



## Bioactivity of *Jatropha curcas* (L.) solvent extracts on key species of mosquitoes

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### Abstract

The larvicidal efficiency of acetone, petroleum ether and ethyl acetate extracts of *Jatropha curcas* (L.) was investigated against three different mosquito species viz., *Culex quinquefasciatus*, *Aedes aegyptii* and *Anopheles stephensi* under controlled conditions. The solvent extracts at 200, 400, 600, 800 and 1000 concentrations have influenced varied level of larvicidal activity against the tested mosquito species. The acetone extract @ 1000ppm have demonstrated the higher level of larvicidal activity (90.00%) than the petroleum ether extract and ethyl acetate extract at the same concentration against *Cu. quinquefasciatus*, *Ae. aegyptii* and *J. curcas*. The estimated LC<sub>50</sub> value assessed from the graded concentrations of acetone, petroleum ether and ethyl acetate extracts of *Jatropha curcas* against *Culex quinquefasciatus*, *Aedes aegyptii* and *Anopheles stephensi* have also demonstrated that the acetone extract was superior than other solvent extracts. The estimated LC<sub>50</sub> exerted by acetone solvent extract against *Cu. quinquefasciatus*, *Ae. aegyptii* and *J. curcas* was 440.42, 445.12 and 469.21 ppm respectively which was the lowest values than the estimated LC<sub>50</sub> exerted by petroleum ether and ethyl acetate extracts.

**Keywords:** *Jatropha curcas*, larvicidal action, mosquito, *Culex quinquefasciatus*, *Aedes aegyptii*, *Anopheles stephensi*

### Introduction

The species of mosquitoes could be approximately analysed as 3,500 grouped under 42 genera of which *Aedes aegyptii* Linn, *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say) were considered as major (WHO, 1992 [17]) The problem of mosquitoes in urban areas are being addressed by many agencies through multiple management strategies. Usage of chemical insecticides viz., organochlorines, organophosphate compounds and synthetic pyrethroid was the main focal point in the management of these dreaded pest in urban localities. These insects were taken care by the herbal products before the introduction of these poisonous synthetic insecticides, The herbal products are based on the pesticidal plants viz., Chrysanthemum, Pyrethrum, Nicotine, Azadirachtin, Quassia, Derris, Turpentine, Hellebore etc (Shaalaa *et al.*, 2005) [14]. Pesticidal plants through co-evolution along with insects, armed with many biochemicals to act against insects (Arivoli *et al.*, 2012) [2]. These pesticidal plants are accounted to be more than 2000 of which ~300 are fortified with plenty of secondary metabolites to act against insects (Remia and Logaswamy, 2009) [11]. In view of these arguments the present investigation was carried out to find out the efficacy of *Jatropha curcas* (L.) against the major species of mosquitoes.

### Material and Methods

#### Mass Production of Mosquitoes

The egg rafts of *Cu. quinquefasciatus* was obtained from CRME (ICMR Centre), Vridhachalam, Tamil Nadu and were brought to the laboratory. The egg rafts were placed in enamel trays (30×24×5 cm) containing 2:1 tap water. The colony was maintained at 75-85% RH, 27±2<sup>o</sup> C temperature and 14:10 light and dark photoperiod cycle (Murugan and Jeyabalan, 1999) [10]. The emerging larvae (wrigglers) were fed with powdered mixture of dog biscuits and baker's yeast in the ratio of 3:1. The trays with pupae were maintained in separate mosquito cages at 26±2<sup>o</sup>C and relative humidity of 85±3% under a photoperiod of 16:8h (L: D) for adult emergence. Cotton soaked in 10 per cent aqueous sucrose solution in a Petri dish and hang over inside the cage to feed adult mosquitoes. A baby chicken was kept inside the cage (3 hours) to offer blood meal for females. A plastic cup or tray filled with water was placed inside to empower the females to lay the eggs (Krishnappa *et al.*, 2012) [9]. The resultant eggs laid by the reared females were directly utilized for toxicity studies. Eggs, larvae and adult females were continuously available for the bioassays from these laboratory colonized mosquitoes. The other two species of mosquitoes *Ae. aegyptii* and *An. stephensi* were also mass cultured in the laboratory using the same methodology.

*Jatropha* leaves were collected from the plant and it was air dried in shady place in order to restore the active ingredients integral. Dried plant materials were powdered in an electrical blender for extraction and each of the powdered plant material (100 g) was soaked in 500 ml of three solvents viz., acetone, petroleum ether and ethyl acetate in a wide mouth conical flask or bottle and the mouth is closed airtight by non-absorbent cotton covered with aluminium foil sheet to avoid evaporation of solvents and kept for three days with shaking it thrice per day

(morning, afternoon and evening). The suspensions were filtered through Whatman filter paper No. 4. The filtered suspension was allowed to evaporate on a hot plate for two hours and the residues obtained was used as stock solution for bioassay experiments (Shivakumar *et al.*, 2013) <sup>[15]</sup>.

### Preparation of Stock Solution

The standard stock solutions were prepared at 1.0 per cent by dissolving the residues (1.0 g) in 100 ml of distilled water. From these stock solutions different concentrations were prepared and these solutions were used for larvicidal assessment.

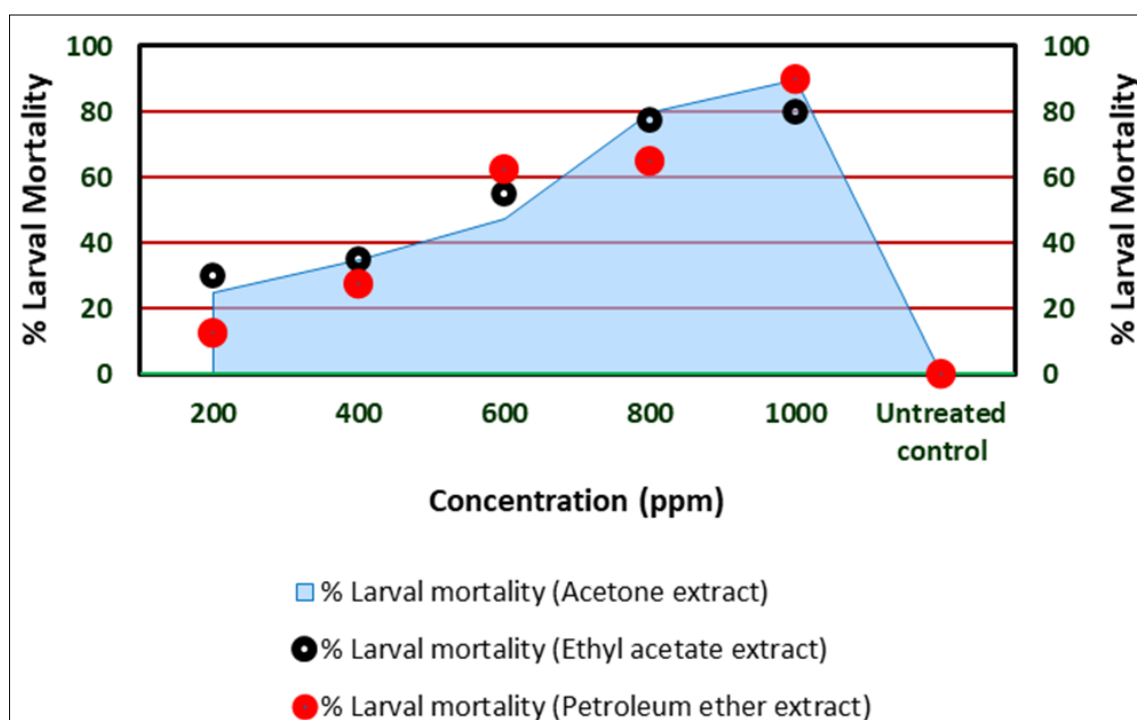
### Larvicidal Activity

Larvicidal activity of *Jatropha curcas* solvent extracts against the three species of mosquitoes was assessed by using the standard method (WHO, 1996) with slight modification. Ten numbers of late third instar larvae of mosquitoes were separately taken on a strainer with fine brush and transferred gently into 250 ml capacity disposable plastic cup containing 100 ml of water to treat in various concentrations (100, 200, 300, 400 and 500 ppm) of respective plant extracts from one per cent stock solution. Stock solutions of the extracts was mixed with Tween 80 (1%) as emulsifier to facilitate the dissolution of the material in water. The control experiment (1 ml distilled water and 1ml of Tween 80 in 100 ml of water) was also carried out parallelly with each replicates. Each experiment was replicated four times under completely randomized design carried out at room temperature of  $27\pm 3^{\circ}\text{C}$ . Mortality of larvae was observed and documented after 24 h of post treatment. The corrected mortality percentage was computed by applying Abbott's formula (Abbott, 1925) <sup>[1]</sup>. The collected data was processed by using probit analysis to arrive the LC50 and LC90 values (Finney, 1971) <sup>[4]</sup>.

### Results and Discussion

Experiments conducted on the larvicidal action of different concentrations of acetone, petroleum ether and ethyl acetate solvent extracts produced the following results.

#### Larvicidal Action of Solvent Extracts of *Jatropha curcas* on *Culex quinquefasciatus*



**Fig 1:** Larvicidal activity of *jatropha curcas* solvent extracts on *Culex quinquefasciatus*

*Jatropha curcas* acetone solvent fractions' (200-1000 ppm) influence on the larva of *Cu. quinquefasciatus* was explored scientifically and the experimental results exhibited positive results wherein the graded fractions exerted corresponding larval mortality within the range of 25.00 and 90.00 per cent with maximum effect by 1000ppm and minimum effect by 200 ppm. Data pertaining to the studies on the influence of petroleum ether extracts of *J. curcas* at different concentrations (200 to 1000 ppm) against *An. stephensi* imparted 12.50 to 90.00 per cent larval mortality with the maximum by 1000 ppm (90.00%) followed by 800, 600, 400 and 200 ppm with 65.00, 62.50, 27.50 and 12.50 per cent mortality respectively. Effect of different concentrations (200-1000ppm) of ethyl acetate extracts of *J. curcas* on *Cu. quinquefasciatus* exhibited larval mortality in the range between 30.00 and 80.00 per cent wherein the maximum (80.00%) was influenced by 1000 ppm concentration followed by 800, 600, 400 and 200 ppm with 77.50, 55.00, 35.00 and 30.00 per cent mortality of mosquitoes respectively.

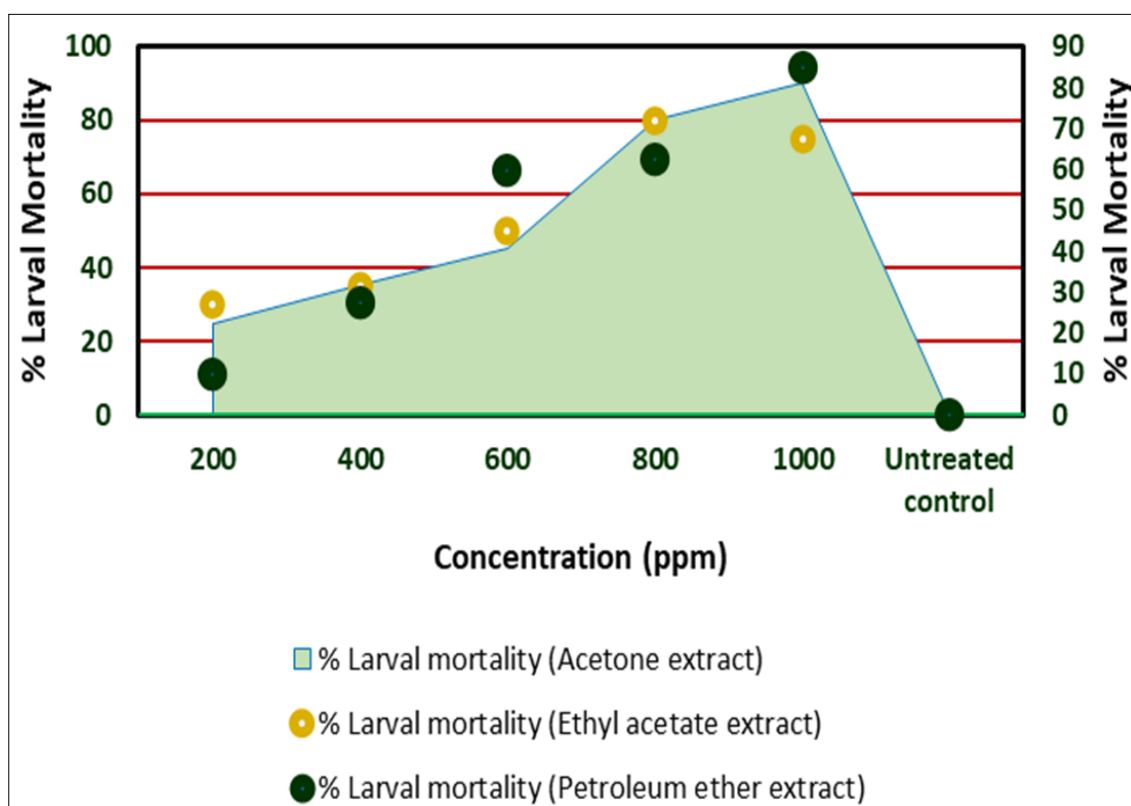
The estimated LC<sub>50</sub> and LC<sub>90</sub> value for the acetone extract of *J. curcas* against *Cu. quinquefasciatus* was 440.42 and 1335.54 ppm respectively and were within the lower and upper confidence limits with significant difference between the treatments statistically at 0.498 (X<sup>2</sup> value) at P<0.05. The estimated LC<sub>50</sub> value of 506.00.53 ppm (Lower Confidence Limit 385.44-664.08 Upper Confidence limit) and LC<sub>90</sub> value of 1272.98 ppm was attained by *J. curcas* petroleum ether extract against *Cu. quinquefasciatus* which showed significant difference between the treatments at P<0.05 (0.758 X<sub>2</sub>). The appraised LC<sub>50</sub> value was 435.52ppm by *J. curcas* ethyl acetate against *Cu. quinquefasciatus* and it was within the lower and upper limit of 291.63ppm and 650.41ppm concentration and showed statistical significance between the concentrations with 0.715 X<sub>2</sub> value at p<0.05 (Table 1).

**Table 1:** Larvicidal activity of three solvent extracts of *Jatropha curcas* against *Culex quinquefasciatus*

Solvent	LCD <sub>50</sub> (LCL & UCL)	LCD <sub>90</sub> (LCL & UCL)	X <sup>2</sup>
Acetone	440.42 (320.85-604.56)	1335.54 (972.95-1833.25)	0.498*
Petroleum Ether	506.00 (385.44-664.08)	1272.98 (969.93-1670.69)	0.758*
Ethyl Acetate	435.52 (291.63-650.41)	1815.50 (1215.68-2711.26)	0.715*

\*Significant at P<0.05 ; LCL=Lower Confidence Limits; UCL=Upper Confidence Limits X<sup>2</sup> = Chi square

#### Larvicidal Action of Solvent Extracts of *Jatropha curcas* on *Aedes aegypti*



**Fig 2:** Larvicidal activity of *Jatropha curcas* solvent extracts on *Aedes aegypti*

Investigations on the effect of acetone solvent extract of *J. curcas* at different concentrations (200 to 1000 ppm) exerted 25.00 to 90.00 per cent mortality of *Ae. aegypti* larva. The maximum mortality was exerted by 1000 ppm (90.00% mortality) followed by 800, 600, 400 and 200 ppm with 80.00, 45.00, 35.00 and 25.00 per cent mortality respectively wherein no mortality was recorded in control treatment. Evaluation experimental data of *J. curcas*' petroleum ether extracts influence on *Ae. aegypti* exhibited 10.00 to 85.00 per cent larval mortality with the maximum by 1000 ppm (85.00%) and minimum (10.00) by 200 ppm. Exploration of graded concentrations of ethyl acetate extract of *J. curcas* for their larval mortality effect on *Ae. aegypti* turned up with the following findings. The larval mortality exerted by the solvent extract ranged between 7.50 and 70.00 per cent.

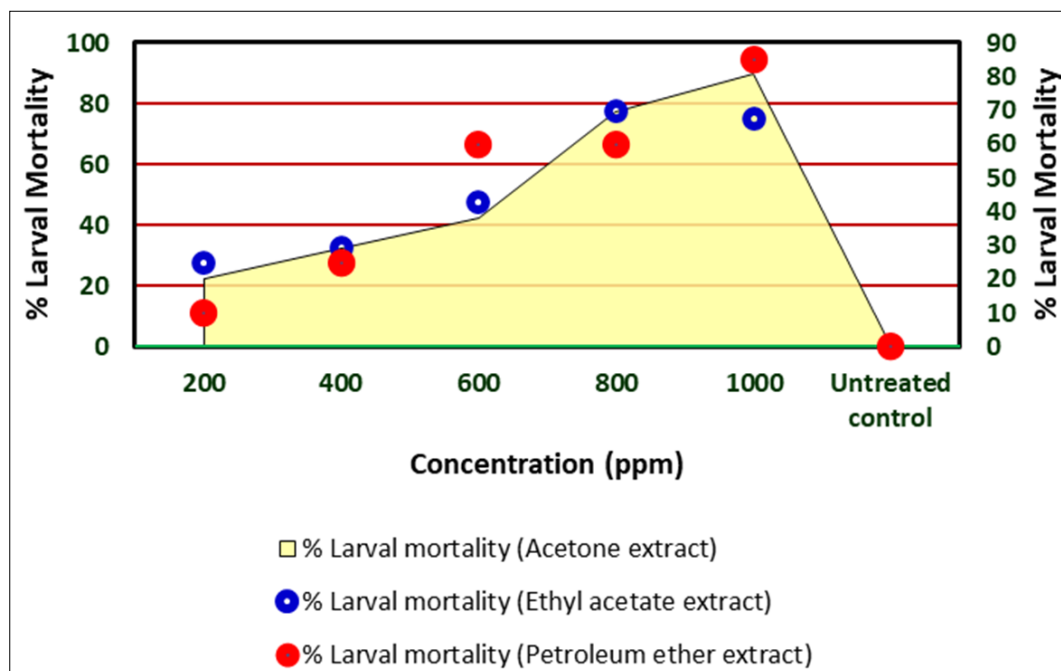
The LC<sub>50</sub> value of 445.12 ppm (Lower Confidence Limit 323.96-611.58 Upper Confidence limit) and LC<sub>90</sub> value of 1361.45 ppm attained by *J. curcas* acetone extract against *Ae. aegyptii* showed significant difference between the treatments statistically at 0.458 as X<sup>2</sup> value at P<0.05. The *J. curcas* petroleum ether extract at different concentrations against *Ae. aegyptii* displayed estimated LC<sub>50</sub> value as 543.21 ppm (Lower Confidence Limit 411.33 and 717.39 Upper Confidence limit) wherein the LC<sub>90</sub> value was 1386.30 ppm which showed significant difference statistically at P<0.05. The estimated LC<sub>50</sub> and LC<sub>90</sub> values for the graded concentrations of *J. curcas* ethyl acetate extracts (100, 200, 400, 600, 800 and 1000ppm) against *Ae. aegyptii* were 454.00 and 2044.30 ppm respectively which exhibited statistical significance at P<0.05 (Table 2).

**Table 2:** Larvicidal activity of three solvent extracts of *Jatropha curcas* against *Aedes aegyptii*

Solvent	LCD <sub>50</sub> (LCL & UCL)	LCD <sub>90</sub> (LCL & UCL)	X <sup>2</sup>
Acetone	445.12 (323.96-611.58)	1361.45 (990.89-1870.59)	0.458*
Petroleum Ether	543.21 (411.33-717.39)	1386.30 (1049.72-1830.80)	0.895*
Ethyl Acetate	454.00 (298.76-689.88)	2044.30 (1345.30-3106.48)	0.683*

\*Significant at P<0.05 ; LCL=Lower Confidence Limits; UCL=Upper Confidence Limits X<sup>2</sup> = Chi square

### Larvicidal action of solvent extracts of *Jatropha curcas* on *Anopheles stephensi*

**Fig 3:** Larvicidal activity of *Jatropha curcas* solvent extracts on *Anopheles stephensi*

Investigations carried out to estimate the larvicidal activity of acetone solvent fractions of *J. curcas* against *An. stephensi* revealed that the larval mortality was in the range of 22.50 to 90.00 per cent wherein the highest concentration exerted the maximum effect (90.00%) followed by 800 ppm with 77.50 per cent while there was no mortality in the control. Experiments conducted to evaluate the *J. curcas*'s petroleum ether extracts on *An. stephensi* larva at different concentrations (200 to 1000 ppm) influenced 10.00 to 85.00 per cent mortality with the maximum by 1000 ppm (85.00%) followed by 800, 600, 400 and 200 ppm with 60.00, 60.00, 25.00 and 10.00 per cent mortality respectively. The ethyl acetate fractions from 200 to 1000 ppm exerted 27.50 to 75.00 per cent larval mortality against *An. stephensi* with the maximum (75.00) effect by 1000 ppm and the minimum effect by 200 ppm (27.50%).

Estimated LC<sub>50</sub> value arrived from the experiment with the testing of acetone solvent fractions (concentrations 200 to 1000 ppm) of *J. curcas* against *An. stephensi* was 469.21ppm which was in between the lower (419.78) and upper confidence limit (733.15) at p<0.05. Research on the evaluation of *J. curcas*'s petroleum ether extracts on *An. stephensi* larva at different concentrations (200 to 1000 ppm) influenced with the LC<sub>50</sub> value of 554.76 ppm (Lower Confidence Limit 419.78-733.15 Upper Confidence limit) and LC<sub>90</sub> value of 1426.25 ppm which showed significant difference between the treatments at P<0.05 (Table 17). Investigation for LC<sub>50</sub> and LC<sub>90</sub> estimation of ethyl acetate fractions of *J. curcas* (200-1000ppm) against *An. stephensi* resulted 502.14 and 2403.50 ppm as their corresponding LC<sub>50</sub> and LC<sub>90</sub> values respectively (Table 3).

**Table 3:** Larvicidal activity of three solvent extracts of *Jatropha curcas* against *Anopheles stephensi*

Solvent	LCD <sub>50</sub> (LCL & UCL)	LCD <sub>90</sub> (LCL & UCL)	X <sup>2</sup>
Acetone	469.21 (344.27-639.70)	1403.15 (1029.36-1912.67)	0.415*
Petroleum Ether	554.76 (419.78-733.15)	1426.25 (1079.22-1884.87)	0.811*
Ethyl Acetate	502.14 (325.68-774.21)	2403.50 (1558.87-3705.78)	0.682*

\*Significant at P<0.05 ; LCL=Lower Confidence Limits; UCL=Upper Confidence Limits X<sup>2</sup> = Chi square

### Discussion

Comparing the acetone, petroleum ether and ethyl acetate extracts of *J. curcas* against three species of mosquitoes viz., *Cu. quiquefasciatus*, *Ae. aegyptii* and *An. stephensi* revealed that the acetone extract at the maximum concentration exerted the highest larval mortality than petroleum ether and ethyl acetate extracts and these results are in accordance with the reports of Selvakumar *et al.* (2015)<sup>[13]</sup> who investigated the mosquitoes'

larvicidal, ovicidal and pupicidal activities with solvent extracts of *Annona reticulata* against *Ae. Aegypti*, *An. stephensi* and *Cu. quinquefasciatus*. In laboratory conditions, these plant extracts evinced strong ovicidal activity against the egg of *Ae. aegyptus*. *An. stephensi* and *Cu. quinquefasciatus* which did not show any hatchability, therefore 200ppm concentration level was considered the best against the mosquitoes. The earliest report of the usage of plants were aplenty wherein the plant alkaloids viz., nicotine and anabasine from tobacco, methyl anabasine and Iupinine from *Anabasis aphylla* utility in mosquito control (*Cu. pipiens*, *Cu. territans*, and *Cu. Uinquefosciaifus*) was credited to Campbell *et al.* (1933) [3]. In line, Haller (1940) [7] illustrated that Amur cork tree fruit (*Phellodendron arnurense*) extract has exhibited mosquito larvicidal activity. Wilcoxon *et al.* (1940) [18] stated that ‘filicin’ a toxic component ‘phloroglucinol propyl ketone’ extracted from male fern (*Aspidium filix-mas*) was found to be toxic against *Cx. quinquefasciatus*. Hartzell and Wilcoxon (1941) [8] investigated that more than 150 plant species for their toxicity against mosquitoes and proved that majority of them are toxic against mosquitoes.

The secondary metabolites present in the botanical plants provided the defense against pests where the later along with various environmental factors created selection pressure to herbivores to withstand against damage. As reported earlier many groups of phytochemicals such as steroids, alkaloids, phenolics, essential oils, terpenoids etc. were demonstrated to have potential anti-insect properties (insecticidal, repellent, antifeedant, oviposition deterrent, insect growth regulator) (Shallan *et al.*, 2005). Different authors listed different number of plants having anti-insect properties, Roark (1947) [17] enlisted 1,200 plant species whereas Sukumar *et al.* (1991) detailed 344 plant species pronouncing mosquitocidal activity. Govindarajan *et al.* (2011) [5] studied about the mosquito larvicidal, ovicidal properties from the botanical plant extracts against the mosquitoes like *An. stephensi*, *Ae. aegypti* and *Cu. quinquefasciatus*. The leaf of *Ervatamia coronia* and *Caesalpina pulcherrima* are used as larvicidal and ovicidal agents against mosquitoes. The benzene extract of *E. coronia* showed highest larvicidal effects to *Ae. aegypti*, *An. stephensi* and *Cu. quinquefasciatus*. Govindarajan *et al.* (2012) [6] opined that *Pithecellobium dulce* Benth larvicidal and ovicidal efficacy results, may be considered to include as a better alternative for controlling *An. stephensi* and *Ae. aegypti*. *Jatropha curcas* has been already used in the medicinal, pharmaceutical and other ayurveda industry for the preparation of drugs and nowadays researches were in producing biodiesel from it. This study proved that it has also anti-mosquito activity which can be further investigated to prepare drugs and botanical pesticides for better pest management in Agriculture.

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