

PUPAL EMERGENCE INHIBITION ACTIVITY OF *ACALYPHA INDICA* LEAF EXTRACT AGAINST DENGUE VECTOR, *Aedes albopictus* MOSQUITO

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Received: 23 Apr 2017 Revised and Accepted: 19 Jun 2017

ABSTRACT

Objective: To investigate the larvicidal activities of six varying extracts of *Acalypha indica* (*A. indica*) leaves from family *Euphorbiaceae* against the dengue mosquito vector, *Aedes albopictus* (*Ae. albopictus*) in laboratory.

Methods: Leaves from the study plants were separated, air dried in room temperature, grounded and extracted with different solvents (petroleum ether, chloroform, ethyl acetate, n-butanol, ethanol and aqueous) by solvent apparatus and aqueous extract by maceration method. The extra solvents were evaporated to obtain crude extracts by using rotary evaporator. The crude extracts of six different solvents were dissolved in dimethyl sulphoxide (DMSO) to prepare test dosages of 1000, 2000, 3000, 4000 and 5000 ppm. Third instar larvae of *Ae. albopictus* were exposed to 1000, 2000, 3000, 4000 and 5000 ppm concentrations of petroleum ether, chloroform, ethyl acetate, n-butanol, ethanol and aqueous extracts of *A. indica* and compared with control to determine the larvicidal effects. Larval bioassays were carried out according to World Health Organisation (WHO) procedures. The rate of larval mortality was recorded after 24h and 48 h of time exposure. Three duplicate trials were made for each tested dosage. IC50 and IC90 values were calculated by using probit analysis.

Results: Based on probit analysis result the 24h and 48h LC50 and LC90 of petroleum ether extract of *A. indica* against *Ae. albopictus* was found to be 2805.43 ppm and 2376.11 ppm, 3825.14 ppm and 3327.8 ppm, respectively. An LC50 and LC90 value of chloroform extracts of *A. indica* against third instar larvae was found to be 2276.5 ppm and 4015.8 ppm (24h), 2213.36 ppm and 3430.43 ppm (48h), respectively. An LC50 value of 4472.17 ppm and 2469.61 ppm, and LC90 value of 4215.84 ppm was obtained on ethylacetate extract treatment against *Ae. albopictus* for 24h and 48h exposure, respectively. The 24h and 48 h LC50 and LC90 values of n-butanol extracts of *A. indica* was found to be 2777.88 ppm and 3628.19 ppm, 2225.61 ppm and 2518.86 ppm, respectively. In the present study, the larvicidal bioassays demonstrated that the n-butanol extract was most effective with 100% mortality against larvae of *Ae. albopictus* at 3000, 4000 and 5000 ppm compared to other extracts. All other extracts (petroleum ether, chloroform and ethyl acetate) of *A. indica* at high concentration (4000 ppm and 5000 ppm) manifested a significant ($P < 0.01$ and 0.05) knock down effect of 100% mortality after 24h and 48h exposure. While the third instar larvae of *Ae. albopictus* were found to be most susceptible and produced no mortality to ethanol and aqueous extract at varying parts per million.

Conclusion: *A. indica* leaf extract was tested for the first time against dengue vector *Ae. albopictus* and the results revealed that *A. indica* can be used to control dengue vector. Further this extract needs to be evaluated under field conditions for proper exploitation of *Ae. albopictus* mosquito larvae. Thus, the present study provided a first report on *A. indica* as a promising mosquito larvicidal activity and can be considered for further investigations such as formulation of bioinsecticides to control *Ae. albopictus* populations.

Keywords: Mosquito, *Ae. albopictus*, Larvae, Medicinal plants, Diseases, Insecticides

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DOI: <http://dx.doi.org/10.22159/ijpps.2017v9i8.19362>

INTRODUCTION

Mosquitoes, the most important agent as vector transmits several serious human diseases like malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya and yellow fever [1-3] and several diseases as a major public health problem in the world. The current mosquito control strategies are mainly focussed on synthetic insecticides considered as the first line of action against mosquito vectors. Besides its toxic nature continuous usage of synthetic insecticides causes ecological imbalance, environmental pollution by contaminating soil, water and air [4], destruction of non-target organisms including humans and animals [5, 6] and development of insecticide resistance in target insects [7]. Because of the undesirable side effects of the synthetic chemical pesticides, natural insecticides development achieved as an alternative way in mosquito control programs. Plants act as an excellent source for the reduction of mosquito population at all the stages due to their excellent larvicidal, pupicidal and adulticidal properties. The use of plant extracts has natural insecticides due to the presence of several appealing features like less hazardous, non-toxic, safer for non-target organisms, target specific activity, biodegradable and rich bioactive chemicals [8-12]. Green larvicides are of great importance and considered now because of the presence of several bioactive components while the conventional

insecticide comprises single active ingredient. These properties have stimulated many investigators to investigate about natural insecticides and also many researchers have reported the effectiveness of various plants against mosquito larvae.

Previous studies have evaluated the potential use of plants such as *C. obtusifolia* [13], *Trachyspermum ammi* [14] against *An. stephensi*, *Piper longum* and *Piper nigrum* [15,16], *Cassia fistula* [17], *Coriandrum sativum* [18] against *Ae. aegypti*, *P. nigrum* against *Culex quinquefasciatus* [19], *Cassia siamea* against *Plasmodium berghei* [20], *Cassia auriculata* against *An. stephensi* and *Culex quinquefasciatus* [21], *Lantana camara* against *Ae. aegypti*, *Culex quinquefasciatus*, *An. culicifacies*, *An. fluviatilis* and *An. stephensi* [22], *L. camara* against *Ae. albopictus* and *Ae. aegypti* [23] as larvicidal activity.

Mosquito mostly belongs to genera *Anopheles*, *Aedes* and *Culex* as vectors for pathogens of various diseases. Among the mosquitoes *Ae. albopictus* (Diptera: Culicidae), a competent vector of *Aedes aegypti* causes chikungunya and dengue viruses, highly dangerous to human health [24]. Very few studies have been carried out on *Ae. albopictus* mosquitoes over a long period [25-28] hence the present study is focused on this species. Keeping these points in view, in this context, the purpose of the present investigation is to explore the larvicidal

activity of *A. indica* leaf extract against *Ae. albopictus* under the laboratory conditions. Therefore, this study provides the first report on the mosquito larvicidal activity effect of *A. indica* leaf extract against third instar larvae of *Ae. albopictus* as target species.

MATERIALS AND METHODS

Chemicals and reagents

All the solvents and other reagents used in the present study are of analytical grade and purchased from Sigma-Aldrich Co.

Collection of plant materials

The leaf of *A. indica* (family: Euphorbiaceae) was collected in and around Velapadi (12°56'5.8"N and 79°8'48.7716"E), Vellore district, India. The collected materials were identified by using the standard taxonomic key. The Voucher specimen (No. 1316) was deposited and kept in our Herbarium for further reference.

Preparation of plant extract

A. indica plant was washed with tap water and then followed by distilled water to remove the sand particles. Leaves were separated, air dried in shade for 20–30 d at environmental temperature. The shade dried materials were grounded into fine powder using a electrical stainless steel blender and stored in air tight bottles until further use.

Extraction method

From the *A. indica* leaf powder 30g of weight quantity was extracted with petroleum ether (250 ml, Qualigens chemicals, India.) Chloroform (400 ml, Qualigens chemicals, India), Ethyl acetate (300 ml, Qualigens chemicals, India), n-Butanol (350 ml Qualigens chemicals, India) and ethanol (500 ml Qualigens chemicals, India) as solvent by employing a soxhlet apparatus separately until exhaustion. The pooled extract were concentrated under reduced pressure in a Vacuum evaporator at 40 °C to get a semi solid residues. Twenty grams of *A. indica* leaf powder was macerated in 200 ml of distilled water on 250 ml Erlenmeyer flasks which were continuously shaken on a rotary at 180 rpm/min for 24h at room temperature. The suspension was filtered using a fine muslin cloth and then through a whatman No: 1 filter paper via a Buchner funnel. The residue was further macerated twice under the same condition. The obtained filtrate of aqueous extract were mixed and concentrated under vacuum, and then dried by using lyophilizers. The obtained extracts was stored at 4 °C in air tight bottle until required for a further analysis.

Larvicidal activity

Stock solution

0.1g of the crude extract (petroleum ether, chloroform, ethyl acetate, n-Butanol, ethanol and aqueous) was dissolved in 1 ml of particular solvent and added 2 drops of Tween 80. Tween 80 (Qualigens) was used as an emulsifier. This was made up 100 ml using distilled water. This solution was considered as stock solution (0.1g/100 ml, 1000 ppm). From the stock solution of all the six solvent extracts different concentration were prepared with dechlorinated water ranging from 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm respectively, and then subjected to Larvicidal bioassay screening.

Collection of larvae

The Larvae were collected from fresh stagnant water (waste pot, plastic container, plant pot) near D. K. M. College, Garden, Vellore. The Larvae was collected in a plastic container and transferred to the Laboratory immediately and identified. Preliminarily, the identification of the mosquito Larvae *Ae. albopictus* collected was done in Vector Controlled Research Center (VCRC), Pondicherry. Third instar Larvae alone were collected for the Larvicidal bioassay. Feed to the mosquito larvae was supplied in the mass ratio of 3:1 (pedigree dog biscuits and yeast) for its growth.

Larvicidal bioassay

The larvicidal assay of the *A. indica* extracts was tested against the *Ae. albopictus* larvae by following the world health organization. The third instar larvae (*Ae. albopictus*) was used for the larvicidal

activity. From the stock solution (1000 ppm of petroleum ether, chloroform, ethyl acetate, butanol, ethanol, aqueous) different dilutions of 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm were prepared in 250 ml of deionized water. Two controlled groups, one setup containing two drop of tween80 mixed with 250 ml of dechlorinated water, and another setup containing 250 ml of dechlorinated water only, were used for testing larvicidal activity. 25 third instar larvae were released into 250 ml of dechlorinated water containing extract solution and control experiment (with tween 80 and without tween 80) were run in parallel. All the container were subsequently nylon mosquito netting to prevent mosquito species from egg laying. The extract water solution was stirred for 30 second with a glass rod after approximately 5 min. 25 larvae were transferred gently to the test medium by dropper. Larval food was not given for the test larvae. After 24h and 48h of exposure mortality rates of larvae were identified when they were unable to move normally after gently touching with needle in the siphon or cervical region. The laboratory test was carried out under the controlled temperature of (26±2 °C) and relative humidity (60±10)%. Each tested concentration was replicated two to five trials and the mean of one replicate was recorded in the study. The control mortalities were corrected by using Abbott's formula

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in Control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC50, LC90 and other statistics at 95% confidence limits of upper fiducial limit (UFL) and lower fiducial limit (LFL), and chi-square values were calculated using the SPSS 16.0 version (software package). The values are expressed as mean±SD of five replicates. Results with P<0.05 were considered to be statistically significant.

RESULTS

The results clearly indicate that Petroleum ether extract of *A. indica* exhibited a potent mortality against 3rd instar larvae of *Ae. albopictus* tested. Petroleum ether extract of *A. indica* was found to be most effective at 5000 ppm (100% mortality, both 24 h and 48 h), where as 4000 ppm found to be more potent and showed 100% mortality at 48 h and 90% at 24hr. Petroleum ether extract at a lowest concentration of 3000 ppm, 2000 ppm and 1000 ppm killed 50%, 40%, 50% respectively, when exposed for 24 h and 40%, 50%, 100% respectively, for 48 h. Petroleum ether extract showed LC50 value of 2805.43 and 2376.11 ppm for 24 h, and 48 h respectively. The LC90 value of extract was found to be 3825.14 ppm and 3327.80 ppm for 24 h and 48 h respectively (table 1,2). The chisquare values were significant at P≤0.05, 0.01 for 24 h and 48 h respectively.

Chloroform extract also recorded a high larval mortality against 3rd instar *Ae. albopictus* larvae. LC50, LC90 and Chisquare value for 24 h and 48 h were also calculated. The data are presented in table 1,2. It was determined that highest concentration caused 100% mortality with LC50 value of 2276.5 ppm and 2213.36 ppm on post exposure at 24 h and 48 h. LC90 value calculated was found to be 4015.8 ppm and 3430.43 ppm at 24 h and 48 h respectively. A statistically significant value was also found at 24 h and 48 h between control and the extract with a value of P ≤ 0.01, P ≤ 0.005 respectively.

Table 1, 2 shows the larvicidal activity of n-Butanol extract of *A. indica* against 3rd instar larvae after 24h and 48h treatment at 1000 ppm-5000 ppm. Among the five different concentration 3000 ppm (48 h), 4000 ppm (24 h and 48 h) and 5000 ppm (24 h and 48 h) recorded 100% larval mortality compared to other concentration recording the LC50 value. The 24h showed the highest LC50 value of about 2276.5 ppm compared to 48 h 2213.36 ppm. The dose dependent result suggested that the value LC90 was identified as 3628.19 and 2518.86 ppm for *Ae. albopictus* at 24 h and 48 h respectively. The "P" values was significant (P 0.01, 0.01) when compared to the control after 24 h and 48 h treatment.

The larval mortality of *Ae. albopictus* after the treatment of ethylacetate extract of *A. indica* was observed. Table 1, 2 provides the result of larval mortality of *Ae. albopictus* (third instar) after the treatment of *A. indica* ethyl acetate at different concentration (1000 ppm-5000 ppm). No mortality was noted by treatment of *A. indica* at 1000 ppm, 10% mortality was noted at 24h at 2000 ppm, whereas it has been increased to 30% at 48h. Similar trend has been noted for 3000 ppm(30%,24h,70%,48h) and 4000 ppm(40%,24h,50%,48h)

and 3000 ppm(60%,24h,100%, 48h). The LC50 and LC90 values were represented as followed LC50 values of third instar larvae was 4472.14 ppm in 24h and the LC90 values of 3rd instar larvae was 2469.61 ppm in 24h and 4215.84 ppm in 48h. The chisquare value significant at 24h ($P \leq 0.01$) 48h ($P \leq 0.01$).

The ethanol and aqueous extract of *A. indica* does not cause any mortality at varying concentrations against the third instar larvae of *Ae. albopictus*.

Table 1: Percentage mortality at different time interval in larvicidal activity

Extract	Concentration	% mortality at different time interval		Mortality percent means (\pm SE) at two intervals	
		24h	48h	24h	48h
Petroleum ether Extract	1000 ppm	20	30	20.33 \pm 0.57	30.33 \pm 0.57
	2000 ppm	40	40	40.33 \pm 0.57	40.66 \pm 0.57
	3000 ppm	40	50	50.66 \pm 0.57	51.00 \pm 0.00
	4000 ppm	90	100	90.33 \pm 0.57	100.00 \pm 0.00
	5000 ppm	100	100	100.00 \pm 0.00	100.00 \pm 0.00
Chloroform extracts	1000 ppm	20	40	19.66 \pm 0.57	40.66 \pm 0.57
	2000 ppm	30	40	30.00 \pm 1.00	40.33 \pm 0.57
	3000 ppm	70	70	70.33 \pm 0.57	70.00 \pm 1.00
	4000 ppm	90	100	89.66 \pm 0.57	100.00 \pm 0.00
	5000 ppm	90	100	90.00 \pm 1.00	100.00 \pm 0.00
n-Butanol extracts	1000 ppm	-	10	0.00 \pm 0.00	10.00 \pm 1.00
	2000 ppm	20	20	19.33 \pm 0.57	19.66 \pm 1.52
	3000 ppm	30	100	30.66 \pm 1.15	100.00 \pm 0.00
	4000 ppm	100	100	100.00 \pm 0.00	100.00 \pm 0.00
	5000 ppm	100	100	100.00 \pm 0.00	100.00 \pm 0.00
Ethyl acetate extracts	1000 ppm	-	-	0.00 \pm 0.00	0.00 \pm 0.00
	2000 ppm	20	30	20.33 \pm 0.57	29.33 \pm 0.57
	3000 ppm	30	70	30.66 \pm 1.15	69.66 \pm 0.58
	4000 ppm	40	50	40.66 \pm 1.15	49.33 \pm 1.15
	5000 ppm	60	100	60.66 \pm 0.557	100.00 \pm 0.00
Ethanol and aqueous extracts	1000 ppm-5000 ppm	0	0	0.00 \pm 0.00	0.00 \pm 0.00
Control	1000 ppm-5000 ppm	0	0	0.00 \pm 0.00	0.00 \pm 0.00

Values represent the mean of three replicates. Values are given as mean \pm Standard derivation, ppm indicates Parts per million, h-hour.

Table 2: Larvicidal activity of *Acalypha indica* leaf extract against *aedes albopictus*

Extracts	95% confidence limits				Regression equation		Chi-square		P value	
	24h		48h		24h	48h	24h	48h	24h	48h
	LC50(LCL-UCL)	LC90(LCL-UCL)	LC50(LCL-UCL)	LC90(LCL-UCL)						
Petroleum ether	2805.43 (2253.13-3493.18)	3825.14 (3688.82-4296.19)	2376.11 (1737.07-3250.23)	3327.80 (2891.27-3690.76)	Y=2.815+0.005x	Y=2.713+0.054x	5.36	6.18	0.05	0.01
Chloroform	2276.5 (1835.23-2823.88)	4015.8 (3919.33-4238.28)	2213.36 (1896.7-2582.89)	3430.43 (3184.72-3846.08)	Y=2.087+0.127x	Y=1.452+0.041x	3.8	7.29	0.01	0.01
Ethyl acetate	4472.14 (3831.54-5219.83)	-	2469.61 (2179.77-2797.98)	4215.84 (4001.19-4675.74)	Y=5.285+0.017x	Y=3.715+0.094x	4.42	8.93	0.01	0.01
n-butanol	2777.88 (2490.72-3098.15)	3628.19 (3320.22-4087.09)	2225.61 (1986.91-2492.99)	2518.86 (2279.61-2994.06)	Y=3.486+0.066x	Y=1.897+0.072x	7.53	10.8	0.01	0.01

LCL-lower confident level, UCL-upper confident level, h-hours, P-Significant, LC50 lethal concentration that kills 50% of the exposed larvae, LC90 lethal concentration that kills 90% of the exposed larvae.

DISCUSSION

In the present study, effect of *A. indica* leaf extract on *Ae. albopictus* third instar larvae were compared in terms of relative potential with control. As the result indicated that *A. indica* extract with petroleum ether, chloroform, n-Butanol, ethylacetate, ethanol and aqueous, applied in different concentrations have a different larvicidal effect against third instar *Ae. albopictus* larvae under laboratory condition. The 3rd instar larvae showed restless movement for short time and then settled at the bottom of the disposable cup and slowly dead.

In the laboratory after 24 h exposure, the n-butanol and chloroform extracts obtained from the leaf of *A. indica* at 5000 ppm have shown higher percentage mortality while the petroleum ether extract and ethyl acetate extract of *A. indica* had moderate percentage mortality.

The mean percentage mortality of 3rd instar larvae on treatment with n-butanol, chloroform extract at 5000 ppm were 100% respectively (48 h). While petroleum ether extract with the sample concentration showed 100% (24 h and 48 h). Where as ethylacetate extract of *A. indica* showed 60% (24 h) and 100% (48 h). The

percentage larval mortality of 3rd instar larvae of *Ae. albopictus* increased as time of exposure increased. Ethanol and aqueous extract of *A. indica* at 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm showed no mortality after 24 h and 48 h exposure under laboratory condition. Alwala et al., [29] reported that the methanolic extracts of *Mangifera indica* exhibited no toxicity while the other extract (aqueous and acetone extract) showed toxicity against *A. aegypti*, dengue vector. The result reveals that the toxic activity may be due to change in the selection of the solvent [30] Similarly, Adebayo et al., [31] examined the bioactivity of methanolic extracts of *Mangifera indica* leaf against *A. aegypti* and found to be more effective against 4th instar larvae.

From previous report it was found that hexane and chloroform extract from bark of *A. squamosa* showed 84 and 100% mortality against 4th instar *An. stephensi* after 24 h exposure. Kamaraj et al., Sukumaret al., [32-33] reported the variation in toxicity against the larval instar may be due to presence of phytochemical compound which varied with plant part, solvent used and the extract concentration.

According to Abbas et al., [34] the larvicidal activity of *Tagetes minuta* was possible due to the effective components like terpenoids, aglycon, flavanoids and saponin. Similarly, this study showed that the larvicidal effect of *A. indica* was possibly because of alkaloids, flavanoids, Tannin and phenolic compounds, steroids and terpenoids. Also Marcard et al., [35] reported that the larvicidal mortality was positively correlated with concentration and duration of exposure. Similarly in this study, as the concentration of extract increases from 1000 ppm to 5000 ppm, the percentage mortality also increases almost five fold, from initial concentration to final concentration. Several investigators also confirmed positive relationship between larval mortality and time factor [36-39]. As the time of exposure increases, the percentage larval mortality of 3rd instar larvae of *Ae. albopictus* were also found to be increases. The mortality of petroleum ether and chloroform extract of *A. indica* at 4000 ppm was 100% after 48 h exposure. While mortality of n-Butanol extract at 3000 ppm was 100% after 48 h exposure. Whereas ethylacetate extract showed 100% mortality at 5000 ppm after 48 h exposure under laboratory condition.

This result also illustrated that all different active chemical compound in the leaf part of *A. indica* was responsible for diverse activity against *Ae. albopictus*. Singh and Prakash, [40] reported that larvicidal activity was observed against *A. stephensi* when six different concentration were used (5, 10, 20, 30, 40 and 50 mg/l). Similarly in the present study six different concentration were used (1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm) against *Ae. albopictus*.

In the present study it can be pointed out that leaf of *A. indica* exerted larvicidal properties. LC50 ranged from 2777.88 ppm-4472.14 ppm when using n-butanol, chloroform and ethyl acetate as solvent within the 24 h exposure. A range of LC50 values between 2225.61 ppm-2469.61 ppm within 48 h exposure period. Sakthivadivel and Thilagavathy, Tang et al., [41,42] has also observed LC50 range value between 30.47 mg/ml and 13.58 mg/ml when using petroleum ether extract at 24 h from seeds of *A. meucana*. However our present investigation observed a low LC50 value (LC50 ranged from 2376.11 ppm (48 h)-2805.43 ppm (24 h)) when using petroleum ether as solvent from leaf of *A. indica*. Similar a low LC50 value (20 mg/ml-50 mg/ml) were observed by when using hexane extract from seeds of *A. mexicana* and stem bark of *P. perniciosum* at 48 h exposure respectively. Bilal et al., [43] successfully tested the larvicidal activity of selected plant extracts against *A. albopictus*. All the extracts showed the moderate activity with a lowest LC50 value at the dose of 363.7, 377.5 and 403.5 mg/l respectively, for 24 h and the value get reduced to 263.95, 300.8 and 342.2 mg/l after 48 h respectively. As similar to previous research our report also documented a LC50 value of 2276.5, 2777.88, 2805.43 and 4472.14 for 24 h exposure and the value get reduced to 2225.61, 2213.36, 2376.11 and 2469.61 ppm for 48 h exposure. Among the tested plant extract n-butanol, chloroform, ethylacetate, and petroleum ether of *A. indica* (leaf) demonstrated remarkable larvicidal activity against *Ae. albopictus*. Thus this plant extract provide the bases to act as alternative to synthetic insecticide in control

programme of mosquito. To avoid the detrimental effects caused by chemical agents against mosquito vector, natural and nontoxic bioactive compound from plant origin can be used as an alternative control agent toward mosquito vectors [44]. Hereby, this study finally proposed a new alternative floral biopesticide in the mosquito management rather than using conventional chemical control. Chemical control is more expensive than the biological control (plant origin) and is also more effective and target specific [45] than conventional chemical control.

CONCLUSION

In conclusion, this study clearly reveals that *A. indica* leaf extract could be one of the new potential bio larvicidal agent against the vector *Ae. albopictus*. Therefore the present result also emphasized the need for further research and investigation to isolate and identify the most bioactive compound and their activity against mosquito vector *Ae. albopictus*.

ACKNOWLEDGEMENT

The third author thank Science and Engineering Research Board (SERB), Govt. of India for the award of SERB N-PDF (File No. PDF/2015/000375/IS) for financial support.

AUTHORS CONTRIBUTION

First Author act as a researcher, Second author did data analysis, Third author contributed some financial support for publication and Fourth author acted as supervisor for this work.

CONFLICTS OF INTERESTS

Declared none

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How to cite this article

- Ashwini U, Taju G, Thirunavukkarasu P, Asha S. Pupal emergence inhibition activity of *Acalypha indica* leaf extract against dengue vector, *Aedes albopictus* mosquito. Int J Pharm Pharm Sci 2017;9(8): 114-118