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Larvicidal activity of *Gmelina asiatica* L. leaf extracts against *Aedes aegypti* and *Culex quinquefasciatus*

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ABSTRACT

Mosquitoes are responsible for the spread of many dreadful human diseases. Phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides. To determine the larvicidal activities of leaf extracts of *Gmelina asiatica* in different solvents against mosquito vector, *Aedes aegypti* and *Culex quinquefasciatus*. Larvicidal efficacy of the crude leaf extracts of *G. asiatica* with four different solvents such as petroleum ether, chloroform, ethanol and acetone were tested against the five day old mosquitoes (*A. aegypti* and *C. quinquefasciatus*). The larvae mortality and the larvicidal efficacy were determined against the mosquito species with different concentrations (ranging from 10-100ppm) under laboratory conditions. Among the tested solvents, maximum efficacy was observed in the chloroform extract of both types of mosquitoes. The LC_{50} , LC_{90} and LC_{95} values of *G. asiatica* against the larvae of *A. aegypti* were 47.32, 81.03 and 86.90 ppm (petroleum ether) 37.39, 64.29 and 69.02 ppm (chloroform) 38.15, 71.71 and 78.31 ppm (ethanol) 45.88, 79.66 and 86.43 ppm (acetone) extracts and the values of *C. quinquefasciatus* were 36.22, 71.93 and 78.69 ppm (petroleum ether) 19.51, 53.51 and 63.09 ppm (chloroform) 39.52, 77.48 and 84.45 ppm (ethanol) 50.98, 85.66 and 89.53 ppm (acetone) extracts respectively. No mortality was observed in controls. These results suggested that *G. asiatica* leaf extract possess larvicidal potential to be used as an ecofriendly approach for the control of vectors *A. aegypti* and *C. quinquefasciatus* and the study provides the first report on the larvicidal activity of *Aedes* and *Culex* mosquitoes.

Keywords: *Gmelina asiatica*, *Aedes aegypti*, *Culex quinquefasciatus*, Larvicide, Crude extracts.

INTRODUCTION

Vector-borne diseases constitute the major cause of morbidity in most of the tropical and subtropical countries and have always been a challenge to the medical professionals struggling for the welfare of humanity [1]. Mosquitoes are the major arthropod vectors causing dreadful and fatal diseases such as dengue, malaria, yellow fever, filariasis, Japanese encephalitis, chikungunya and create allergic responses to humans which include local skin and systemic reactions such as angioedema [2].

In India, malaria is one of the most important causes of direct or indirect infant and adult mortality with approximately 2 or 3 million new cases arising each year, is transmitted by mosquitoes [3]. Control of such mosquito borne disease is becoming more difficult because of increasing resistance to pesticides, lack of vaccines, suitable drugs etc. Biological control at the larval stage of development of mosquitoes is one of the cheap, ecofriendly treatments of malaria control. One of the methods to control the vectors in order to bring interruption in disease transmission, and the control of mosquitoes in larval stage has been efficient way in the integrated vector

management [4]. The herbal medicines are used as larvicides, they are efficient and do not harm other organism in the environment [5,6]. Phytochemicals from plants, especially from leaves and oils are severe larvicidal effect [7,8]. Among them *Gmelina asiatica* L. (Verbenaceae) is a temperate plant; popularly known as 'Nilakkumil' in Tamil and Asian Bushbeech in English, is a large straggling shrub found in peninsular India [9]. The whole plant of *G. asiatica* is medicinally important and many reports claim to cure several diseases. The aerial parts and roots of this plant has been used to anticancer [10] antibacterial [11] anti-inflammatory [12] antidiabetic [13] nematocidal [14] antioxidant and hepatoprotective [15] properties. Hence the medicinal plant *G. asiatica* selected to study the larvicidal effect against *A. aegypti* and *C. quinquefasciatus* vector.

MATERIALS AND METHODS

Collection and extraction of plant materials

Leaves of *G. asiatica* were collected from Scott Christian College Campus, Nagercoil, Kanyakumari District, South Tamilnadu, India. The freshly collected healthy mature leaves are thoroughly washed with distilled water and kept in shade at room temperature for about two weeks to dry. They were made into powdered with the help of a mechanical grinder and sieved. The dried powdered sample (100g) of *G. asiatica* was extracted with 1000 mL of solvents such as petroleum ether, chloroform, ethanol and acetone by a Soxhlet apparatus separately. The resultant filtrate was concentrated in powdered form by evaporation of the solvents using Rotary evaporator. The solid residue was designated as the extract, which was stored in a refrigerator at 4°C until further analyses.

Collection and maintenance of eggs

The eggs of *Aedes aegypti* and *Culex quinquefasciatus* were collected from the Centre for Research in Medical Entomology (ICMR), Madurai, Tamilnadu, India. The collected eggs were brought to the laboratory and transferred to 18 × 13 × 4 cm size enamel trays containing 500 mL water and kept for hatching. The freshly hatched larvae were fed with dog biscuits and yeasts in 3:1 ratio. They were added to the culture medium 24 h before adding the eggs. The feeding was continued till it reached the pupal stage. Homogenous population of larvae was produced (5days old and 5 mm in length) from five to seven days later.

Larvicidal bioassay

Three trials were carried out against *A. aegypti* and *C. quinquefasciatus* [16]. Toxicity assays of the crude extract was conducted separately using the third instar larvae of *A. aegypti* and *C. quinquefasciatus*. Stock solution (1000ppm) was prepared by dissolving 100 mg of crude extract in 1 mL DMSO and volume raised to 100 mL with distilled water. From this, different dilutions of 10-100 ppm were prepared in 200 mL deionized water in 250 mL beaker and third instar larvae 20 numbers were released in it and mortality was scored after 24 h. The beakers were kept in room temperature and the larvae were exposed to 200 mL water containing 0.1 mL of DMSO which served as control. The larvae in each solution were then left for 24 h and the number of dead larvae was counted after, and the percentage mortality was calculated by Abbott's formula [17].

$$\text{Morality(\%)} = \frac{\% \text{ mortality treated group} - \% \text{ mortality control group}}{\% \text{ mortality control group}} \times 100$$

Statistical Analysis

Three replicates of each sample were used for statistical analysis and the values are reported as mean ± standard deviation (SD). The percentage of mortality values for the third instar larvae *A. aegypti* and *C. quinquefasciatus* treated with various concentrations (ranging from 10 to 100 ppm) of the leaf extract of *G. asiatica* was recorded and the percentage mortality was calculated and the data was analyzed using curve expert software for finding the LC₅₀, LC₉₀ and LC₉₅ values. The third degree polynomial fit was used as a suitable mathematic model in the curve expert software.

RESULTS

Larvicidal activities of plant extracts were investigated in the laboratory, against the mosquito species *A. aegypti* and *C. quinquefasciatus*. The efficacy of *G. asiatica* leaf extract on the third instar larvae of *A. aegypti* and *C. quinquefasciatus* is given in Table 1. The larvicidal effect of the plant extract was clearly depending on the concentration of the extracts. All the larvae maintained in the control medium survived for 24 h, thus no mortality was observed in the control. The percentage mortality values of 3rd instar larvae of *A. aegypti* treated with different

concentrations (ranging from 10 to 100 ppm) of the plant extract of *G. asiatica* after 24h exposure. Chloroform extracts of *G. asiatica* showed 100% mortality at 70 ppm. Ethanolic extracts of *G. asiatica* showed 100% mortality at 80 ppm whereas petroleum ether and acetone extracts of *G. asiatica* showed 100% mortality at 90 ppm.

The regression equation (based on the probit analysis), the concentration of various extracts against 3rd instar larvae of *A. aegypti* and *C. quinquefasciatus* after 24 h exposure are shown in Table 1. The correlation coefficient (r^2) of petroleum ether, chloroform, ethanol and acetone extracts were 0.9516, 0.9558, 0.9455 and 0.9549 and their relationship were expressed as $Y = -2.21$, $Y = -2.15$, $Y = -3.22$ and $Y = -1.63$ respectively against the larvae of *A. aegypti*. The LC_{50} , LC_{90} and LC_{95} were recorded as 47.32ppm, 81.03ppm and 86.90ppm for petroleum ether extract; 37.39ppm, 64.29ppm and 69.02ppm for chloroform extract; 38.15ppm, 71.71ppm and 78.31ppm for ethanol extract and 45.88ppm, 79.66ppm and 86.43ppm for acetone extract respectively (Table 2). Experimental results showed that chloroform extracts of *G. asiatica* leaves were highly effective against third instar larvae of *A. aegypti*.

Table 1. Mortality of 3rd instar larvae of *Aedes aegypti* exposed for 24h in different concentrations of *G. asiatica* leaf extract

Solvents	Concentration of the extract (ppm)										
	Control	10	20	30	40	50	60	70	80	90	100
Petroleum ether	0	20	33	41	53	61	73	95	100	100	100
Chloroform	0	35	53	66	75	85	95	100	100	100	100
Ethanol	0	18	30	40	41	63	73	85	93	100	100
Acetone	0	16	20	26	31	48	58	71	90	100	100

Table 2. Lethal concentration of leaf extracts of *G. asiatica* against *Aedes aegypti*

Sl. No.	Solvents	LC_{50} (ppm)	LC_{90} (ppm)	LC_{95} (ppm)	Regression analysis	R	R^2	SE
1	Petroleum ether	47.32	81.03	86.90	$Y = -2.21 + 5.44x$	0.975	0.951	8.47
2	Chloroform	37.39	64.29	69.02	$Y = -2.15 + 8.17x$	0.977	0.955	8.64
3	Ethanol	38.15	71.71	78.31	$Y = -3.22 + 1.22x$	0.972	0.945	9.18
4	Acetone	45.88	79.66	86.43	$Y = -1.63 + 5.94x$	0.977	0.954	8.21

In the present work, 3rd instar larvae of *C. quinquefasciatus* in all the experimental cohorts exposed to organic solvent extracts exhibited effective mortality for all the extracts. The mortality rate was observed as dose dependent with different concentration (ranging from 10 to 100ppm) of the plant extract of *G. asiatica* after 24h exposure. Petroleum ether extracts of *G. asiatica* showed 100% mortality at 80ppm. Chloroform extracts of *G. asiatica* showed 100% mortality at 70ppm whereas ethanolic and acetone extracts of *G. asiatica* showed 100% mortality at 90ppm (Table 3).

The correlation coefficient (r^2) for petroleum ether, chloroform, ethanol and acetone extract was 0.9586, 0.9747, 0.9547 and 0.9619 their relationship can be expressed as $Y = -4.14$, $Y = -3.64$, $Y = -3.62$ and $Y = -5.23$ respectively against the mosquito larvae of *C. quinquefasciatus*. The LC_{50} , LC_{90} and LC_{95} was recorded as 36.22ppm, 71.93ppm and 78.69ppm for petroleum ether extract; 19.51ppm, 53.51ppm and 63.09ppm for chloroform extract; 39.52ppm, 77.48ppm and 84.45ppm for ethanol extract; 50.98ppm, 85.66ppm and 89.53ppm for acetone extract respectively (Table. 4). It was clear from the results that, among the four solvents tested chloroform extract showed maximum toxic effect against the third instar larvae of *C. quinquefasciatus*.

Table 3. Mortality of 3rd instar larvae of *Culex quinquefasciatus* exposed for 24h in different concentrations of *Gmelina asiatica* leaf extract

Solvents	Concentration of the extract (ppm)										
	Control	10	20	30	40	50	60	70	80	90	100
Petroleum ether	0	20	33	41	53	61	73	95	100	100	100
Chloroform	0	35	53	66	75	85	95	100	100	100	100
Ethanol	0	18	30	40	41	63	73	85	93	100	100
Acetone	0	16	20	26	31	48	58	71	90	100	100

Fig 1. Polynomial fit showing the effect of petroleum ether extract of *Gmelina asiatica* on the mortality of *Aedes aegypti* larvae

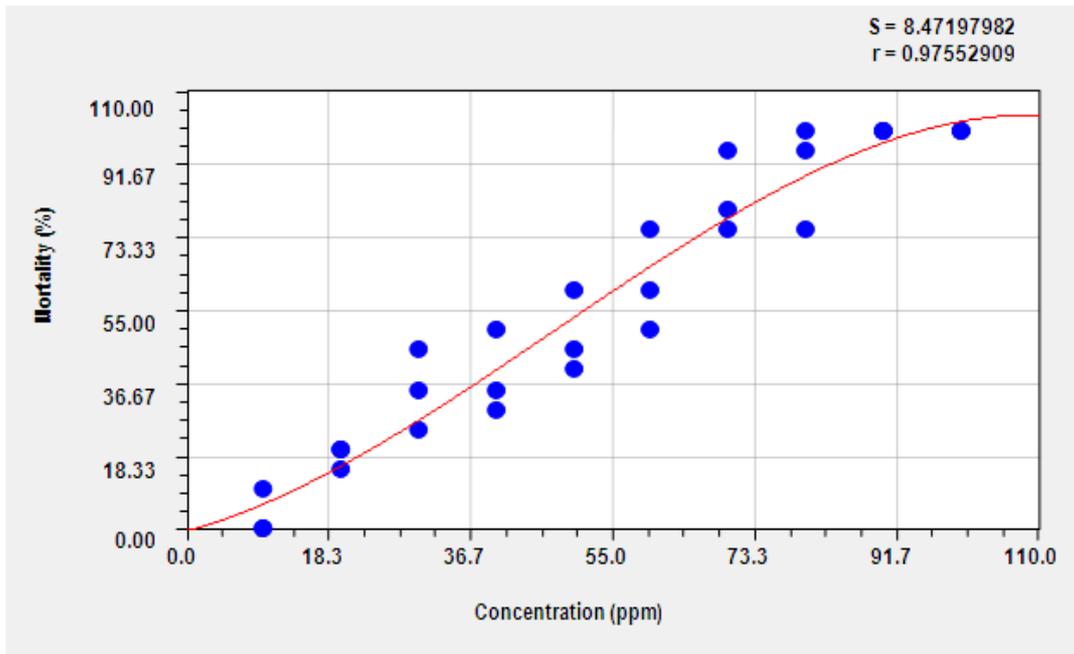


Fig 2. Polynomial fit showing the effect of chloroform extract of *Gmelina asiatica* on the mortality of *Aedes aegypti* larvae

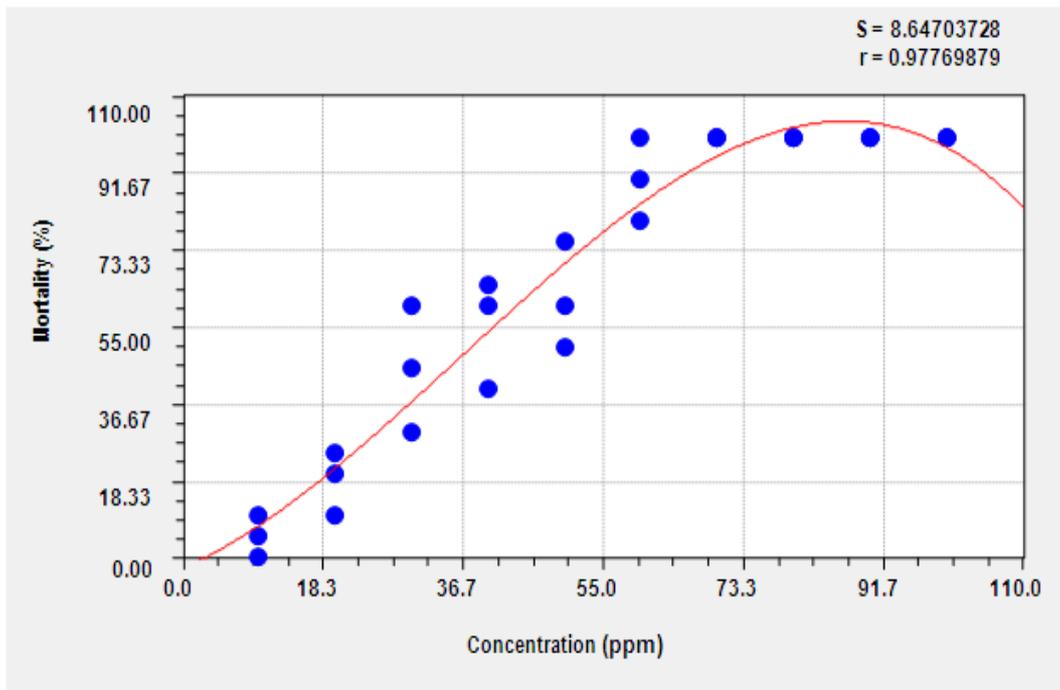


Table 4. Lethal concentration of leaf extracts of *Gmelina asiatica* against *Culex quinquefasciatus*

Sl. No.	Solvents	LC ₅₀	LC ₉₀	LC ₉₅	Regression analysis	R	R ²	SE
1	P. ether	36.22	71.93	78.69	Y= -4.14+1.10x	0.979	0.958	7.40
2	Chloroform	19.51	53.51	63.09	Y= -3.64+2.91x	0.987	0.974	5.34
3	Ethanol	39.52	77.48	84.45	Y= -3.62+1.01x	0.977	0.954	7.65
4	Acetone	50.98	85.66	89.53	Y= -5.23+3.15x	0.980	0.961	7.10

Fig 3. Polynomial fit showing the effect of ethanolic extract of *Gmelina asiatica* on the mortality of *Aedes aegypti* larvae

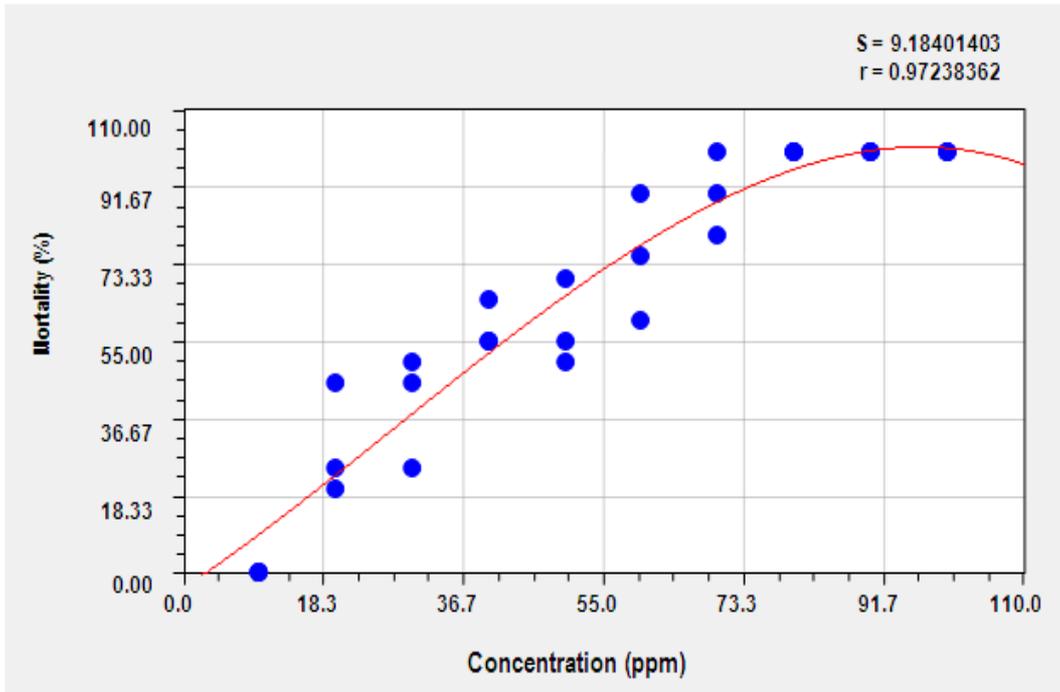


Fig 4. Polynomial fit showing the effect of acetone extract of *Gmelina asiatica* on the mortality of *Aedes aegypti* larvae

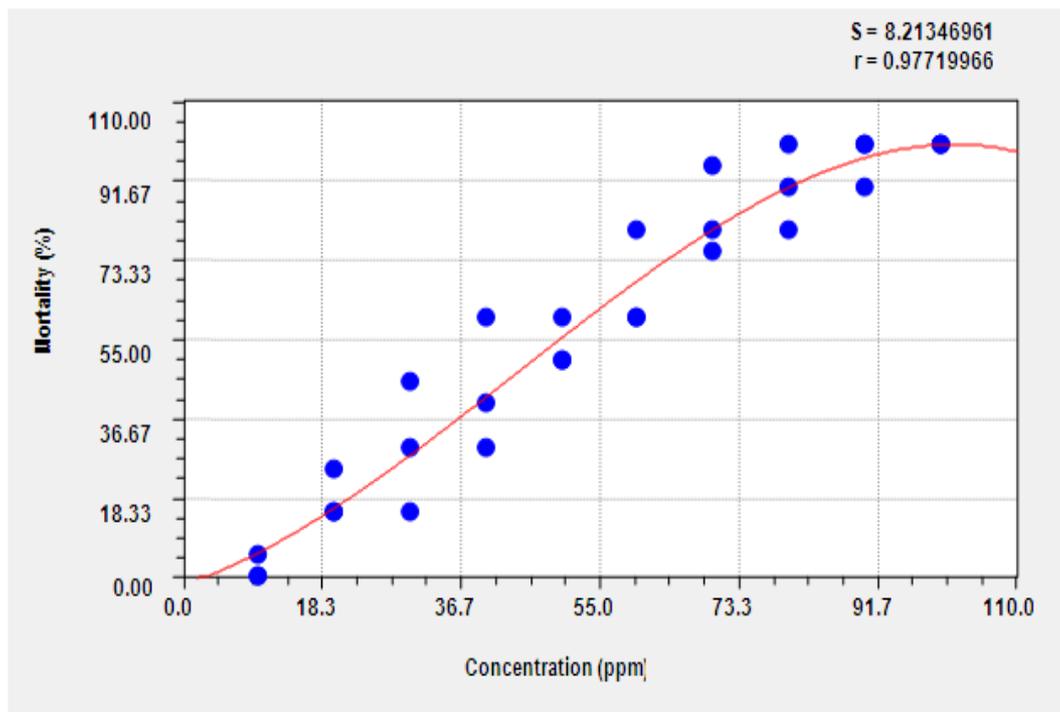


Fig 5. Polynomial fit showing the effect of petroleum ether extract of *Gmelina asiatica* on the mortality of *Culex quinquefasciatus* larvae

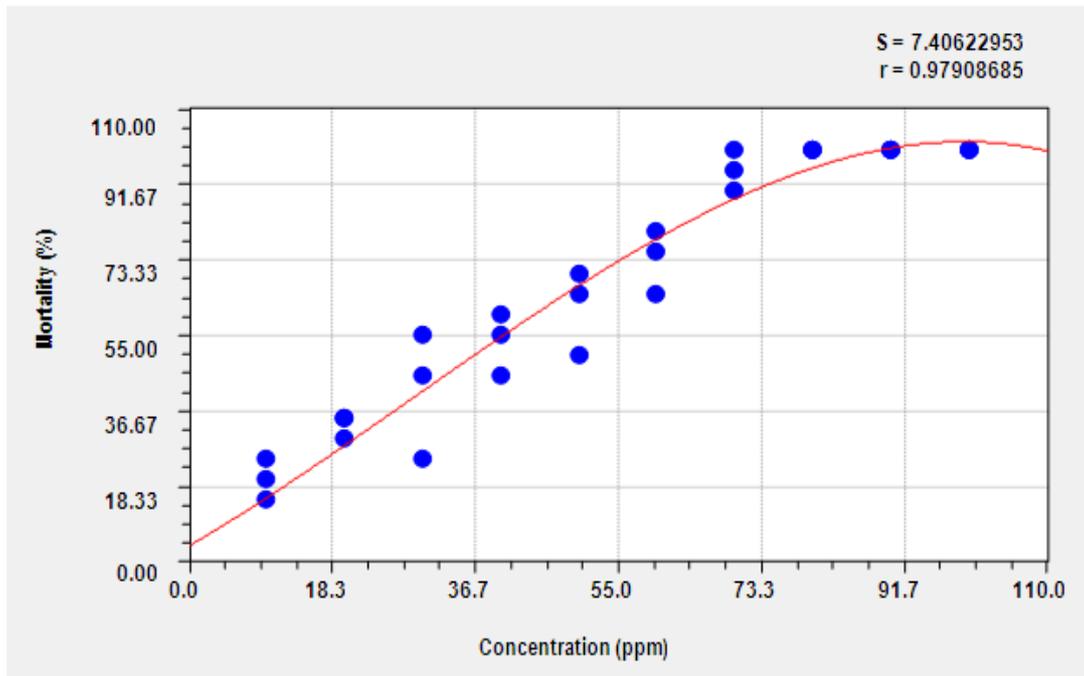


Fig 6. Polynomial fit showing the effect of chloroform extract of *Gmelina asiatica* on the mortality of *Culex quinquefasciatus* larvae

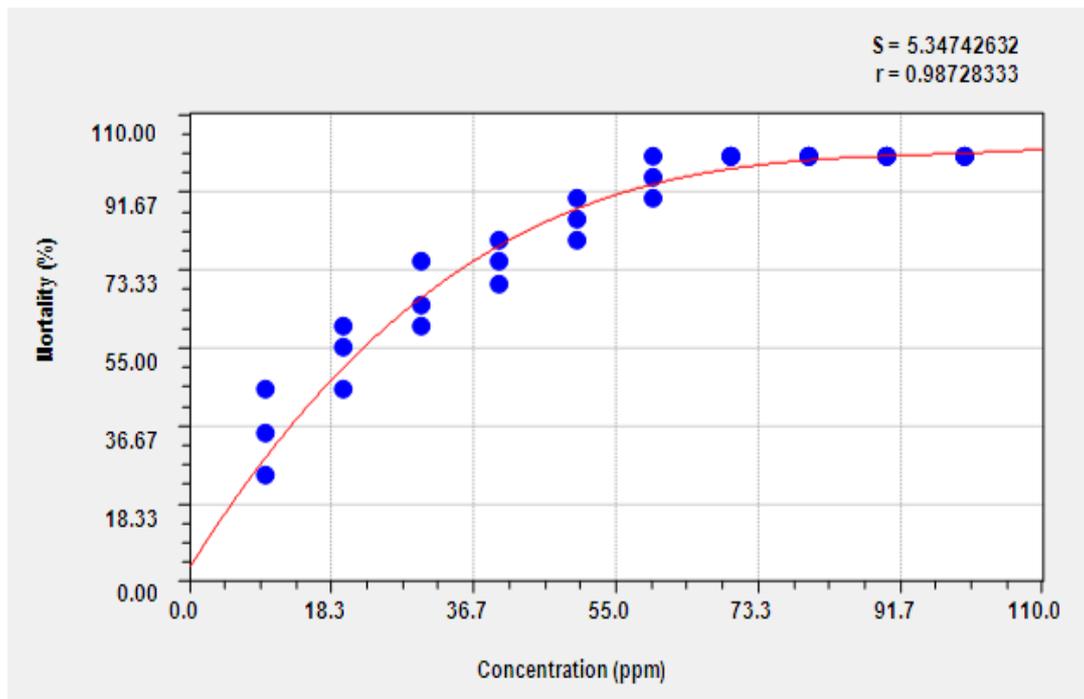


Fig 7. Polynomial fit showing the effect of ethanolic extract of *Gmelina asiatica* on the mortality of *Culex quinquefasciatus* larvae

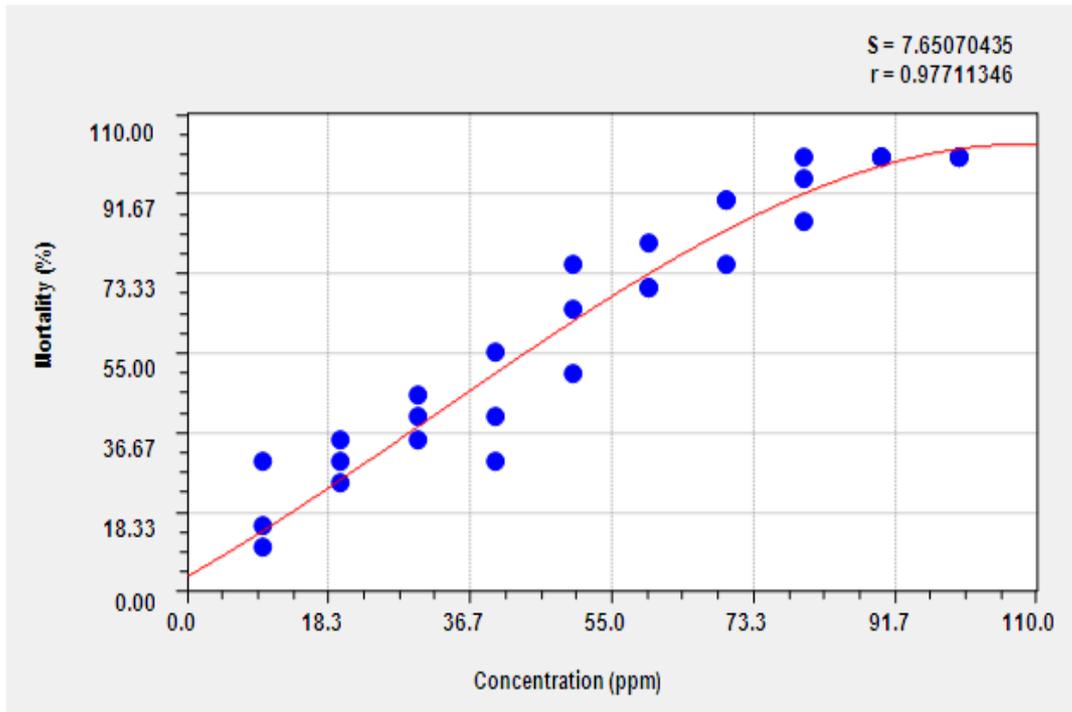
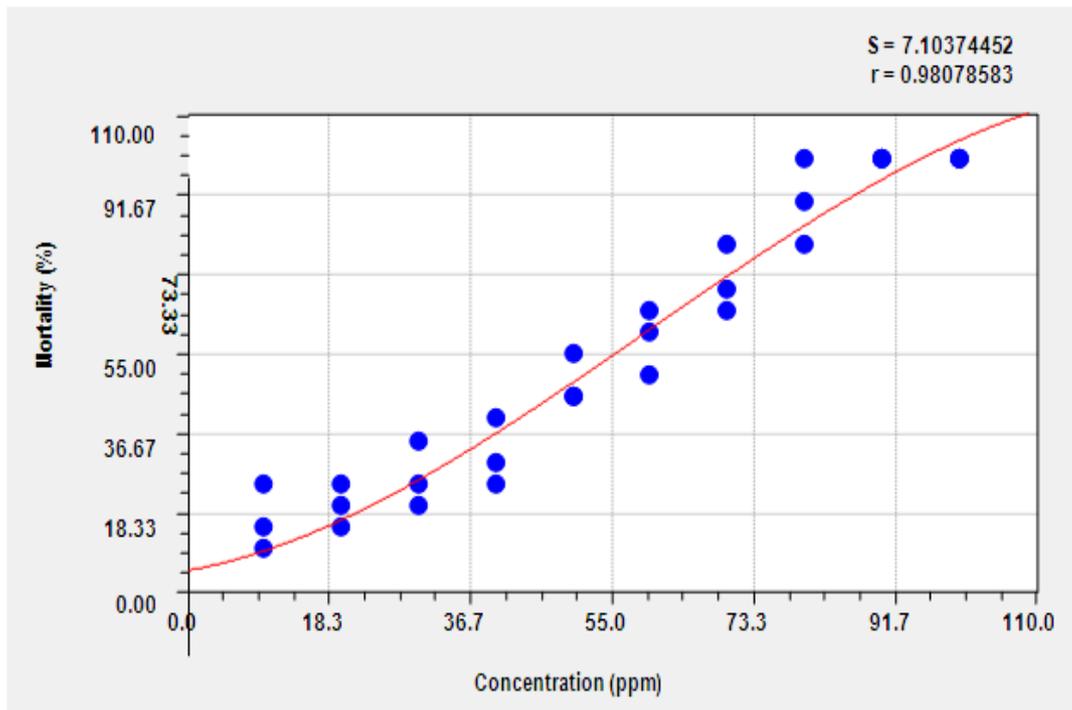


Fig 8. Polynomial fit showing the effect of acetone extract of *Gmelina asiatica* on the mortality of *Culex quinquefasciatus* larvae



DISCUSSION

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting counter measures for development of newer insecticides [18]. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat [19]. Many researchers have reported the effectiveness of plant extracts against mosquito larvae [20, 21].

The percentage mortality values of third instar larvae of *A. aegypti* and *C. quinquefasciatus* treated in different concentration (ranging from 10 to 100ppm) of the leaf extract after 24h exposure was recorded (Table 1 and 3). The chloroform extracts of *G. asiatica* showed 100% mortality at 70ppm in both tested larvae. The results of larvicidal activity clearly indicate that the percentage of mortality being directly proportional to the concentration of the extract. The results obtained in the present study are in accordance with the observations of Kovendran and Murugan [22] who studied the chloroform extracts of *C. inermis* that showed good larvicidal activity (91% mortality at 100ppm) against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*. Similar studies carried out by Nikkon *et al.* [23] suggested that the chloroform fraction of *Duranta repens* stem showed highest larval mortality (100%) against *C. quinquefasciatus*.

The larvicidal activity of plant extracts might be due to the presence of various secondary metabolites and also the fatty acids such as hexadecanoic acid and 9,12-octadecadienoic acid are probably the active principle responsible for the activity of ethanol extract of *G. asiatica* leaf that are previously reported as effective larvicidal agents [24,25]. Carbohydrates, saponins, phytosterols, phenols, flavonoids, tannins, alkaloids and terpenoids in the plant extracts were previously reported to have mosquito larvicidal activity [26-28]. Sukumar *et al.* [29] suggested the existence of variations in toxicities of phytochemical compounds on target species depending on the plant part which they are extracted. In addition, Jeybalan *et al.* [30] noted that other variations were due to the responses and developmental stages of species to the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of compounds in the extract, effect on growth and reproduction and other factors.

CONCLUSION

The findings of the present investigation revealed that the tested extracts of *G. asiatica* leaf controls the development of *A. aegypti* and *C. quinquefasciatus* larvae at different concentration and also by the different solvents and it opened a new way for further investigations of larvicidal properties of natural product extract.

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