaves were steam distilled for 60 min in a full glass apparatus. The extraction was carried out for 120 min in 500 ml of water. All the oils were stored in the dark at 4°C until required for further work.

2.3. Bioassays and larval mortality

The larvicidal activity of the essential oils were tested according to the WHO procedure (18). As the oils do not dissolve in water, they were first dissolved in ethanol. Three-fold serial dilution of essential oils (2.5-400 ppm) was prepared. After about 15 min, 12 larvae taken on a strainer with fine mesh, and then transferred gently to the test medium by tapping and simultaneously each replicate was incubated in separate petri dishes at 27°C and 80-90% relative humidity for 24 h. Two control groups were set up including negative (distilled water) and positive (1 ppm Cypermethrin) controls (19).
Mortality was recorded after an exposure of 24 h. During the exposure period, no food was given to the larvae. The dead larvae were counted after 24 hours and percentage of mortality was reported from the average of the 3 replicates. The larvae considered dead were those that did not move when touched with a needle in the siphon or cervical region (20). The mortality rates were corrected using the Abbott’s formula (21).

2.4. Statistical analysis

The lethal concentrations (LC50 and LC90) were calculated using Probit analysis (22).
All data were analyzed using statistical analysis system: analysis of variance (ANOVA) and post hoc Tukey test (SPSS, 23) and $P<0.05$ was used as the significant difference between the means.

3. Results

The toxicity of oils of *Z. multiflora*, *E. caesia Benth*, and *M. piperita* against *Culex* larvae were noted and the LC_{50}, LC_{90}, 95% confidence intervals of LC_{50} and LC_{90}, and chi-square were also calculated (Table 1).

Table 1. Larvicidal activity of essential oils of *Z. multiflora*, *E. caesia Benth, and M. pierita* against *Culex*

Specimens	LC_{50}	95% confidence interval	LC_{90}	95% confidence interval	X^{2*} (df)
Z. multiflora	5.09429	3.9191-6.3431	26.9919	20.610-38.7016	9.7024 (6)
E. caesia Benth	3.66376	2.68021-4.67246	35.3173	25.072-56.389	24.0233 (6)
M. piperita	8.3115	5.3855-11.7674	218.888	134.65-433.21	9.7024 (6)

Total mortality after 24 h . LC50 = lethal concentration (ppm) that kills 50% of the exposed larvae, LC90 = lethal concentration (ppm) that kills 90% of the exposed larvae. df= degree of freedom, * Chi-square value, significant at P < 0.05 level.

As shown in table 1, E. *caesia Benth* has the lowest LC50 between all specimens. The essential oils of Z. *multiflora*, E. *caesia Benth*, and M. *pierita* showed significant repellency against *Culex* (Figures 1-3). 1-3).

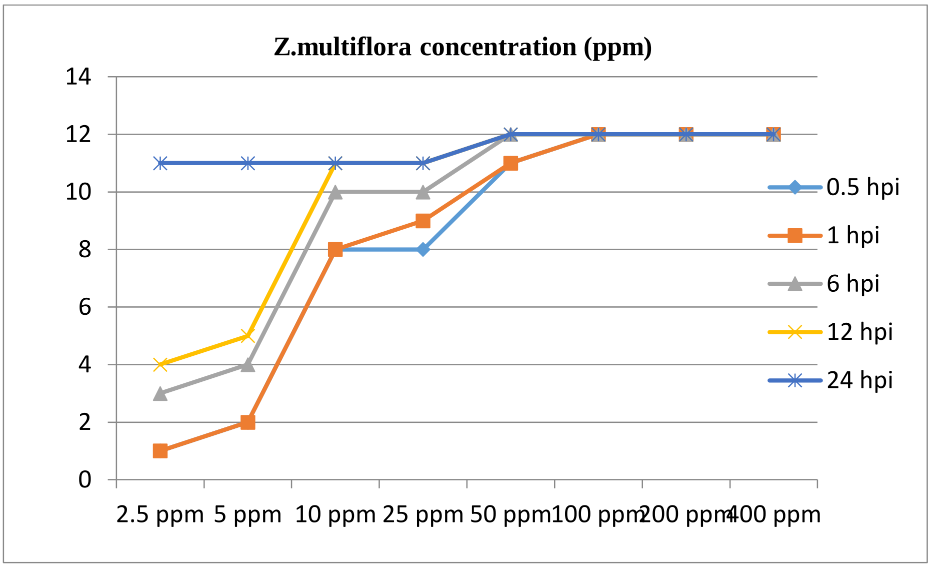

Fig 1. Toxicity of *Z. multiflora* EO against *Culex* larvae in consecutive concentrations and times

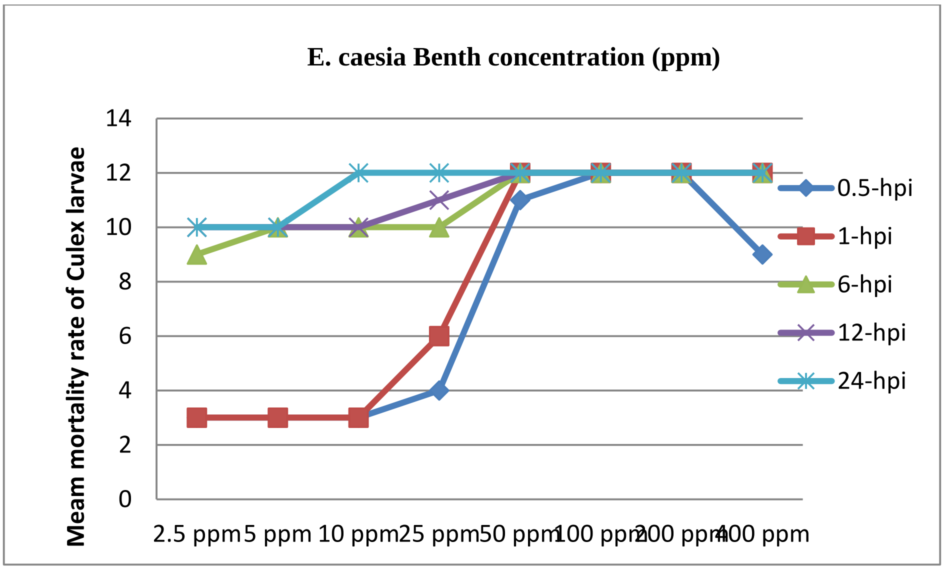

Fig 2. Toxicity of *E. caesia Benth* EO against *Culex* larvae in consecutive concentrations and times

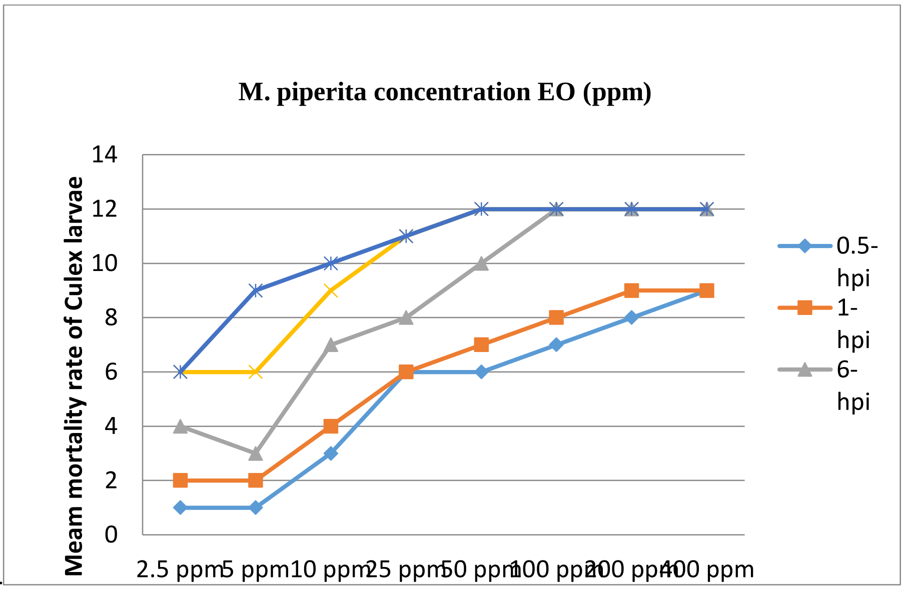

Fig 3. Toxicity of *M. piperita* EO against *Culex* larvae in consecutive concentrations and times

As shown in Figures 1-3, the essential oils have concentration-dependent larvicidal activity. The mortality rate of *Culex* larvae at concentrations of 100, 200, and 400 ppm of *Z. multiflora* and *E. caesia Bent*h EOs, at 24 hours post incubation is equal to each other and causes 100% mortality. About other concentrations of the EOs, the mortality rate was increased by elevating incubation time. Fig. 3 shows that the mortality rate at concentrations of 100, 200, and 400 ppm of *M. piperita* EO, at 6, 12 and 24 hpi is completely equivalent (100% mortality) and about other concentrations, by elevating concentration and incubation time, mortality rate was increased.

4. Discussion

Using the herbal essential oils in vector control is an alternative way for minimizing side effects of chemical insecticides in the environment (23). Several studies have been done in this field. The larvicidal activity of leaf essential oils of three species of genus *Atalantia* (*A. monophylla*, *A. racemose* and *A. wightii*) was evaluated against *Culex*, Anophele, and Aedes recently. Among the three, essential oil of *A. racemosa* showed maximum activity against the three selected mosquito species so that at 100 and 200 ppm showed 100% mortality (24). In another study by Pavela et al (25), larvicidal activity of 20 samples of essential oils of different species of *Mentha L.* and *Pulegium* against *Culex* was evaluated and proved that the lowest (the most potent) estimated LC_{50} (17 mg/L) is attributed to *Mentha longifolia* and *Mentha suaveolens*.

It is indicated that bioactive secondary metabolites act as insecticide (26,27). According to the most important constituents of the essential oils in the present study (monoterpenoids), the larvicidal properties of *Z. multiflora*, *E. caesia Benth*, and *M. piperita* EOs can be related to these secondary metabolites, as previous studies have shown that terpenoids have the most effective larvicidal, pupicidal or adulticidal activities against different species of mosquito (28). The results of the present study also showed that mortality rate of *Culex* larvae is dependent on essential oils concentration and also exposure duration. Also, larvicidal activity of *Zanthoxylum limonella* Alston was assessed against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say) by Soonwera and Phasomkusolsil (28). At 24 h, the mortality rate of the EO was 100% too and a positive correlation was found between the EO concentration and mortality. According to the LC_{50} and LC_{90} of these Eos, especially E. caesia Benth, these botanical insecticides can be considerable in comparison to chemical toxin such as Cypermethrin. By attention to the mortality rate of these EOs, it can be concluded that using these herbal EOs leads to the rapid removal of contamination (the larvae) with the least residual effect in the environment.

Conclusion

This study showed that *Zataria multiflora*, Eucalyptus *caesia Benth*, and *Mentha piperita* essential oils are potent botanical insecticide. Due to the abundant growth of these medicinal plants, as well as their adaptation to the climatic conditions of most regions of Iran, the EOs can be used as a potential alternative to the *Culex* mosquito biological control.

Conflict of Interest

The authors declare they have no conflict of interest

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