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Preliminary Phytochemical Analysis and Larvicidal Property of *Chromolaena odorata* (L.) against *Culex quinquefasciatus*

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Abstract
Mosquitoes are most dangerous insect in the world and they cause many diseases to birds, animals and humans. The control of mosquitoes using plant product is more effectiveness. The current study evaluates the larvicidal bioassay of *Chromolaena odorata* leaf extracts carried out against fourth instar larvae of *Culex quinquefasciatus*. The extracts were obtained from solvent such as Petroleum ether, Chloroform, and Ethanol. Extracts from *C. odorata* were tested for larvicidal activity at levels ranging from 0.625ppm, 1.25 ppm, 2.5 ppm, and 5 ppm. The mortality rate of the larvae was determined 24 hours, 48 hours, and 72 hours. The calculation of LC_{50} value of extracts was calculated after 48hrs exposure. After a 48-hrs exposure, the LC_{50} value of the extracts was calculated. After 48 hrs of incubation, the *C. odorata* Petroleum ether extract showed 100% mortality. *C. quinquefasciatus* was more susceptible to plant extracts. The crude extracts of *C. odorata* found to better results on vector control it may be active phytochemicals present in this extract to be explored and also plant-derived compounds have significant potential in mosquito vectors control programme.

Keywords: *Chromolaena odorata*, *Culex quinquefasciatus*, larvicidal, leaf extracts

Introduction
Mosquitoes are unavoidable insects in terms of its nuisance behavior, known for harmful anthropronootic and zoonootic disease carrier hence denoted as medically important vector. Throughout the World about 3541 species of mosquitoes have been identified and in India 404 mosquito species has been recorded in the country. The mosquitos are categorized in Class: Insecta, Order: Diptera and Family: Culicidae. The mosquito species such as *Aedes*, *Culex*, and Anopheles are the significant genus groups which is responsible for spreading perilous diseases among human population.

Worldwide, vector mosquitoes are increasingly responsible for the transmission of numerous diseases to both humans and animals. The most prevalent insect in the world, the mosquito, is the cause for the majority of human illnesses. Mosquitoes are avowed as “Public enemy number one” stated by WHO (Deepalakshmi and Jeyabalan, 2017) [8]. Mosquitoes consist of forty one genera and approximately 3,500 kinds of mosquitoes are on the world (Azhari *et al*, 2009) [4] and they are acting as vectors of many vertebrates hence act as blood parasites. Mosquito controlling strategies relies upon different life phase of mosquito. Adults are controlled by spraying chemical insecticides, excess usage would results in development of resistance and it also act as a source for delivering hereditary protection to the mosquitoes against these insecticides. Larval phases of mosquitoes are controlled through synthetic larvicides or plant extracts as larvicides (Okumu, 2017) [17].

A herbaceous perennial plant Chromoleana odorata, commonly named Eupatorium odoratum, can be found as tangled, dense bushes (Anyasor *et al*., 2011) [3]. This plant is referred to as a weed and carried by a number of common names, including Siam weed, Devil weed, French weed, and Communist weed (Vaisakh and Pandey, 2012) [24]. *Chromoleana odorata* is known to as "Obuinenawa" by the Igbo and "ewe awolowo" by the Yoruba in Nigeria. Traditional uses of *C. odorata* include a number of therapeutic uses. Plant leaves are extracted and used as a cold and cough medicine, as a hot water bath for skin diseases, and as a general effective treatment for diarrhoea, malaria, fever, toothaches, diabetes, and colonitis. (Phan *et al.*, 2001; Ajao *et al.*, 2011) [18, 1].

According to earlier research, this plant contains several early preliminary phytochemicals including steroids, triterpenes, flavonoids, alkanoids, and essentisal oils. Vaisakh and Pandey, 2012) [24]. Most of these phytochemicals have been reported to act as both anti-oxidants and larvicidal properties (Kishore *et al*. 2011; Rattan, 2010 Albaba *et al*. 2015) [14, 19, 2]. They used plant parts to separate compounds to control mosquitoes. Phytochemical screening is used to identify the secondary metabolites, and they use controlling. Mosquito control by many methods includes larvicidal, pupicidal, ovicidal, adulticidal, and repellent activity. They use many activities to control mosquitoes. They are used to defeat the medically important problems of plant utilization by making plant parts safe, having fewer side effects, being effectively degradable in nature, and reducing and improving the resistance of mosquito vectors (Jeyasankar and Ramar, 2016) [10]. To enhance knowledge about the replacement of chemical pesticides with botanical, the objectives of the current study was to evaluate the preliminiary phytochemicals analyses and the larvividal properties of chromolaena odarata against *Culex quinquefasciatus* with applying different solvent extracts.

Materials and Methods

Plant Extraction: The pristine leaves, *C. odarata*, was collected during the month of January 2023 from Kerampara, Chitturtaluk, Palakkad district, Kerala, India. The plant was dried in shadow at room temperature /relative humidity (27.0 °C and 75±5% RH). Once plant leaves dried, the whole plant material was processed using an electric blender. Powder samples of 100 gram of *C. odorata* were immersed in 250 ml of petroleum ether, chloroform, and ethanol using the maceration method. The sample was then concentrated using a rotary evaporator after being filtered using Whatman's No. 1 filter paper. (Yamoto Scientific Co., Ltd., RE 600, Japan), and then air dried. The concentrations were collected in novel borosilicate vials and kept in the fridge-freezer for utilization in further investigations against significant mosquito vectors.

Preliminary Phytochemical Studies

The leaves extracts of *C. odarata* was analyzed for the occurrence of major phytochemicals like alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, and glycosides according to standard methods.

Mosquito species and vector rearing

The mosquito's larvae of *C. quinquefasciatus* were procured from the NCDC (National Centre for Disease Control) in Mettupalayam. The mosquito species *C. quinquefasciatus* was selected for the present investigation. The collected larvae were reared in plastic plates that contained tap water in a laboratory condition Yeast and dog biscuits were given as initial feeding for the development of later stages. The pupae were taken out from the culture, placed in a container along with tap water, and then placed into the oviposition cage (44 x 44 x 43). Emerging adults were kept in a cage containing a 10% sucrose solution. Early on the third day, breed broiler chickens (*Gallus gallusdomesticus*) were used to provide blood meals to the adults that had emerged. Small plastic dishes for adult oviposition that were lined with filter paper and contained tap water were set inside the cage. During the 14:10 light and dark cycles, the entire setup was kept at 28±2 °C and 70–80% relative humidity (Kamaraj *et al*., 2009) [12].

Larvicidal activity: The fourth-instar larvae of various mosquito species that had just undergone a moult (0–6 hours) were used as samples for the crude extracts, which were made at various concentrations. For the dissolution of plant extracts, 2 drops of Tween 20 were added, and then they were diluted with 100 ml of dechlorinated water to get the desired concentrations. The control was prepared with 2 drops of Polysorbate 20 (Tween 20) in 100 ml of dechlorinated water. 250-ml transparent cups were used for the bioassay, and five replications were maintained.Ten newly moulted fourth-instar mosquito larvae have been introduced at various extract concentrations. The results were observed and recorded after 24 and 48 hours of treatment. The LC50 value was calculated using probit analysis (Finney, 1971). The LC50, LC90, and other statistics chi-square values were calculated by using the statistical package for social science (SPSS) version 16.0 for Windows. The significance level was set at p 0.05.

Corrected mortality

$$= \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \text{X } 100$$

$$\text{Percentage mortality} = \frac{\text{Numbere of dead larvae}}{\text{No of larvea introduced}} \text{X100}$$

Statistical analysis

Using Microsoft Excel 2007, an examination of the profit data was conducted. Lethal concentration (LC50) is characterized as the concentration of the test substance that results in 50% of test organism's mortality during the specified exposure time. By exposing the mosquitoes at various stages of their development to ranging extract concentrations, a solution was identified. By using a probit analysis and SPSS 16.0 (Statistical Package of Social Sciences) software, LC50 and LC90 were estimated along with their fiducial limits at a 95% confidence level based on the test organisms' mortality that was observed in these bioassays. Results with a p value of 0.05 were marked as statistically significant.

Results

Phytochemical analysis: The primary phytochemical analysis was carried out with petroleum ether, chloroform and ethanol extract of *C. odorata*. Investigation on leaf extracts showed the presence of active phytochemical groups such as alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, and glycosides showed positive results in ethanol extracts as showed in Table 1.

Table 1: Preliminary phytochemical screening of *Chromolaena odorata*

S. No	Phytochemicals	Petroleum ether	Chloroform	Ethanol
1	Alkaloids	+	-	+
2	Flavonoids,	+	+	+
3	Tannins	+	+	+
4	Steroids	+	-	+
5	Triterpenoids	+	+	+
6	Saponin	-	-	+
7	Glycosides	-	+	+

+ Presence of compound - Absence of compound

In the larvicidal bioassay, petroleum ether, chloroform, and ethanol crude extracts from *C. odorata* was tested against *C. quinquefasciatus*, and the findings are presented in Table 2-4. The LC_{50} and LC_{90} values for the plant extract at 24 hours, 48 hours, and 72 hours of larval mortality are given in table 2 - 4. Findings of the present results revealed that as the duration of exposure time increases, the rate of mortality also increases,

respectively. Maximum larvicidal activity was observed at 72 hours when compared to 24 hours, which depicts that the plant extract slowly affects the developmental system of larvae. The extract showed larval mortality increasing sequentially from a lower concentration of 25 ppm to a maximum activity of 100 ppm mortality at a higher concentration. Larvae of *C. quinquefasciatus* was more susceptible to the pertroleum ether extract; it showed that 50% mortality was observed at 0.625 ppm at 48 hours and 100% mortality was obtained at 2.5 ppm (LC_{50}) 0.461 (LC_{90}) 0.953.

Table 2: Larvicidal activity of *Chromolaena odorata* against *Culex quinquefasciatus* at 24 hrs exposed

Extracts	Concentration (%)	Larval Mortality (%)	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2 (df=3)
Petroleum Ether	Control	0	7.933	247.2	0.034
	0.625	10			
	1.25	20			
	2.5	30			
	5	40			
Chloroform	Control	0	10.456	404.29	0.288
	0.625	10			
	1.25	20			
	2.5	20			
	5	40			
Ethanol	Control	0	10.456	404.29	0.288
	0.625	10			
	1.25	20			
	2.5	20			
	5	40			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

Table 3: Larvicidal activity of *Chromolaena odorata* against *Culex quinquefasciatus* at 48 hrs. exposed

Extracts	Concentration (%)	Larval Mortality (%)	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2 (df=3)
Petroleum ether	Control	0	0.694	9.496	0.123
	0.625	50			
	1.25	60			
	2.5	80			
	5	90			
Chloroform	Control	0	0.514	254.1	0.084
	0.625	50			
	1.25	60			
	2.5	70			
	5	70			
Ethanol	Control	0	1.234	100.78	0.0479
	0.625	40			
	1.25	50			
	2.5	60			
	5	70			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

Table 4: Larvicidal activity of *Chromolaena odorata* against *Culex quinquefasciatus* at 72 hrs exposed

Extracts	Concentration (%)	Larval Mortality (%)	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2 (df=3)
Petroleum ether	Control	0	0.155	9.191	0.1301
	0.625	70			
	1.25	80			
	2.5	90			
	5	90			
Chloroform	Control	0	0.235	52.296	0.1202
	0.625	60			
	1.25	70			
	2.5	80			
	5	80			
Ethanol	Control	0	0.643	38.745	0.0046
	0.625	50			
	1.25	60			
	2.5	70			
	5	80			

LC_{50}=Lethal Concentration brings out 50% Mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

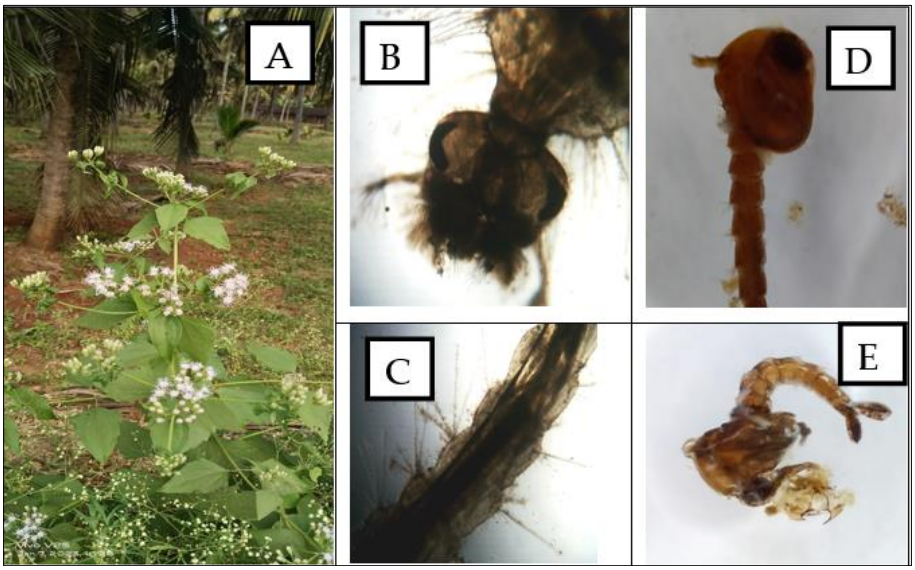

Fig 1: A) Plant, *Chromolaena odorata*, B), C), D) & E): Morphological deformities of *Culex quinquefasciatus* larva and pupa treated with plant extracts

Discussion

The utilization of bio active plant-based products through insecticidal activities has more concerned great awareness from scientists of all over the world. Hence, they are easily eco-friendly in nature and are comparatively safer to mammals and non-target organisms. A widespread review of the flora was undertaken to explore for possible plant crude extracts or bio active compounds that may be used in the management of important human vector mosquitoes. In the present investigation, mosquito species such as *C. quinquefasciatus* were tested for their larvicidal activity. The leaves of *C. odorata* were extracted with petroleum ether, chloroform, and ethanol and tested for mosquitocidal properties against selected mosquito species. Results revealed that as the duration of exposure duration period increases, the rate of mortality also increases. The maximum larval mortality was found in 48 hours. From these findings, *C. quinquefasciatus* was more susceptible to petroleum ether extract when compare to other solvent extracts.

The obtained results are corroborates with earlier reports. The investigation of the larvicidal efficacy of the crude leaf ethyl acetate extract of *T. procumbens* was tested against *Cx.tritaeniorhynchus* showed promising larvicidal activity (Kamaraj *et. al.*, 2011) [13]. Larvicidal activity of acetone, ethyl acetate, chloroform and butanol dried leaf extract of *Meliaazedarach* tested against 3rd instar *Culexquinquefasciatus* and *Aedesaegypti*. The result suggested that the ethyl acetate of *M. azedarach* leaf extract was an excellent larvicidal potential in controlling mosquito vectors. (Ravichandran and Kanayairam, 2014) [20]. Jeyasankar *et al.* (2012) [11] have reported that the ethyl acetate extract of *Phyllanthusemblica*, Exhibited more than 90% larval mortality at 250ppm on *C. quinquefaciatus.*

Efficiency of leaf chloroform extract of *Nyctanthesarbortristis* have been reported with LC50 value of 526.3 780.6ppm (24 hours) and 303.2, 518.2 (48h) for *Ae. Aegypti*and *An. Stephensi* (Mathew *et al.*, 2009) [25]. Previous studies showed that ethanol extracts from fruit endocarps of *Meliaazedarach* and *Azadirachtaindica*, Dash, (2007) [6]. Moreover, ethanolic extracts derived from three species of the Piperaceae (pepper) family, *Piper longum*, (Deepa *et al.*2014) [7]. *P. ribesoides*and *P. sarmentosum* had toxic effect on *Ae. Aegypti*4th instar larvae. Their LC_{50} values ranged from 2.23 to 8.13 ppm (Chaithong *et al*, 2006) [5].

Findings of the present works, it has been concluded that, the selected plant possess mosquitocidal properties against the vector mosquitoes. Thus, hypothesis proposed in the present study is accepted since the hexane extract of *C. odorata* showed significant activities against the mosquito vectors.

Furthermore, generally the phytochemicals are eco-friendly in nature and safer to non-target organisms and the utilization of the selected plant phytochemicals after a thorough screening to the cause by various vector mosquitoes in the near future.

In conclusion, based on the results, plant-derived compounds have significant potential in mosquito vectors control program. The diversified use of plant derived compounds and their active formulation in the mosquito control program of human health importance across the world. Therefore, future research work should be directed towards the practical application of bioactive compounds.

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