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Evaluation of Synergistic Effects of Two Monoterpenoids of Geraniol and B-Citronellol on Culex Larvae in Vitro

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ABSTRACT

BACKGROUND AND OBJECTIVE: Insects are the cause of excessive spread of parasites and pathogens. Insect population control faces the challenges of increasing resistance to chemical pesticides and the environmental hazards posed by them. Therefore, the use of natural insecticides is important. The aim of this study was to investigate the synergistic effect of geraniol and betacitronellol on the larvae of Culex Pippins complex.

METHODS: In this cross-sectional study, samples were taken from the Insectarium of Babol University of Medical Sciences. Yield of geraniol, betacitronellol and their combination in ratios of 4:1, 3:2, 2:3 and 1:4 in a total of 6 groups on the larvae of the third stage of the mosquito culex Pippins complex at concentrations of 0.5, 1, 2, 5, 10 and 20 µg/ml were evaluated with 5 replications. Needle test was used to determine whether the larvae were dead or alive. The LC50 of each group was then recorded after 24 hours.

FINDINGS: The highest mortality of larvae belonged to the geraniol group with LC50 equal to 48.1 µg/ml. While betacitronellol with LC50 equal to 49.10 µg/ml had the lowest toxic dose. Among the four combined ratios of geraniol-betacitronellol, the highest toxicity was related to the ratio of 1:4 and the lowest toxicity was related to the ratio of 4:1 with LC50 of 3.32 and 7.71 µg/ml, respectively, and antagonistic effect was observed in all ratios ($p<0.05$).

CONCLUSION: The results of the present study showed the antagonistic effects of the combination of geraniol with betacitronellol on mosquito larvae of Culex Pippins complex.

KEY WORDS: *Geraniol, β-citronellol, Culex, Insecticide.*

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Introduction

Mosquitoes are carriers of various types of human, animal and parasite pathogens. Among them, the genus Culex is the second most transmitter disease among vertebrates in countries with tropical and subtropical climates (1). The population of Culex Pippins has a wide geographical distribution and is found all over the world in urban and non-urban, tropical regions, and in these areas it can be the main carrier of diseases.In fact, Culex Pippins plays an important role in the transmission of a number of pathogens and parasites that infect humans, including malaria, West Nile virus, dengue fever, St. Louis encephalitis virus, filariasis, etc. (2).

The prevalence of dengue fever has quadrupled since 1970, and almost half of the world's population is at risk. In 1990, it was estimated that approximately 30% of the world's population, or 1.5 billion people, lived in areas where the risk of dengue fever was above 50% (3). One of the first ways to deal with mosquitoes is to use synthetic chemicals, but overuse of these substances can cause resistance in these organisms. In addition, chemical insecticides have toxic and harmful effects on humans and the environment by polluting the soil, climate (4). These problems require extensive research to produce new products that, in addition to being safe and harmless to the environment, can also be degraded in the environment (5).

Essential oils and plant extracts seem to be a good alternative to insecticides because they are effective, degradable and inexpensive without adverse environmental effects. Essential oils of aromatic plants contain a wide range of biologically active compounds, most of which are terpenoids. Terpenoids have been reported as acute toxic compounds against insects (6). Accordingly, several studies have been conducted in the field of insect control and have evaluated the toxicity of essential oils and terpenoids on different species of mosquitoes and tried to use them as a potential and new source in order to control diseases through mosquito population control (7).

The population of mosquito larvae is the target of most control programs, as the extermination of adults may temporarily reduce their population and cost more (8). Targeting mosquito larvae is more effective and will help prevent the spread of vectors (9). In 2011, Zahran et al. evaluated the lethal effect of 12 monoterpenes, including geraniol, on adult mosquitoes of Culex Pippins (10). Abouhosseini Tabari et al. further evaluated the effect of aromatic geranium essential oil on adult mosquitoes and culex eggs. Youssefi et al. also investigated the synergistic effects of carvacrol and thymol monoterpenes on larvae and eggs of Culex mosquitoes (12). Monoterpenes are the main constituents of most plant essential oils and have pesticidal activities, including insecticidal, antifungal and antibacterial properties (13). Numerous studies have been performed on the insecticidal properties of essential oils against different species of mosquitoes. However, few studies have shown the insecticidal effect of monoterpenes against mosquitoes. Geraniol and betacitronellol are monoterpenes that are abundant in the essential oils of plants such as Pelargonium roseum and Rosa damascena Mill (11) and are widely used in the perfume industry due to their pleasant odor (14). In addition, geraniol has been implicated in the treatment of prostate cancer (15) and betacitronellol as a repellent causes repulsion of repisphalus and amblyoma mites (16) and due to its high lethality, these two monoterpenes can be good as insecticides (11).

The aim of this study was to investigate the synergistic effects of geraniol and betacitronellol combination and to find the most effective combination ratio between geraniol and betacitronellol on Culex larvae in vitro.

Methods

This cross-sectional study was performed on the larvae of Culex Pippins complex after obtaining permission from the ethics committee of the Army University of Medical Sciences of the Islamic Republic of Iran with the code IR.AJAUMS.REC.1397.093. The species was diagnosed by Dr. Karami, an entomologist at Babol University of Medical Sciences. Geraniol with code 106-24-1 and beta citronellol with code 106-22-9 were purchased from Sigma Company. Mosquitoes were kept in cages with a temperature of 25±2 °C, relative humidity of 80±2% and light period: darkness 12:12 in the insectarium of Babol University of Medical Sciences. Adult female mosquitoes laid eggs in petri dishes 9 cm in diameter and 1.5 cm deep containing fresh water. The eggs were collected daily and transferred to trays for hatching eggs. 5% yeast suspension was used as food source. The larvae generating activity of geraniol and betacitronellol groups was evaluated according to the WHO protocol. The compounds tested in these two groups were dissolved in 6 concentrations of 0.5, 1, 2, 5, 10 and 20 μg/ml to investigate the effects of larvae generating in fresh water. Each compound was first dissolved in 1 ml of DMSO (dimethyl sulfoxide) as an emulsifier and then

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diluted in 249 ml of fresh water to reach each of the desired concentrations. Control was prepared using 1 ml DMSO in 249 ml water. 20 larvae of the third stage of Culex Pippins complex mosquitoes were placed in each of the desired concentrations. 5 replications were performed for each concentration. After 24 hours, by tapping the needle tip into the respiratory siphon or the neck of the larvae and observing their reaction or non-reaction to the stimulus, they were confirmed dead or alive and the results were recorded (17).

In the second stage of experiments, to determine the synergistic or antagonistic effect of the combination of geraniol and betacitronellol, based on the fixed ratio method, four ratios of geraniol -betacitronellol 4: 1, 3: 2, 2: 3 and 1: 4 was tested to evaluate the toxicity of the compounds on the larvae of Colex Pippins complex. For each of the binary ratios, the dilution steps were performed according to the previous step in 6 concentrations so that the final concentrations from the range of 0.5 to 20 µg/ml could be used. For each combined ratio, 5 replications were performed and the minimum concentration that killed 50% of the population (LC50) and the FLC index for each ratio were calculated according to quantitative correction (18). The FLC index was calculated based on the following formula:

FLC index= LC50 A in combination / LC50A alone + LC50 B in combination / LC50B alone

A FLC index of 0.1 indicates an enhancing effect, a FLC< 0.1 indicates a synergistic effect, and a FLC> 0.1 indicates an antagonistic effect (19). The obtained information is given in the isobologram according to the dilutions studied.

Statistical analysis: SPSS software version 16 was used for statistical analysis. Probit regression test was used to obtain the lethal concentrations of 50 (LC50) and 90 (LC90). Data were analyzed using one-way analysis of variance (One way-ANOVA) and Tukey post hoc test and $p<0.05$ was considered significant.

Results

During this study, 80% of larval mortality was observed after 24 hours of exposure to 2 µg/ml geraniol, while no mortality was observed in betacitronellol at the same concentration and in the control group ($p<0.05$). The results of probit analysis on larvae generating showed that LC50 and LC90 values of geraniol were 1.48 and 2.19 µg/ml, respectively, while LC50 and LC90 for betacitronellol were 10.49 and 15.82, µg/ml respectively (Table 1). The results of quantitative analysis of LC50 toxicity of geraniol and betacitronellol compounds and their FLC index are presented in Table 2. The trend of interaction between beta-citronellol-geraniol combination against the larvae of the third stage of culex Pippins was analyzed by the method of fixed ratios and is shown in the isoblogram diagram (Figure 1).

There was an antagonistic effect in all ratios. The ratio of 4: 1 betacitronellol-geraniol with FLC index= 1.85 and LC50 equal to 3.32 µg/ml had the highest effect compared to the others but showed a weaker effect against geraniol against Culex Pippins larvae ($p<0.05$), so that with increasing the amount of betacitronellol in combination, its antagonistic effect increased.

Table 1. Comparison of beta-citronellol and geraniol larvae generating activity against Culex Pippins larvae

Compounds	Concentration (µg/ml)	Mortality rate±SE (%)	Mortality rate In the control group	LC50 (µg/ml) (LCL-UCL 95%)	LC90 (µg/ml) (LCL-UCL 95%)
Beta Citronellol	0.5	0±0	0	10.49 (8.93-13.85)	15.82 (12.88-23.86)
	1	0±0	0		
	2	0±0	0		
	5	13.33±3.33	0		
	10	43.33±6.60	0		
	20	100±0	0		
Geraniol	0.5	0±0	0	1.48 (1.29-1.7)	2.19 (1.92-2.65)
	1	26.66±3.33	0		
	2	80±10	0		
	5	100±0	0		
	10	100±0	0		
	20	100±0	0		

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Table 2. Larvae generating results obtained from geraniol-betacitronellol combination by constant ratio method

Ratios Geraniol-beta citronellol	Larvae FLC index*	LC50 (µg/ml) (LCL-UCL 95%)
1:4	1.85[a]	3.32 (3.21-4.11)
2:3	1.7[a]	3.85 (2.8-4.8)
3:2	1.26[b]	5.37 (4.31-6.21)
4:1	1.62[a]	7.71 (6.59-8.34)

Mismatched letters indicate significant differences between treatments. p<0.05 is considered significant.

***FLC index= LC50 geraniol in combination / LC50 geraniol alone + LC50 β-citronellol in combination/ LC50 β-citronellol alone**

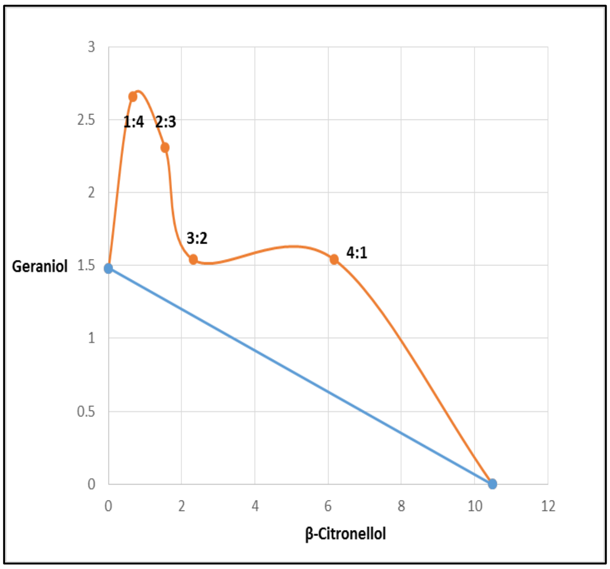

Figure 1. Isoblogram of betacitronellol-geraniol combination

Discussion

In this study, it was found that the combination of geraniol and betacitronellol showed less effect on the larvae of the third stage of Culex compared to geraniol. Cheng et al. showed that the essential oil of Cinnamomum osmophloeum has larvae generating effects on Aedes Aegypti mosquito. They showed that the main component of this essential oil is Cinnamaldehyde, which has the greatest effect against Aedes larvae (20). A similar study was performed by Mukandiwa et al. on an extract from C. anisata and showed the mortality of third stage larvae of Aedes egyptian mosquitoes with LC50 values of 68.3 and 59.7 ppm after 24 and 48 hours, respectively. Pyrano coumarin and ceslin isolated from the same extract in the same study had LC50 with values of 13.9 and 10 ppm after 24 and 48 hours, respectively (21). While in the present study, the 1: 4 combination ratio of geraniol-betacitronellol showed a better effect on Culex larvae with LC50 equal to 32.3 µg/ml, which is probably due to the greater sensitivity of this species. Mavundza et al. showed that the ethanolic extract extracted from the leaves of Clausena anisata killed the larvae of Anopheles arabiensis mosquitoes with LC50 values equal to 7.112 ppm (22).

In the present study, the lethality of the combination of geraniol and betacitronellol on the mosquito larvae of culex Pippins complex has been shown. According to our findings, Traboulsi et al. showed the Larvae generating effect of essential oils of Myrtus communis, Origanum syriacum, Mentha microphylla, Pistacia lentiscus and Lavandula stoechas against fourth instar larvae of Culex Pipins mosquitoes, with LC50 values between 16 and 89 mg/L (23).

Regarding other species of Culex, the larvae generating effect of Plectranthus barbatus essential oil against Culex tritaeniorhynchus with LC50 and LC90 values of 94.34 and 179.58 µg/ml, respectively, was evaluated. Also, the toxicity of the main components of this essential oil, which included eugenol, alphapinene and β-caryophyllene, was evaluated against the larvae of Colex tritaeniorhynchus with LC50 values of 30.80, 36.75 and 48.17 µg/ml for these compounds (24). However, in the present study, the combination of geraniol with betacitronellol in a ratio of 1: 4 showed better and stronger effects than the essential oils used in the above studies.

Abouhosseini Tabari et al. showed that aromatic geranium essential oil had a good larvae generating effect with LC50, 49.5 µg/ml against the larvae of stage 3 Culex Pippins (11). The present study showed that among the tested compounds, the most effective is geraniol with LC50 equal to 48.1 µg/ml and its combination with betacitronellol reduces their toxicity, so that by increasing the amount Beta-citronellol reduces the effectiveness. This may be due to the antagonistic effect between the two compounds, which is why geraniol alone is more effective than aromatic geranium essential oil. The method of fixed ratios and isoblogram diagrams obtained from the combination of geraniol-betacitronellol is presented in the present study and it is shown that the toxicity of geraniol is reduced in the presence of betacitronellol. In comparison with our findings, Karpouhtsis et al.

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showed that the combination of carvacrol and thymol, the main components of oregano essential oil, reduced insecticidal activity and concluded that the two phenols had an antagonistic effect on each other (25). Overall, the present study showed the antagonistic effects of the combination of geraniol with betacitronellol on the larvae of the culex mosquito, so it is suggested that the combined effect of these two monoterpenes with other monoterpenes be evaluated to investigate the agonist and antagonist effects.

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