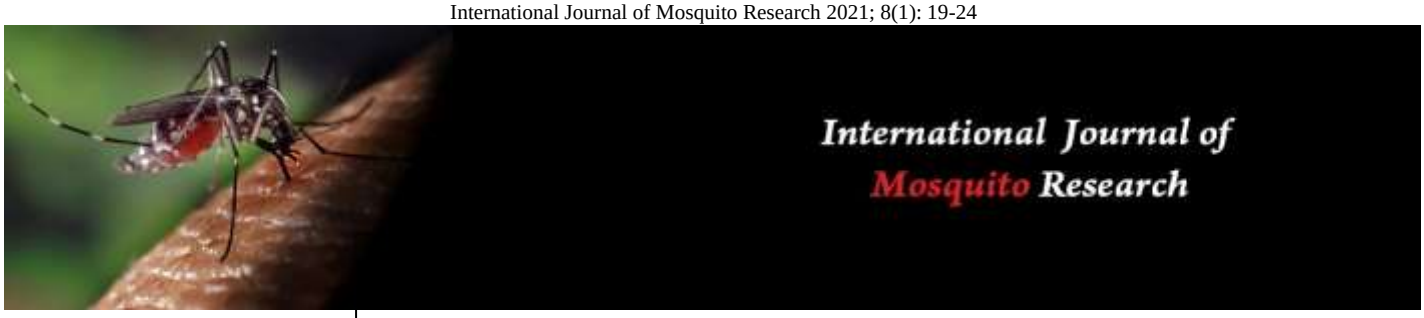

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Sterculia guttata Roxb. As insect growth regulatory agent and its influence on developmental indices of filarial vector, *Culex quinquefasciatus* Say

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Abstract
The extensive and repeated use of synthetic insecticides disrupted natural systems leading to resistance and resurgence in target populations and destruction of non-target beneficial fauna. The present study took an effort to contribute to control or reducing the mosquito population of *Culex quinquefasciatus* Say by employing different column fractions of the methanolic seed extract of *Sterculia guttata* Roxb. The higher dose treatments caused mortality in a dose-dependent manner and it also produced various growth inhibiting effects on the developmental stages when, exposed at lower dose treatments. MeOH: EA-4:1 column fraction of the methanol extract of *S. guttata* exhibited significant insect growth regulatory activities on *Cx. quinquefasciatus* as can be evidenced from the effective interference with the life cycle stages of the filarial vector.

Keywords: *Sterculia guttata*, *Culex quinquefasciatus*, filarial vector, juvenile hormone analogue and insect growth regulators (IGRs)

1. Introduction
Insecticides are commonly used in the field of agricultural, industrial and public health applications and are classified based on their structure and mode of action. Owing to many reasons, insecticides obtained from plants and plant derived products attract more attention of Integrated Vector Management (IVM) programs. The vector-borne infectious diseases contribute the major fraction of the global infectious disease burden, nearly half of the World's population gets infected with at least one type of vector-borne disease [1]. These diseases profoundly restrict socio-economic status and development in countries with the highest rates of infection; many of which are located in the tropics and subtropics.
In the 20th century, large scale vector control programs successfully brought vector-borne disease transmissions under control over huge areas by the use of broad spectrum conventional/synthetic insecticides. However, these chemical controls have not generally produced sustainable control of vector population and it also generates various environmental issues. Besides all these conventional practices, control agents with features like more selective, less harmful and more compatible with biological controls and Insect Growth Regulator (IGRs) effects have become an attractive alternative to broad-spectrum insecticides. Scientific literature provides the information only about the efficacy of the synthetic IGRs, so attention need to be focused on botanical IGRs. Treatment with *Ipomea carnea* extract influence the growth and development of *Culex quinquefasciatus* and *Anopheles stephensi* by reducing the growth index [2]. Morphogenetic abnormalities are reported in *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* when treated with different plant based extracts [3, 4, 5].

2. Materials and Methods
2.1 Collection and Extraction of plant material
Seeds of *Sterculia guttata* Roxb. Were collected from in and around Thalassery, Kerala, India. Collected plants were taxonomically identified by the experts from the Department of Botany, University of Calicut. The seeds of *S. guttata* were washed with dechlorinated water, shade dried under room temperature, powdered and was extracted with methanol using Soxhlet

apparatus. The extracted content was then subjected to rotary vacuum evaporation until the solvents were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained was stored at 4 ºC. Crude extract was dissolved in appropriate solvents for making 1% stock solution. From the stock solution, different desired concentrations were prepared and used for bioassays on larval instars of *Cx. quinquefasciatus*.

2.2 Maintenance of Laboratory Culture of *Cx. quinquefasciatus*

Culex quinquefasciatus larvae collected from the open drains of Kalluthan Kadavu Colony, Calicut, were brought to the laboratory and maintained at 27±2 °C and 75-85% relative humidity. The larvae were kept in plastic or enamel trays containing tap water and fed with a diet of fine powder of dog biscuits and Brewer's yeast in the ratio of 3: 1 respectively. The pupae were kept inside standard emergence cages and after emergence the mosquitoes were identified and species confirmed before rearing. The adults were fed with 10% sucrose solution and an additional blood meal was provided (immobilized quail) to adult females in order to facilitate the development of egg. A bowl containing water was kept in the emergence cages to facilitate oviposition. The eggs laid were removed from the cage and after hatching, the larvae were reared in the laboratory at room temperature.

2.3 Larvicidal Bioassay

Bioassay for the estimation of larvicidal activity using WHO protocol [6] with slight modifications was adopted for the study. Larvicidal bioassay of selected plant extracts was conducted against freshly hatched first instar larvae of *Cx. quinquefasciatus* using desired concentrations of the plant extract.

2.4 Fractionation of the crude extract using Column Chromatography

Column chromatography was conducted according to the protocol of Harwood and Moody [7], with slight modifications. Column of size 50cm x 2.5cm were used for the study. The column was eluted with different combinations of solvents like Methanol: Ethyl acetate (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) with increasing polarity respectively. The coloured bands/different fractions travel down the column as the compound was eluted. The different column fractions were eluted and evaporated to dryness, prepared 1% stock solution and stored in the refrigerator.

2.5 Developmental Indices

Selected Column fractions of the different plant extracts were tested for Insect Growth Regulating (IGR) activity according to the protocol of Mehdi *et al*. [8] with slight modifications against freshly hatched I instar larvae of *Cx. quinquefasciatus.* Desired sub lethal concentrations of the different column fractions of the selected plant extract of *S. guttata* (MeOH: EA-4:1) was tested for the estimation of Effective concentration (EC_{50}) to allow emergence of 50% of the treated larval population. Triplicates and controls were also maintained for each experiment. For the accurate determination of IGR activity, extension of total developmental duration, deformities and mortality rates were observed and recorded every day until adult emergence.

2.6 Data Management and Statistical analysis

Mortality was corrected according to Abbott's formula [9]. The LC_{50} values and Chi- square tests were calculated according to Finney's Probit analysis [10]. P value was calculated using t-test and $p<0.05$ were considered to be statistically significant.

3. Results

3.1 Larvicidal Bioassays

The results of the initial assay of extracts showed that crude methanolic extract of *S. guttata* induced 96.67% mortality at 100ppm (table 1). On the basis of preliminary screening on larvicidal toxicity, 24 hr LC_{50} and LC_{90} of *S. guttata* seed is 24.02 ppm and 73.8ppm respectively (table 1). Positive correlation was found between the concentrations and percentage larval mortality in methanolic extract of *S. guttata.* The Coefficient of determination (R^2) was 89.5% and 98.9% respectively i.e., more than 89% and 98% of the larval mortality could be explained by the independent concentrations (figure 1).

3.2 Effect of different Column fractions of selected plant extracts on I instar larvae of *Culex quinquefasciatus*

Apart from the nine gradients of methanol: Ethyl acetate (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) column fractions of *S. guttata*, Methanol: Ethyl acetate (4:1) was the only fraction which produced 80.00% adult emergence with concentrations of 1ppm. Treatment with 0.1, 0.3, 0.5, 10 and 50 ppm of the Methanol: Ethyl acetate (4:1) column fraction of *S. guttata* on I instar larvae of *Cx. quinquefasciatus* provided 20.00±0.0, 36.67±6.67, 53.33±3.33, 70.0±0.0 and 90.00±0.0 per cent mortality respectively after 24 hrs of exposure (table 2). 24 hrs LC_{50} and LC_{90} and associated statistics of the column fractions of the selected plants extracts are provided in table 3. 24 hr LC_{50} and LC_{90} of Methanol: Ethyl acetate, 4:1 column fraction of *Sterculia guttata* is 24.25ppm and 94.162ppm respectively.

3.3 Percent emergence of the first instar larvae of *Culex quinquefasciatus* using different Column fractions of the selected plant extracts

The percent emergence of *Cx. quinquefasciatus* after treatment with 1, 5, 20, 40 and 60 ppm of the methanol: Ethyl acetate 4:1 column fraction of *Sterculia guttata* is 80.00±5.77, 53.33±3.33, 40.00±5.77, 10.00±0.0 and 0.0±0.0% respectively (table 4). There was 100% emergence recorded in the control set.

3.4 Insect growth regulating activity

The experimental results revealed that the selected plant extract could induce prolonged larval and pupal periods, when compared to control (Table 5). Larval duration significantly increased in the treatment of *S. guttata* (MeOH: EA-4:1) to 17 days and total developmental period extended to 21.33±0.33 days at 6.419ppm concentration. Whereas in control, total developmental duration was 13.67±0.33 days and larval duration was 10.67±0.33 days.

3.5 Morphogenetic deformities associated with *Cx. quinquefasciatus* larvae exposed to selected plant materials

It was observed that the treatment with column fraction (MeOH: EA- 4:1) of *S. guttata* exhibited 15.34% larval-larval intermediates, 12.00% larval-pupal intermediates and 2.66%

pupal-adult intermediates. Microscopic examination of dead larvae revealed certain morphological deformities like sclerotization of larval cuticle, which may be characteristic feature of pupal cuticle. Changes in morphological features of larval-larval intermediates consists ecdysal suture. Even though, the presence of ecdysial suture, the larvae could not molt into next instar **(**plates 1, 2 & 3**)**. The dead pupa on the other hand, showed a variety of malformations like partial demelanisation with straight abdomen of larval-pupal intermediates (plate 4) and partly emerged pupae with attached head capsule as pupal-adult intermediates (plates 5 & 6) and melanized pupae with straight abdomen and head capsule with antennae, which is a characteristic feature of adult mosquitoes.

4. Discussion

Recently the mosquito control programs using synthetic insecticides is facing set back due to the development of resistance in mosquitoes. Phytochemicals are considered to be suitable alternatives to synthetic insecticides as they are comparatively inexpensive, safe and are readily available around the globe [11]. The larvicidal efficacies of the plant *Sterculia guttata* in mosquitoes have not been well established as a plant species with insecticidal properties. Present study investigated the larvicidal efficacies of separately extracted crude methanolic extracts of the seeds of *S. guttata* on I instar larvae of *Cx. Quinquefasciatus* and found to possess promising larvicidal activity with LC_{50} value of 24.02ppm for Crude methanolic extract after 24hrs of exposure. The plant extracts caused larval mortality in a dose-dependent manner and was above 89% for crude methanolic extracts. The percentage of larval mortality was found to be significantly different ($P < 0.05$) from that of control and untreated groups. The larvicidal activity of seeds of *S. guttata* was also comparable with different solvent extracts on different species of mosquitoes [12]. The results obtained from the larvicidal bioassays using column fractions of crude methanolic extracts of *S. guttata*, revealed that only the fraction of Methanol: Ethyl acetate-4:1 produced above 80% adult emergence from the treated larvae of *Cx. quinquefasciatus*. Median lethal dose of *S. guttata* (MeOH: EA-4:1) column fraction exhibited 91.2% adult emergence with EC_{50} value 12.84ppm**.**

Despite the toxic effects and reduced adult emergence, IGRs exhibited combined effects on the developmental period and adult emergence with extended developmental duration of exposed larvae [13]. Present study also evidences the proof of IGR activity exerted by the plant extracts on *Cx. quinquefasciatus* based on the developmental process. During the developmental progress of *Cx. quinquefasciatus* exposed to selected plant extract exhibited prolonged larval and pupal periods, when compared with the control. *S. guttata* showed significant changes in the larval duration with the total developmental period extending up to 21.33± 0.33 days.

The current results are comparable with other research findings, as it produced growth regulatory responses in *Cx. quinquefasciatus.* The treatment of three indigenous plant extracts *Mimusops elengi*, *Pongamia pinnata* and *Erythrina variegata* [14] produced growth regulatory responses in the dengue vector *Aedes albopictus* Skuse. At sublethal doses of 50ppm and 100ppm of crude methanolic extract of *Ageratum conyzoides* on *Anopheles gambiae* sensu stricto and *Anopheles arabiensis* greatly affected the development in the immature stages such as prolongation of larval instar stages, pupal durations, inhibition of larval and pupal molting, morphological abnormalities and mortality especially during molting and melanization processes [15]. According to Kuwano *et al.* [16] pyriproxifen extended the length of larval developmental period, which may be due to the increased presence of JH in the haemolymph of insect larval stage. Mwangi and Mukiama [17] recorded an extension of larval periods and inhibition of pupal duration in *An. Arabiensis* treated with extracts from the plant *Melia volkensii.*

Apart from IGR activity, the tested plant extract produced adult emergence inhibition activity in *Cx. quinquefasciatus*. The sub-lethal dose treatments inhibited growth and caused mortality in a dose-dependent manner and also induced growth inhibiting effects on various developmental stages. Furthermore, the developmental progress was affected by showing several deformities including moulting inhibition, morphological abnormalities and mortality at the time of moulting and melanisation processes. Deformities that developed in the treatment of *S. guttata* (MeOH: EA-4:1), attributed to the dechitinizing effect of extracts with the formation of Larval-larval intermediates, larval-pupal intermediates and Pupal-adult intermediates. Sub-lethal doses of *S. guttata* (MeOH: EA-4:1) resulted in appearance of highest number of larval-larval intermediates, larval-pupal intermediates and lowest number of pupal-adult intermediates (Plate 1 to 6). In the case of larval-larval intermediates, death has occurred with molting sutures on head of the larvae, inhibiting the molting into next instars whereas, the formation of larval-pupal intermediates resulted in the death of treated larvae at an early stage of pupation. Based on aforementioned data, the column fractions of *S. guttata* (MeOH: EA-4:1) seems to have IGR activity, as it caused more profound harmful effects in larvae and pupae during molting.

5. Conclusion

From the findings, it is clear that the column fractionated seed extracts of *Sterculia guttata* (MeOH: EA-4:1), might be acting as JH analogue and as well as ecdysone agonist, as it disrupts the metamorphosis or normal developmental processes in *Cx. quinquefasciatus*. Moreover, the crude methanolic extract also showed comparable larvicidal effects on larvae of *Cx quinquefasciatus*. Since a large population was threatened by the filarial vector *Cx. quinquefasciatus*, the results of the present study using plant derived compounds could help to control these vector mosquitoes and offer immense scope for those plant derived compounds to be utilized in integrated vector management programs as a better alternative to rather expensive and environmentally hazardous synthetic insecticides.

Table 1: Percent mortality observed after 24hrs treatment with the Crude Methanol extract of *Sterculia guttata* tested against the first instar larvae of *Cx. quinquefasciatus*

SI No	Extracts	Concentration (ppm)	Mortality (%)	Corrected %	P- Value	Controls	
						Methanol	Water
1	Methanol	5	23.33 ± 3.33	23.33	0.0001*	0.00 ± 0.00	0.00 ± 0.00
2		10	50.00 ±5.77	50.00			
3		50	73.33 ± 3.33	73.33			
4		70	90.00 ± 5.77	90.00			
5		100	96.67 ± 3.33	96.67			
6	Estimated 24 hrs LC_{50} (LC_{90}) ppm				24.02 (73.8)		

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.
The values are expressed as mean ± SD for 10 animals (n=10) per group
*Significant at $P<0.05$ with Control experiments.

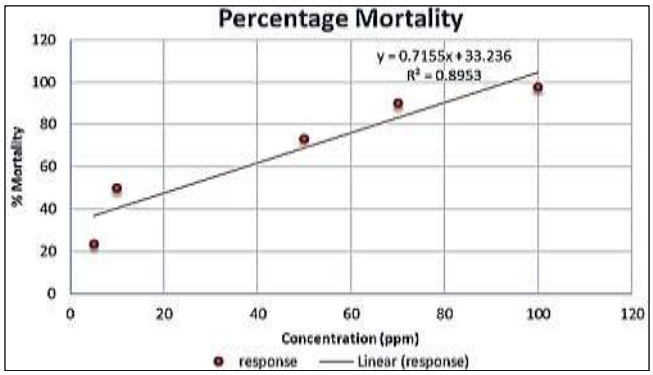

Fig 1: Correlation and Regression analysis of Percentage mortality of methanol extract of *Sterculia guttata* against the first instar larvae of *Culex quinquefasciatus*

Table 2: Percent mortality of column gradients of *S. guttata* (Methanol: Ethyl acetate, 4:1) tested against the first instar larvae of *Culex quinquefasciatus*

SI No.	Concentration (ppm)	Mortality (Mean± SE)	P- Value
1	0.1	20.00±0.00	0.0056*
2	0.3	36.67±6.67	
3	0.5	53.33±3.33	
4	10	70.00 ±0.00	
5	50	90.00 ±0.00	
6	Control	0.00±0.00	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
*Statistically Significant at $P<0.05$ with Control experiment.

Table 3: 24 hr LC_{50} and LC_{90} (ppm) and associated statistics of Methanol: Ethyl acetate, 4:1 column fraction of *Sterculia guttata* tested against I instar larvae of *Cx. quinquefasciatus*

Plant/Column fraction	24 hrs LC_{50} (LC_{90})	Lower Fiducial Limit (LFL)	Upper Fiducial Limit (LFL)	X^2	Regression	Significance
S. guttata Methanol: Ethyl acetate (4:1)	24.254 (94.162)	-16.467 (58.973)	60.009 (317.420)	16.479	y= 1.111x + 38.65 R^2= 0.691	0.001*

*Statistically Significant at $P<0.05$

Table 4: Data on percent emergence of the column fraction Methanol: Ethyl acetate, 4:1 of *Sterculia guttata* seed extract. The experiment commenced from the first instar and observations were made till emergence

Concentration (ppm)	Emergence (Mean±SE)	Corrected % (Mortality)	EC_{50} (ppm) (U L-LL)	EC_{90} (ppm) U L-LL	X^2	Regression Equation
1.0	80.00±5.77	20.00	12.838 (21.960-1.899)	-12.769 (-0.817-47.932)	11.047	y= -1.254x + 68.26 R^2= 0.912
5.0	53.33±3.33	46.67				
20	40.00±5.77	60.00				
40	10.0 ± 0.00	90.00				
60	0.00 ±0.00	100.00				
Control (+ve)	93.33±3.33	6.67				
Control (-ve)	96.67±0.00	3.33				

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
*Significant at $P<0.05$.

Table 5: Data on larval and total developmental duration of *Cx. quinquefasciatus* when treated with different concentrations of column fraction of the selected plant extract

SI No.	Name of the plant/ Column gradient	Conc. (ppm)	Extension of larval duration (days)				Extension of Pupal duration (days)	Total developmental duration (days)	P Value
			I instar	II instar	III instar	IV instar			
1.	*S. guttata* MeOH: EA- 4:1	6.419	4.33± 0.33	4.00±0.58	4.00±0	4.67±0.33	4.33±0.88	21.33± 0.33	0.0001*
	Control	----	2.67± 0.33	3.00±0	2.33±0.33	2.67±0.00	3.00± 0	13.67±0.33	

Note: Number of individuals per sample= 50
*Significant at the level $P<0.005$

Morphogenetic deformities associated with the exposure of column fractionated seed extract of *S. guttata* (M: EA- 4:1) on freshly hatched I instars larvae of *Cx. quinquefasciatus* at half of the median lethal dose (6.419ppm) treatment (Plates- 1, 2, 3, 4, 5 & 6).

Plate 1: Larval-larval intermediates

Plate 2: Larval-larval intermediates

(Larval moulting arrested with ecdysial suture)

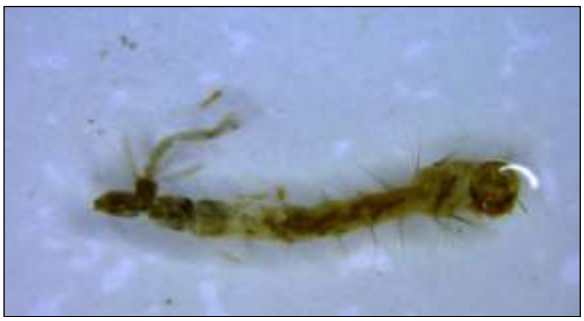

Plate 3: Deformed larva with demelanized abdomen

Plate 4: Larval-pupal intermediate (Pupa with straight abdomen)

Plate 5: Pupal-adult intermediate (Partially emerged adult with attached head capsule with antennae)

Plate 6: Pupal-adult intermediate (Partially developed adult with head capsule enclosed within the pupal case)

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