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LARVICIDAL, PUPICIDAL, OVIPOSITION DETERRENT ACTIVITY AND SMOKE TOXICITY OF MATURE LEAF EXTRACTS OF *ANNONA RETICULATA* LINN. AGAINST FILARIAL VECTOR *CULEX QUINQUEFASCIATUS* SAY

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ABSTRACT

Crude and ethyl acetate extracts of mature leaves of *Annona reticulata* Linn. (*A. reticulata*) were investigated to establish their biocontrol potentialities under laboratory condition against larvae (different instars) and pupae of *Culex quinquefasciatus* Say, 1823 (*Cx. quinquefasciatus*) at different concentrations. Crude as well as ethyl acetate extracts showed excellent larvicidal activity at remarkable low concentrations. Oviposition deterrent activity of ethyl acetate extract and smoke toxicity effect of mature leaf powdered coils on adult mosquitoes were also examined and showed an excellent result. Pupae also showed motality at low concentrations. LC_{50} and LC_{90} values of larvicidal and pupicidal activity gradually decreased with increased exposure period. Statistical justifications were done through Log-probit, regression and ANOVA analyses. No adverse effect has been found on tested non target organisms. Photochemical analyses of leaf extracts revealed the presence of different secondary metabolites. So, crude and ethyl acetate leaf extracts of *A. reticulata* can effectively be used as larvicide, pupicide, oviposition deterrent activity and smoke toxicity against *Cx. quinquefasciatus* mosquito species.

KEYWORDS: ***Annona reticulata, Culex quinquefasciatus,*** **larvicidal, pupicidal, oviposition deterrent activity, smoke toxicity, phytochemicals**

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INTRODUCTION

Mosquito (Culicidae) is the most important blood sucking group among insects. Blood feeding female mosquitoes are the major vectors and transmit many diseases like filariasis, malaria, dengue fever, yellow fever, chikungunya, Japanese encephalitis etc. and cause millions of deaths every year [1]. Lymphatic filariasis is caused by *Wuchereria bancrofti* transmitted by female *Cx. quinquefasciatus* mosquitoes. About 120 million people are infected worldwide and 44 million people are with chronic manifestation [2]. To control mosquitoes, various organophosphates like temephos, fenthion etc. and insect growth regulators like diflubenzuron, methoprene etc. are used. Synthetic pesticides initiate development of resistant varieties, ecological hazards and harm to mammals including humans [3]. Insecticides of botanical origin are easily degradable and safe for the environment and there is no report about development of resistance varieties against the plant products [4]. Extracts from different parts of plants were found to be effective for mosquito larvicidal activity [5,6,7,8,9,10]. *A. reticulata* is small, evergreen tree belonging to the family Annonaceae. Its native land is West Indies and South America. It is cultivated throughout India for its fruits [11]. It is also known as ramphal, bullock's heart, sitaphal, sarifa, custard apple. In Ayurveda *A. reticulata* is used for the treatment of cancer, dysentery, epilepsy, cardiac problem, worm infestation, constipation, haemorrhage and also has antifertility, antitumour and aborfacient properties [12,13]. Roots have antiproliferative activity on human cancer cell lines [14].

MATERIALS AND METHODS

2.1 Preparation of crude extract

Fresh mature and green leaves of *A. reticulata* were collected during September and October, 2013 from Burdwan town, West Bengal, India and the voucher specimen (no. GCZSM-4) was deposited in the Department of Zoology, The University of Burdwan, West Bengal, India. 50 gm of fresh mature leaves of *A. reticulata* were crushed with mechanical grinder and mixed with 200 ml of distilled water. The mixture was then filtered through muslin cloth and filtrate was lyophilized. The dried crude extract was stored in refrigerator at 4^0 C for further bioassay experiment. 0.1 gm of dried crude extract was mixed with 25 ml of distilled water to make stock test solution. From stock solution, required graded concentrations i.e. 2, 4, 6, 8 and 10 ppm were prepared through dilution of tap water.

2.2 Preparation of solvent extracts

For the preparation of solvent extracts fresh mature leaves of *A. reticulata* dried in shade (for 10-12 days) were chopped finely. 200 g finely chopped leaves were put in a soxhlet apparatus and the plant extracts were prepared using solvents like, petroleum ether (2000 ml), benzene (2000 ml) and ethyl acetate (2000 ml.), one after another using same plant material. The period of extraction for each solvent was 72 hours. The final extract of each solvent was concentrated by rotary evaporator. Only the semi solid ethyl acetate extract was selected for preparation of graded concentrations of 0.5, 1, 2, 3, 4 and 5 ppm. 0.045 g semi solid ethyl acetate extract was mixed with 5 ml of ethanol and then added 85 ml distilled water to get 90 ml of stock test solution, so stock test solution was made on 5.55 % ethanol. From stock solution, required graded concentrations i.e 0.5, 1, 2, 3, 4 and 5 ppm were made through dilution of tap water.

2.3 Mosquito species

The present study was conducted at Burdwan ($23^0$16' N, 87°54' E) West Bengal, India, in the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan. *Cx. quinquefasciatus* larvae were collected from drains surrounding the university campus and larvae were kept in plastic trays and fed with artificial food i.e. mixture of dog biscuits and dried yeast powder in the ratio of 3:1. Mosquito colonies were kept free from exposure to pathogens, insecticides or repellents.

2.4 Larvicidal bioassay

The bioassay experiments were conducted according to standard WHO procedure (2005) with slight modification [15]. All instars larvae were used during bioassay experiment with crude and solvent extracts. Twenty five larvae were put in different plastic bowls (12 cm diameter/ 225 ml capacity) containing each with 100 ml of test solution of different concentrations of crude extract (i.e. 2, 4, 6, 8 and 10 ppm) and ethyl acetate extract (i.e. 0.5, 1, 2, 3, 4 and 5 ppm) to investigate the mortality percent. 100 ml of tap water was only used in the control treatment for larvicidal bioassay of crude extract and 100 ml of tap water with 0.5 ml ethanol was used in the control treatment for larvicidal bioassay of ethyl acetate extract. Larval mortalities were recorded after 24, 48 and 72 h of exposure. The data of mortality in 48 and 72 h were expressed by addition of the mortality at 24 and 48 h respectively. Dead larvae were identified when they failed to move after probing with a fine brush in the siphon or cervical region. The experiments were replicated three times on separate three days under laboratory conditions at 25-30°c and 80-90% relative humidity.

2.5 Pupicidal bioassay

Twenty five pupae were kept in different plastic bowls (12 cm diameter, 225 ml capacity) containing each with 100 ml of test solution of different concentrations of ethyl acetate extract (30, 60, 90 and 120 ppm) to investigate the mortality percent of pupae. 500 mg of ethyl acetate leaf extract of *A. reticulata* dissolved in 5 ml of ethanol and then 95 ml of distilled water added to get 100 ml of stock test solution, so, stock solution was made on 5% ethanol. From stock solution, required volume was taken and mixed with required volume of water to make 100 ml of final test solution of different concentrations. 100 ml of tap water with 0.5 ml of ethanol was used in the control treatment. Pupal mortalities were recorded after 24 h of exposure.

2.6 Oviposition deterrent activity

The oviposition deterrent test was performed using the method of Xue *et al.* [16] with slight modification. Adults were provided with 10% glucose solution and were periodically blood fed on immobilized pigeon. Adults were 6 days old when fed blood and four days later were used for oviposition deterrent activity. Twenty two gravid female *Cx. quinquefasciatus* were (10 days old, 4 days after blood feeding) transferred to mosquito cage (30×30×30 cm^3) covered with a mosquito net, with a glass top and a cloth sleeve for access. A cotton ball soaked in 10% glucose solution was available at all times. From stock solution (mention earlier for pupicidal bioassay) required volume were taken and mixed with required volume of water to get 25, 50, 75 and 100 ppm concentrations of test solutions of ethyl acetate extract. Four plastic bowls containing different concentrations of test solution (i.e. 25, 50, 75 and 100 ppm) and one plastic bowl containing 100 ml of tap water with 0.5 ml of ethanol served as control were placed in a cage. The position of the bowls was alternated between the different replicates so as to nullify any effect of position on oviposition. Three replicates were done. All experiments were run at ambient temperature (25-30°C) with relative humidity of 80 - 90%. After 24 h, the number of eggs laid in treated and control bowls was recorded. The percent effective deterrence for each leaf extract concentration was calculated using the following formula ER (%) = (NC-NT/NC) ×100% Where ER= percent effective deterrence; NC= number of eggs in control; and NT= number of eggs in treatment**.**

2.7 Preparation of mosquito coils

Mosquito coils were prepared using the method of Saini *et al.* [17] with suitable modifications. Mosquito coils were prepared by 2 g of shade dried *A. reticulata* leaf powder, 2 g of sawdust, and 2 g of charcoal powder. All the materials were thoroughly mixed with distilled water to form a semi solid paste. From this paste 0.4 cm thick mosquito coils were prepared. The mosquito coils were dried in shade for the experiment.

2.8 Smoke toxicity test

The smoke toxicity experiment was conducted in a glass chamber measuring 71 cm x 62 cm x

62 cm with a door at the front of the chamber. 100 adult mosquitoes were released into the chamber and the mosquitoes were exposed to the smoke of two burning coils for 60 minutes and the data of mortality were recorded after every 15 minutes. The data of adult mosquitoes mortality percent at 30, 45 and 60 minutes were expressed by the addition of the mortality percent at 15, 30 and 45 minutes respectively.

2.9 Effect on non target organisms

The effect of the crude and ethyl acetate extracts of *A. reticulata* leaves were tested against non target organisms like *Diplonychus annulatum* (predatory water bug), *Chironomus circumdatus* larvae (insect) and tadpole larvae of toad. Those were exposed LC_{50} of crude and ethyl acetate solvent extracts against 3rd instar larvae of *Cx. quinquefasciatus* at 24 h to observe the mortality and other abnormalities such as sluggishness and reduced swimming activity up to 72 h of exposure.

2.10. Phytochemical analyses of the plant extracts

Phytochemical analyses of the plant extracts were carried out according to the methodology of Harbone, (1984) [18] and of Trease and Evans, (1989) [19]. The qualitative phytochemical analyses revealed the presences of different phytochemicals and those were terpenoids, alkaloids, steroids, tannins, flavonoids, phenols, aminoacids and anthraquinones.

2.11. Statistical analyses

Experimental data was performed by using the computer software 'STAT PLUS 2009' and MS EXCEL 2007 to calculate the $LC_{50,}$ LC_{90}, regression equation (Y= mortality, X = concentration), coefficient of determination (R^2), mean mortality percent, Standard error and ANOVA. The percentage of corrected mortality was analyzed by Abbott's formula if necessary [20].

RESULTS

Mortality percent of larvicidal activity of crude leaf extract of *A. reticulata* were presented in table 1. Mortality percent increased with increase in period of exposure and concentration of crude extract. No larval mortalities were observed in control treatments. LC_{50}, LC_{90} values (95% confidence level), regression equations and coefficient of determinations (R^2) of crude extract was presented in table 2. From table 2, it was observed that LC_{50} and LC_{90} values gradually decreased in period of exposures and there is a positive correlation between mortality percent (Y) and concentration (X) of crude extract as coefficient of determination (R^2) values close to one in each case. Mortality percent of larvicidal activity of ethyl acetate leaf extract was presented in table 3. From Table 3, it was noticed that mortality percent increased with increase in concentration and time of exposure. No larval mortality was observed in control treatments. From table 4 it was observed that LC_{50} and LC_{90} values gradually decreased in period of exposures and coefficient of determination value (R^2) close to one in all cases. Table 5 shows the mortality percent of pupae which increased with the increase in concentrations of ethyl acetate extract. No pupal mortality was observed in control treatments. LC_{50} and LC_{90} values were 38.0731 and 196.4458 ppm and regression equation and R^2 value were Y=22.67+0.5422X and 0.9526 respectively against pupae after 24 h of exposure. Oviposition deterrent activities were observed which increased with the increase of concentration of ethyl acetate leaf extract of *A. reticulata* solution**.** At 100 ppm concentration, effective deterency is 82.64 % and at 25, 50 and 75 ppm concentrations, eggs lying were reduced by 43.47%, 56.52% and 65.27% respectively. Smokes from burning of *A. reticulata* leaf powder coils demonstrated smoke toxicity effect on adult *Cx. quinquefasciatus.* 82.33±1.45%, 100±00 % and 6.66±.88% mortality was observed after 60 minutes of exposure to smokes by burning two coils of *A. reticulata* leaf powder coils, commercial coils and negative coils respectively. Mortality percent of different smoke exposed to adult mosquitoes were recorded in the following sequences: Commercial mosquito coils> mosquito coils containing powder of *A. reticulata* leaf>

mosquito coils without any plant materials (table 6). No change in survivality was observed when treatment was done to non target organisms at LC_{50} of 24 h of exposure of both crude and ethyl acetate extracts against 3rd instar larvae of *Cx. quinquefasciatus.* Phytochemical analysis of *A. reticulata* leaves revealed the presence of many secondary metabolites such as terpenoids, alkaloids, steroids, tannins, flavonoids, phenols, amino acids and anthraquinones (table 7). Three ways factorial ANOVA established statistical significance of larval mortality ($p<0.05$) in terms of concentrations of ethyl acetate extract, instars of *Cx. quinquefasciatus* and time of exposures collectively (Table 8).

Table 1
Efficacy of crude leaf extract of Annona reticulata Linn. on different larval instars of Culex quinquefasciatus

Instars	Conc.(ppm)	Mean mortality percent ± Standard error		
		24 hours	48 hours	72 hours
1st	2	69.33±5.8	78·66±5.81	86.66±2.67
	4	78·67±5.81	84.00±4.00	90.67±2.67
	6	86.67±3.52	89.00±1.33	92.00±2.31
	8	100.00±00.00	100.00±00.00	100.00±00.00
	10	100.00±00.00	100.00±00.00	100.00±00.00
2nd	2	53.33±13.33	62.67±10.66	69·33±9.33
	4	64.00±10.58	72.00±8.33	80.00±4.61
	6	72.00±8.32	80.00±6.92	86.67±3.52
	8	89.33±1.33	90.67±1.33	92.00±2.31
	10	100.00±00.00	100.00±00.00	100.00±00.00
3rd	2	54.66±2.66	56.00±4.00	65·33±2.66
	4	65·33±2.66	73·33±6.66	80.00±6.93
	6	80.00±6.93	86.67±3.53	89.33±1.33
	8	100.00±00.00	100.00±00.00	100.00±00.00
	10	100.00±00.00	100.00±00.00	100.00±00.00
4th	2	38.67±10.67	49.33±9.33	60.00±10.06
	4	65.33±9.33	73·33±6.67	78.67±5.81
	6	72.00±4.00	82.67±2.67	88.00±2.31
	8	84.00±4.00	89.33±1.33	100.00±00.00
	10	100.00±00.00	100.00±00.00	100.00±00.00

Control – No mortality (For all instars)

Table 2
Log probit analyses and regression analyses of larvicidal activity of crude leaf extract of Annona reticulata Linn. against different larval instars of Culex quinquefasciatus

Larval instars	Period of exposure (h)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equations	R^2 value
1st	24	1.4230	5.0529	Y= 62.1330+4.1335X	0.9496
	48	0.9562	4.2039	Y= 72.7280+ 2.9340X	0.9432
	72	0.5150	3.0098	Y= 83.063+1.8005X	0.9204
2nd	24	2.2372	8.7305	Y=40.1310+5.9335X	0.9866
	48	1.6348	7.4146	Y=53.0690+4.6665X	0.9983
	72	1.2073	5.9546	Y= 63.5980+3.6670X	0.9859
3rd	24	2.1840	6.4233	Y=42.3930+6.2675X	0.9480
	48	2.0025	5.5087	Y=48.7990+5.7335X	0.9300
	72	1.5849	4.8410	Y= 60.1300+4.4670X	0.9259
4th	24	2.8076	8.8752	Y=29.6010+7.0665X	0.9587
	48	2.1630	7.0838	Y=43.7300+5.8670X	0.9343
	72	1.7917	4.8486	Y=54.9350+5.0665X	0.9139

R2 = Coefficient of determination, LC = Lethal concentration

Table 3

Mortality percent of different instars of Culex quinquefasciatus exposed to different concentrations of ethyl acetate leaf extract of Annona reticulata Linn.

Instars	Conc.(ppm)	mean mortality percent ± Standard error		
1st		24h	48h	72h
	0.5	25.33±9.33	38.67±9.33	56.00±4.00
	1.0	42.66±11.85	58.67±5.81	76.00±4.00
	2.0	85.33±1.33	86.67±1.33	100.00±00.00
	3.0	92.00±2.32	100.00±00.00	100.00±00.00
	4.0	100.00±00.00	100.00±00.00	100.00±00.00
	5.0	100.00±00.00	100.00±00.00	100.00±00.00
2nd	0.5	14·67±6.67	33·33±6.67	41·33±6.67
	1.0	22·67±9.33	42·67±9.33	56.00±8.33
	2.0	85·33±2.67	86.67±1.33	89.33±1.33
	3.0	86.64±1.32	100.00±00.00	100.00±00.00
	4.0	100.00±00.00	100.00±00.00	100.00±00.00
	5.0	100.00±00.00	100.00±00.00	100.00±00.00
3rd	0.5	12.00±4.00	29.33±5.81	38.67±5.81
	1.0	16.00±4.00	32.00±4.00	41·33±6.67
	2.0	32.00±4.00	45·33±2.67	56.00±4.00
	3.0	56.00±4.00	62·67±2.67	72.00±4.00
	4.0	76.00±6.08	82.67±2.67	89.33±1.33
	5.0	96.00±4.00	100.00±00.00	100.00±00.00
4th	0.5	9.33±5.81	32.00±8.33	41·33±8.11
	1.0	20.00±00.00	41.33±6.67	46·67±6.67
	2.0	22·66±2.67	41.33±1.33	52.00±4.00
	3.0	25·33±2.67	42.66±2.66	52.00±4.00
	4.0	62·67±9.33	70.33±2.67	85·33±2.67
	5.0	85·33±6.67	89.33±5.81	92.00±4.00

Control – No mortality (For all instars)

Table 4

Log probit analyses and regression analyses of larvicidal activity of ethyl acetate leaf extract of Annona reticulata Linn. against different larval instars of Culex quinquefasciatus

Larval instars	Period of exposure (h)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equations	R^2 value
1st	24	0.9498	2.4083	Y=31.4867+ 16.5419X	0.8072
	48	0.6730	1.8843	Y=46.5282 +13.2155 X	0.7800
	72	0.4944	1.1983	Y=66.6411 +8.5260 X	0.6348
2nd	24	1.2197	2.7220	Y= 16.7157+19.9364 X	0.7959
	48	0.8467	2.0725	Y=36.7787 +12.6603 X	0.7778
	72	0.7011	1.8454	Y= 47.4567+13.0271 X	0.7701
3rd	24	2.1805	6.8182	Y=-2.1372 +19.5802 X	0.9910
	48	1.4556	6.6383	Y=16.7646 +16.2202X	0.9877
	72	1.0590	5.2804	Y=28.9284 +14.4361 X	0.9965
4th	24	3.1428	13.2510	Y=-3.4224 +15.8616 X	0.8717
	48	1.6364	15.4868	Y=22.2337+12.2953 X	0.8433
	72	1.1073	11.2419	Y=31.9049+11.4774X	0.8690

R^2 = Coefficient of determination, LC = Lethal concentration

Table 5

Mortality percent of pupae of Culex quinquefasciatus exposed to different concentrations of ethyl acetate leaf extract of Annona reticulata Linn.

Conc.(ppm)	Mean mortality percent± standard error
	24h
30	36±4
60	62.67±2.67
90	65.33±2.67
120	89.33±5.81

Control – No mortality

Table 6

Smoke toxicity effect of Annona reticulata leaf powder mosquito coils, commercial mosquito coils and mosquito coils without any plant materials on Culex quinquefasciatus adult mosquitoes.

Time of observation after burning of mosquito coil	*A. reticulata* leaf powder mosquito coil		Commercial mosquito coil		Control mosquito coil(Coil without leaf powder)
	No. of dropped down mosquitoes	No. of dead mosquitoes	No. of dropped down mosquitoes	No. of dead mosquitoes	No. of dead mosquitoes
After 15 minutes	36.66±1.66	12.33±1.45	81.66±1.66	19±2.08	2.33±.33
After 30 minutes	47±1.52	30±1.15	92.33±1.45	47.33±1.20	3.33±.33
After 45 minutes	58±1.15	47.66±1.45	94.33±2.33	83.66±2.33	5.33±.33
After 60 minutes	97.33±1.20	82.33±1.45	100±00	100±00	6.66±.88

Table 7

Result of qualitative analyses of phytochemicals from leaves of Annona Reticulata

Phytochemicals	Presence (+)	Absence (-)
Terpenoids	+	
Alkaloids	+	
Steroids	+	
Tannins	+	
Flavonoids	+	
Phenols	+	
Amino acids	+	
Anthraquinones	+	
Saponins		_

Table 8

Completely randomized three ways ANOVA analyses using instars (I) of Cx. quinquefasciatus, hours (H), and Concentrations of ethyl acetate leaf extract of Annona reticulata (C) as three independent parameter

Source of variation	Sum of squares(SS)	Degree of freedom(df)	Mean of squares(MS)	F value	p-level
Instars(I)	2081.44	3	693.81	163.97	0
Time(H)	682.06	2	341.03	80.59	0
Conc.(C)	649.69	5	1529.92	361.56	0
I×H	53.97	6	8.99	2.13	0.05
I×C	1153.33	15	76.89	18.17	0
H×C	219.99	10	21.99	5.20	0
I×H×C	64.42	30	2.15	0.51	0.98
Within groups	609.33	144	4.23	-	-
Total	2515.15	215	58.21	-	-

DISCUSSION

Insecticides of plant origin are safe, biodegradable as well as suitable alternative source for control of almost all mosquito species. Present study reveals that crude extract as well as solvent extract (ethyl acetate) of *A. reticulata* leaf are very much effective as larvicide, pupicide and oviposition deterrent activity of *Cx. quinquefasciatus.* Smoke emerged from leaf powder mosquito coils has also an amazing effect on *Cx. quinquefasciatus* because it is almost similar to that of smoke of commercial coils. Rawani *et al.,* (2010) reported ethyl acetate extract of *Solanum nigrum* leaf against larvicidal activity of *Cx. quinquefasciatus* and showed 100% mortality at 50 ppm dose against 3rd instar larva of *Cx. quinquefasciatus* having LC_{50} value 17.04 ppm after 24 h of exposure [21]. In this study, 100% mortality was observed only at 5 ppm concentration of ethyl acetate extract of leaves of *A. reticulata* against 3rd instar larvae of *Cx. quinquefasciatus* having LC_{50} value 1.4556 ppm after 48 h of exposure and 96±4% mortality was observed at 5 ppm concentration with LC_{50} value 2.1805 ppm after 24 h of exposure. Kamaraj *et al.,* (2011) reported many plants having mosquito larvicidal activity, of which leaf ethyl acetate extract of *Chrysanthemum indicum* against the 4th instar larvae of *Anopheles subpictus* with LC_{50} value after 24 h of exposure is 39.98 mg/L and for *Cx. tritaeniorhynchus* LC_{50} value after 24 h of exposure is 42.29 mg/L respectively [22]. But in case of leaf ethyl acetate extract of *A. reticulata*, LC_{50} value is 3.1428 ppm (mg/L) against 4th instar larvae of *Cx. quinquefasciatus* after 24 h of exposure. Kundu *et al.,* (2013) reported the efficacy of ethyl acetate extract of mature seed coat of *Casia sophera* against *Cx. quinquefasciatus* and showed 100% mortality at 520 ppm against 1st instar larvae after 24 h of exposure [23]. But only at 4 ppm concentration of leaf ethyl acetate extract of *A. reticulata* demonstrated 100% mortality against 1st instar larvae of *Cx. quinquefasciatus* after 24 h of exposure. Nayak, (2014) worked with only methanol extract of leaves of *A. reticulata* with concentrations, 5, 10, 25, 50, 100, 200 ppm against 4th instar larvae of *Cx. quinquefasciatus* and reported 100% mortality of larvae occurred at 5 ppm concentration after 48 h exposure [24]. But in this study, ethyl acetate extract of leaves of *A. reticulata* showed 100% mortality at 3 ppm concentration against 1st and of 2nd instars larvae after 48 h of exposure and for 3rd instar larvae, 100% mortality was noticed at 5 ppm concentration after 48 h of exposure and 92% mortality was noticed at 5 ppm concentration against 4th instar larvae after 72 h of exposure. In this study, 89.33±5.81% pupal mortality at a concentration 120 ppm having LC_{50} value 38.0731 ppm after 24 h of exposure was recorded. Rawani *et al.* recorded highest pupal mortality at dose 150 ppm of petroleum ether extract of *Carica papaya* having LC_{50} values 86.53 and 72.16 ppm after 24h of exposure for *Cx. quinquefasciatus* and *An. stephensi* respectively [25]. Highest percent effective deterency of oviposition in this study at a dose 100 ppm of ethyl acetate extract of *A. reticulata* was recorded 82.64%. Swathi *et al.,* (2010) evaluated oviposition deterrent activity of ethanolic extracts of *Pongamia pinnata, Coleus froskohlii and Datura stramonium* leaves and showed reduced eggs laying by 97.62%, 77.3%, 100% against *Ae. aegypti* and 59.10%, 39.22%, 82% against *Cx. quinquefasciatus* at higher concentration (0.1%) [26]. Rajkumar and Jebanesan, (2005) tested acetone extract of leaf of *Solanum trilobatum* for oviposition deterrent activity of *An. stephensi* and showed that concentrations 0.01, 0.025, 0.05, 0.075 and 0.1% reduced egg laying by gravid females are 18.4%, 44.0%, 66.6%, 89.8% and 99.4 % respectively compared to ethanol treated controls [27]. In this study, 82.33±1.45% mosquitoes died after 60 minutes of exposure by smoke of burning of *A. reticulata* leaf powder mosquito coils. Adhikari and Chandra, (2014) established smoke toxic effect of *Swietenia mahagoni* on *An. stephensi* [28]. Singha *et al.,* (2011) reported smoke toxicity of *Mesua ferra* leaves on *Cx. quinquefasciatus* [29]. Further, non-responsiveness of the non target organisms, which share the same habitat with mosquito, to crude and solvent extracts of *A. reticulata*

emphasize as an eco-friendly mosquitocidal agent.

CONCLUSION

In conclusion *A. reticulata* crude and ethyl acetate extracts can be used effectively as mosquito larvicide, pupicide, and oviposition deterrent and repellent of *Cx. quinquefasciatus.* Further investigations are needed to know this activity against different mosquito's species and the chemical structure of the active principle involved in larvicidal, pupicidal and oviposition deterrent activity.

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CONFLICT OF INTEREST

We have no conflict of interest.

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