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Assessment of larvicidal properties of bio-pesticide (*Cymbopogon citratus)* plant extracts against *culex* mosquitoes larva

P Muthukumar[1], Suvarna V P[2], Poovizhi K V[2]

[1] Assistant Professor, PG and Research Department of Zoology, Arulmigu Palaniandavar College of Arts and Culture, Palani, Tamilnadu, India

[2] PG Students, PG and Research Department of Zoology, Arulmigu Palaniandavar College of Arts and Culture, Palani, Tamilnadu, India

Abstract

Objective: Assessment of larvicidal properties of *Cymbopogon citratus* (Lemon grass or Malabar grass or Cochin grass) plant extracts against *Culex* mosquitoes at PG and Research Department of Zoology, Arulmigu Palaniandavar College of Arts and Culture, Palani.

Methods: The III and IV instars of *Culex* larvicidal activity of Malabar grass extracts was tested the protocol recommended by WHO. Methanol and water extracts were prepared at 50, 100, 150, and 200 mg/L concentrations and tested against III and IV instars larvae of *Culex* mosquitoes. The mortality rate of mosquito larvae's was recorded after 24, 48 and 72 h exposure period continuously.

Results: III instar larvae after 24 h exposure period, maximum mortality of 84.60% was recorded at 200 mg/L concentration of methanol extract. After 48h exposure period, maximum mortality of 90.20% and 72 h exposure period, maximum mortality of 94.20% was recorded in methonal extract in all the tested concentration. The maximum mortality of fourth instar larvae was 89.30% and 92.20% in methanol extract at 150 and 200 mg/L concentrations respectively. The percentage of mortality in III and IV instars of mosquitoes was higher in methanol extract followed by water extract.

Conclusions: The mortality percentage of different concentration of the metnanol and water extracts tested and exposure period. This experiments confirmed larvicidal activity of *Cymbopogon citratus* leaf extracts against *Culex* mosquitoes. This leaf extract can be used by applying on drainage water treatment plant containing tanks or *culex* breeding places to prevent adult emergence.

Keywords: leaf extracts, *Cymbopogon citratus*, *Culex* mosquito larvae, larvicidal activity

Introduction

Insect-transmitted diseases impose an enormous burden on the world's population in terms of loss of life (millions of deaths per year) and morbidity. These diseases are also responsible for massive economic losses; both in terms of health-care costs and lost productivity, mostly in countries that can least afford them. Mosquitoes are plays important role in the spread of vector-borne diseases like malaria, dengue, chikungunya, filariasis and Japanese encephalitis which cause thousands of deaths per year (Wilson *et al*, 2020) [18]. Mosquitoes are the majority of important and abundant pest in urban, sub-urban and rural surroundings. Many plant products have been tried in earlier days before the discovery of chemical pesticides (Nicolopoulou-Stamati *et al*., 2016) [10]. Although, chemical control provides quick mortality, resistance of mosquito against the use of insecticides (Malathion, Permethrin, etc...) have been widely reported (Ferreira *et al.*, 2019; Richards *et al.*, 2018, Francis *et al*., 2017) [5, 16, 6]. Moreover, chemical mosquito repellents have toxic synthetic pyrethroids as active ingredients whose exposure to food and water is hazardous to health. Current scenario, several researches are searching locally available plant materials in order to find out eco-friendly products to manage different mosquito species (Oladipupo *et al*., 2019; Asadollahi *et al*, 2019; Girmay *et al.*, 2014) [12, 7] *Cymbopogon citratus*, generally known as lemon grass and other *Cymbopogon* species is a tall, coarse grass with a strong lemon taste. Lemon grass is a perennial herb widely cultured in the tropics and sub-tropics places. It designates two different species; East Indian *Cymbopogn flexuosus* and West Indian, *Cymbopogon citratus* (Avoseh *et al*, 2015) [3]. It is a tropical plant, grown as an ornamental in several temperate areas with maximum a height of about 1.8m and its leaves 1.9cm wide covered with a whitish bloom (Adegbegi *et al.*, 2017) [2]. Biologically active compounds derived from selected plants species such as *C.citratus*, *O. gratissiumum*, *Hyptis sauveolen*, *Acarcia arabica*, *Azadirachta indica* and *Eleusive indica* have been commonly used in the past to control insects in many tropical countries (Jitendra Kumar *et al.*, 2021). The essential oils obtained from the leaves and stems of *C. citratus* are used as treatment for several health problems like fever, throat inflammations, ears or eyes a typical example is the use of the leaves in the Eastern Nigeria to treat various heart disorders (Ojewumi *et al.*,2017) [11]. Ethanol extract of *C.citratus* was most effective against *Anopheles arabiensis* mosquito larvae and also had strong repellent activity against adult mosquitoes (Ojewumi *et al.*,2017) [11]. In our area, *C.citratus* is locally called as "Lemon grass" in perennial grass and growing extensively in Hills regions of the country. Previously some works on *C.citratus* extracts against *Anopheles* mosquitoes. But there

is no much work on this potential plant extract against *Culex* mosquitoes. Therefore, present study was initiated to evaluate solvent extract of *C.citratus* leaves against immature stages of the *Culex* mosquitoes.

Materials and Methods

Mosquito larvae collection and maintenance

The III and IV instars *Culex* larvae were collected in between the Iduban and Palani hills swamps of the stagnant water polluted with organic wastes. The collected larvae were brought to the college laboratory and maintained in a plastic container by providing powdered yeast and dog biscuits (3:1 ratio) as a source of feed. For the identification of the *Culex* species was carried out with the aid of a microscope and further comparison with the preserved specimen in the Department of Zoology laboratory of our College. After which breeding was continued in the laboratory of Department of Zoology.

C. citratus collection and processing for solvent extraction

Lemon grass leaves were collected from Iduban Hills, Palani, Dindigul District, Tamilnadu. Green leaves of grass were collected and washed with water to remove unwanted debris attached to the grass and dried under room temperature in order to prevent chemical denaturation. After complete drying, grass leaves were cut into small pieces and powdered using grinding Mixer. The powdered grasses were sieved to obtain fine powder for solvent extraction.

Solvent extraction of plant powder

The methanol-leaf extracts from the sieved fine grass leaf powder was obtained by using Soxhlet apparatus. About 250 g of fine grass powder was taken into 200 mL Erlenmeyer flask of methanol (as a solvent) and extracted in the Soxhlet apparatus for 16 hrs over a mantle heater at 80°C. Similar manner water extract also prepared. The methanol extracts were concentrated using a vacuum evaporator at 45°C under low pressure. After complete evaporation of the solvents, the concentrated extracts were collected and stored in a refrigerator for further use.

Preparation of stock and working concentration

From the concentrated solvent extracts stock, solution of 1000 mg/L was prepared by adding 150 mg of grass extract mixed with 1 mL of methanol and 1 mL of soap solution (added for the purpose of emulsification). The final volume of 150 mL was prepared by adding distilled water. From the stock solution, working concentrations of 50, 100, 150, and 200 mg/L were prepared by serial dilution method. The four concentrations prepared from the stock solution were tested against third and fourth instar larvae of the *Culex* larvicidal activity. The mortality rate of mosquito larvae was recorded after 24, 48 and 72 h exposure period continuously.

Evaluation of larvicidal activity of plant extract

Larvicidal activity of methanol and water extract of *C.citratus* grass was tested by following WHO (2005) [19], protocol with modifications. The larvicidal activity of lemon grass extract was conducted by using 250 mL plastic container. In each container, 10 larvae of third and fourth instar larvae were released. In each beaker, concentration of methanol and water extract was maintained at 50, 100, 150, 200 mg/L in 100 mL of final water volume. In control, except grass extracts, remaining materials were added as mentioned in preparation of concentration. The number of dead larvae was recorded continuously after 24, 48 and 72 h exposure period from replication. The larval mortality was corrected and calculated by using Abbott's formula (1925).

$$\text{Corrected percent of mortality}=\frac{\text{Mortality in test \%}-\text{Mortality in control \%}}{\text{100 \%}-\text{Mortality in control \%}}\text{X100}$$

Statistical analysis

The percentage of mortality of *Culex* larvae obtained from the replications at different concentrations and exposure periods was subjected to statistical analysis. The calculations were carried out by using Microsoft Excel program in order to obtain mean percentage values and standard deviation. The past software was used to confirm statistical significant at 5% level ($P < 0.05$) by using Chi-square ($\chi 2$) analysis. The LC_{50} and LC_{90} values and 95% upper confidence limit (UCL) and lower confidence limit (LCL) were also calculated.

Results

Mortality of third instar larvae of *Culex* mosquitoes exposed 24, 48, 72 h in *C.citratus* plant extracts

Mean percentage of mortality of III instar larvae of *Culex* mosquitoes exposed to *C.citratus* grass extracts after 24, 48, 72 h exposure period was present in Table 1. 24 h exposure period results revealed that maximum percentage of mortality of 32.20% was observed in methanol extract at 200 mg/L. The calculated LC_{50} and LC_{90} values were 197.6 and 341.4 mg/L respectively. The $\chi 2$ analysis results showed statistically significance at 5% level ($\chi 2 = 16.0$; $P = 0.00$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 162.5–394.2 mg/L and 263.4–878.2 mg/L respectively. The mean percentage of mortality in water extract was maximum (36.20%) at 200 mg/L concentration. The calculated LC_{50} and LC_{90} values were 276.7 and 499.8 mg/L respectively. The $\chi 2$ analysis results showed statistically significance at 5% level ($\chi 2 = 8.632$; $P = 0.396$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 206.2–804.5mg/L and 347.5–918.7mg/L respectively. After 48 h exposure period of methanol extract, maximum percentage of mortality of 92.22% was observed at 200 mg/L. The calculated LC_{50} and LC_{90} values were 136.4 and 242.6 mg/L respectively. The $\chi 2$ analysis results showed statistically significance at 5% level ($\chi 2 = 14.562$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 112.8–194.8 mg/L and 186.7–352.6 mg/L respectively. The mean percentage of mortality in water extract was maximum (76.64%) at 200 mg/L concentration. The calculated LC_{50} and LC_{90} values were 174.4 and 263.6 mg/L respectively. The $\chi 2$ analysis results showed statistically significance at 5% level ($\chi 2 = 18.162$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 134.6–223.6 mg/L and 223.5–413.9 mg/L respectively.

Table 1: Mean percentage of mortality of third instar larvae of *Culex* mosquitoes after 24, 48, 72 h exposure in *C.citratus* grass extracts. %.

Concentrations (mg/L)	Extracts tested after 24 hours		Extracts tested after 48 hours		Extracts tested after 72 hours	
	Methanol	Water	Methanol	Water	Methanol	Water
50	9.46 ± 2.36	9.46 ± 2.36	10.06 ± 2.41	10.06 ± 2.41	92.52 ± 3.41	10.09 ± 2.18
100	9.46 ± 2.36	9.46 ± 2.36	12.96 ± 4.57	10.06 ± 2.41	92.52 ± 3.41	10.09 ± 2.18
150	11.40 ± 7.27	9.46 ± 2.36	74.41 ± 15.33	31.36 ± 4.62	92.52 ± 3.41	31.66 ± 2.18
200	32.20 ± 8.41	36.20 ± 9.09	92.22 ± 5.26	76.64 ± 4.62	92.52 ± 3.41	84.42 ± 4.16
LC50 (LCL-UCL)	197.6 (162.5–394.2)	276.7 (206.2–804.5)	136.4 (112.8–194.8)	174.4 (134.6–223.6)	116.8 (96.4–127.82)	156.7 (104.4–198.2)
LC90 (LCL-UCL)	341.4 (263.4–878.2)	499.8 (347.5–918.7)	242.6 (186.7–352.6)	263.6 (223.5–413.9)	182.56 (164.2–296.3)	253.1 (212.4–428.8)
$\chi 2$	16.0	8.632	14.562	18.162	15.96	21.124
P value	0.000	0.396	0.006	0.004	0.00	0.00

Values were presented as mean ± SD of three replications.

After 72 h exposure period of methanol extract, maximum percentage of mortality of 92.52% was observed at 200 mg/L. The calculated LC_{50} and LC_{90} values were 116.8 and 182.56 mg/L respectively. The $\chi 2$ analysis results showed statistically significance at 5% level ($\chi 2 = 15.96$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 96.4–127.82mg/L and 164.2–296.3 mg/L respectively. The mean percentage of mortality in water extract was maximum (84.42%) at 200 mg/L concentration. The calculated LC_{50} and LC_{90} values were 156.7 and 253.1 mg/L respectively. The $\chi 2$ analysis results showed statistically significance at 5% level ($\chi 2 = 21.124$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 104.4–198.2 mg/L and 212.4–428.8 mg/L respectively.

Mortality of fourth instar larvae of *Culex* mosquito exposed 24, 48, 72 h in *C. citratus* grass extracts

Mean percentage of mortality of fourth instar larvae of *Culex* mosquitoes exposed to *C.citratus* grass extracts after 24, 48, 72 h exposure period was present in Table 2. After 24 h exposure period results revealed that maximum percentage of mortality of 77.80% was observed in methanol extract, maximum percentage of mortality of 31.64% was observed at 200 mg/L. The calculated LC_{50} and LC_{90} values were 213.6 and 322.8 mg/L respectively. The $\chi 2$ analysis results showed statistically significance at 5% level ($\chi 2 = 14.6$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 136.8–372.4mg/L and 252.6–836.4mg/L respectively. The mean percentage of mortality in water extract was minimum (9.46%) in all the tested concentrations. The range of 95% LCL and UCL of LC_{50} and LC_{90} values was not calculated by SPSS software due to no variation in percentage of mortality.

After 48 h exposure period results revealed that maximum percentage of mortality of 96.74% was observed in methanol extract at 200 mg/L concentration. The calculated LC_{50} and LC_{90} values were 126.4 and 242.6 mg/L respectively. The $\chi 2$ analysis results did not show statistical significance at 5% level ($\chi 2 = 2.24$; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 112.8–194.8 mg/L and 166.6–382.4 mg/L respectively. The mean percentage of mortality in water extract was maximum (66.24%) at 200 mg/L. The calculated LC_{50} and LC_{90} values were 194.6 and 252.4 mg/L respectively. The $\chi 2$ analysis results showed statistical significance at 5% level ($\chi 2 = 10.256$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 141.6–315.2mg/L and 233.6–463.1mg/L respectively. After 72 h exposure period of fourth instar larvae of *Culex* mosquitoes mean percentage of mortality, when exposed to *C.citratus* grass leaves extract results revealed that maximum percentage of mortality of 90.90% was observed in methonal extract in 150 and 200 mg/L concentrations. The calculated LC_{50} and LC_{90} values were 44.6 and 234.6 mg/L respectively. The $\chi 2$ analysis results did not show statistical significance at 5% level ($\chi 2 = 4.56$; $P > 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 6.4–68.8 mg/L and 198.4–292.6 mg/L respectively. The mean percentage of mortality in water extract was maximum (64.76%) at 200 mg/L. The calculated LC_{50} and LC_{90} values were 172.2 and 266.4 mg/L respectively. The $\chi 2$ analysis results showed statistically significance at 5% level ($\chi 2 = 11.42$; $P < 0.05$). The calculated range of 95% LCL and UCL of LC_{50} and LC_{90} value was 134.2–214.4 mg/L and 222.8–386.2 mg/ L respectively.

Table 2: Mean percentage of mortality of fourth instar larvae of *Culex* mosquitoes after 24, 48, 72 h exposure in *C.citratus* grass extracts. %.

Concentrations (mg/L)	Extracts tested after 24 hours		Extracts tested after 48 hours		Extracts tested after 72 hours	
	Methanol	Water	Methanol	Water	Methanol	Water
50	9.46 ± 2.36	9.46 ± 2.36	10.06 ± 2.41	9.46 ± 2.36	46.32 ± 2.66	9.46 ± 2.36
100	9.46 ± 2.36	9.46 ± 2.36	22.96 ± 2.27	9.46 ± 2.36	68.44 ± 3.24	9.46 ± 2.36
150	9.46 ± 2.36	9.46 ± 2.36	56.21 ± 10.22	19.16 ± 2.86	90.90 ± 0.00	34.42 ± 1.98
200	31.64 ± 3.54	9.46 ± 2.36	96.74 ± 2.28	66.24 ± 3.12	90.90 ± 0.00	64.76 ± 2.26
LC50 (LCL-UCL)	213.6 (136.8–372.4)	Not calculated	126.4 (112.8–194.8)	194.6 (141.6–315.2)	44.6 (6.4–68.8)	172.2 (134.2–214.4)
LC90 (LCL-UCL)	322.8 (252.6–836.4)	Not calculated	242.6 (166.6–382.4)	252.4 (233.6–463.1)	234.6 (198.4–292.6)	266.4 (222.8–386.2)
$\chi 2$	14.6	Not calculated	2.24	10.256	4.56	11.42
P value	0.12	Not calculated	0.004	0.001	0.286	0.006

Values were presented as mean ± SD of three replications.

Discussion

Different verities of mosquitoes were multiplies in water habitat, which are nuisance to humans, and also spread various diseases from organism to human and animal. Controls of mosquitoes are vital to reduce the vector-borne disease incidence. *Culex* mosquito are highly irritable and disease causing organisms, which control in the larval stage is worthwhile to minimize the emergence of adult mosquito inhabitants and also easy to handle in small breeding habitats. Those mosquitoes breeding in small ponds,

marshes, ditches, pools, drains, water containers and any other utensils holding water is easily manageable. Synthetic chemicals are proved to be effective, but they cause adverse effects on the environment and human health (WHO, 2016) [17]. In this situation, eco-friendly alternatives are important for safer control of mosquitoes. The phytochemicals from plant origin were proved to be effective due to multiple modes of action (Kasim Roba *et al.,* 2015) [9]. To complement in this research program, solvent extracts of *C.citratus* were tried in the laboratory against immature stage of *Culex* mosquitoes.

In the present study, IIIrd and IVth instars larval mortality was varied significantly. Among the two solvents used, maximum mortality was observed in methanol extract followed by water. The chemical substances of the *C.citratus* may dissolve maximally in methanol extract compared to water. This will guide selection of solvent which is important for grass extraction. The percentage of mortality was varied among the stage of the immature mosquitoes. The maximum percentage of mortality was observed in third instar larvae compared to fourth instar larvae. This will further suggest that before application it is important to monitor the stage of the larvae to determine the concentration of the biopesticides. The percentage of mortality of all the stages of immature mosquitoes was increased in increased concentration and period of exposure. This highlights that selection of appropriate concentration and exposure period is important for maximum benefits of plant extract in mosquito control program. Several studies also reported that dose depended on mortality of mosquito species (Yu *et al*., 2013; Girmay *et al*., 2014; Ramkumar *et al*, 2016; Karthi *et al*, 2020; Prakash *et al*, 2021) [20, 7, 14, 8, 13].

Conclusion

In our study, *C.citratus* plant extracts cause significant mortality on immature *Culex* mosquitoes. These plant parts contain alkaloids, flavonoids, terpenoids, steroids, saponin, quinine and several compounds like limonene, xanthatin, myrcene, xanthinin. Any of these compounds or mixtures may be toxic to the immature *Culex* mosquitoes. These plants are growing well in our country and it can be used to reduce the mosquito population by applying on small man-made breeding places. Further, isolation and characterization of the active molecules will lead to development of novel botanical pesticide for vector control program.

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