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# Larvicidal and pupicidal effect of ethanolic extract of the aerial roots of *Rhaphidophora aurea* intertwined over four different host trees against *Culex quinquefasciatus* say

**P Arulpriya,[1] P Lalitha[1]* and N Aarthi[2]**

[1]Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women University (Estd. u/s 3 of UGC Act 1956), Coimbatore – 641043.
[2]Department of Zoology, School of Life Science, Bharathiar University, Coimbatore-641046.

## ABSTRACT

Phytochemicals have proven that they are potential mosquito control agents and also alternative to synthetic insecticides. Different concentration of the ethanolic extract of aerial roots of *Rhaphidophora aurea* (Money plant) intertwined over *Lawsonia inermis* (Mehandhi)*, Areca catechu* (Betal nut tree), *Cocos nucifera* (Coconut tree) and *Azadirachta Indica* (Neem tree) have been tested on the first(I), second(II), third(III), fourth(IV) instar larvae and pupae of *Culex quinquefasciatus* say. Lethal concentration ($LC_{50}$ and $LC_{90}$) were also worked for the different larval and pupal stages. The larval and pupal density decreased after the treatment with the extract. The results obtained indicate better activity of the ethanol extract of aerial roots of *Rhaphidophora aurea* climbed over *Lawsonia inermis* than the ethanol extract of aerial roots of *Rhaphidophora aurea* climbed over *Areca catechu*, *Azadirachta Indica* and hence these extracts can be suitable alternatives to synthetic insecticides. The ethanol extract of aerial roots of *Rhaphidophora aurea* climbed over *Cocos nucifera* showed zero mortality in all the stages and also no significant repellent bioassay.

**Keywords:** Larvicidal assay. Pupicidal assay. *Culex quinquefasciatus*. *Rhaphidophora aurea.*

***Corresponding author***

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## INTRODUCTION

Mosquitoes are the dreadful insects of mankind of all the diseases that transmit diseases, mosquitoes represent the greatest menace. *Culex* species are most significant vector for the transmission of *Wuchereria bancrofti* which is responsible for lymphatic filariasis [1, 2]. Lymphatic filariasis represents a major, vector borne public health and put at risk more than a billion people in more than 80 countries. It is estimated that 1.2 billion (20% of the world's population) are at risk of acquiring infection, one third of these infected live in India, one third in Africa and most of the remainder in Asia, the Pacific and Americas [3].

Chemical control is an effective strategy used extensively in mosquito control program. Many kinds of toxic chemical compounds to mosquitoes include organochlorine, organophosphorus, carbamates, pyrethroids, respectively. However, indiscriminate usage of chemicals in the breeding site and also used in the form of adulticides, fumigants, repellents and residual spray the rate of mosquito breeding are increased [4]. The continuous usage of chemicals disrupts natural enemies and also let to outbreaks of some insect species [5]. The problems of high cost and development of resistance in many vector mosquito species to several synthetic insecticides have revived interest in exploiting the pest control potential of plants [6].

Plant derivatives are highly toxic to many insect species without being phytotoxic. More than 2000 plant species are known to possess some insecticidal properties [7]. A considerable number of plant derivatives have shown to be effective against mosquitoes with a safe manner. Though several plant species from different families have been reported for mosquitocidal activity, only a few botanicals have moved from laboratory to field use which might be due to the presence of phytochemicals when compared to synthetic insecticides [8].

Prohibitive expenditure to meet the challenges of getting higher resistance in insects, resurgence of pests and escalating environmental pollution caused by synthetic pesticides call for the discovery of less-expensive, nonhazardous alternatives in the management of insect-pests. Obviously naturally occurring insecticides play a more prominent role in pest control programs in the future [9].

*Rhaphidophora aurea* is a popular ornamental foliage plant belonging to *Araceae* family. It is very efficient in removing indoor pollutant such as formaldehyde, xylene and benzene. This plant has a special characteristic of host gust relationships, so the roots are great concern. The roots of the plant are used traditionally for the treatment of all types of eruptive boils and the juice is used for quicker healing of accidental wounds, filariasis and toxic viral fevers [10].

The conventional chemical pesticides have resulted in the development of resistance, undesirable effects on non-target organism and fostered environmental and human health concern. An alternative approach for mosquito control is the use of natural products of plant origin. Hence the present study, an attempt was made to establish the larvicidal, pupicidal and

repellent properties of ethanol extract of aerial roots of *Rhaphidophora aurea* intertwined over four different host trees against *Culex quinquefasciatus* as target species.

## MATERIALS AND METHOD

### Plant collection and extraction

Aerial roots of *Rhaphidophora aurea* intertwined over the *Lawsonia inermis* (MM) and *Azadirachta indica* (MN) were collected from Coimbatore and *Areca catechu* (MB) and *Cocos nucifera* (MC) was collected from Palakkad District. The botanical identification (BSI/SC/5/23/09-10/Tech- 1534) was carried by Dr G.V.S.Murthy, Joint Director, Botanical survey of India, Coimbatore. The defatted aerial roots of *Rhaphidophora aurea* were extracted with ethanol by refluxing with suitable volume. The extracts were distilled separately by using rotary flash evaporator and kept in a refrigerator than used for the studies.

### Stock solution

Stock solution (1%) was prepared with 200 mg residue in 20 mL ethanol and was kept in a screw-cap vial coverd with aluminum foil over its mouth. The stock solution was then serially diluted ten-fold in methanol (2 mL solution to 18 mL solvent) and test concentrations were obtained by adding 0.1–1.0 mL of the appropriate dilution to 100 mL distilled water [11]. One gram of the plant residue was dissolved in 100 ml of methanol (stock solution) considered as 1% stock solution. From this stock solution different concentrations were prepared ranging from 30, 50, 100, 125 and 150 ppm respectively.

### Larvicidal and pupicidal bioassay

*Culex quinquefasciatus* was used to test the larvicidal and pupicidal activity. The eggs were collected from in and around Coimbatore districts (Sewage water bodies) with the help of 'O' type brush. It was maintained at conditions 27$\pm$ 2$00^0$C and 80% $\pm$ 5 relative humidity less than 12 L: 12 D cycles. These eggs were brought to the laboratory and transferred to 18 X 13 X 4 cm size enamel trays containing 500 ml of water for larval hatching. The mosquito larval and pupal culture was maintained in the laboratory. The plastic jars will be kept in 90 X 90X 90 cm size mosquito cage for adult emergence. The cage is made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part as covered with a muslin cloth bottom of the cage is fitted with 10% sugar solution for a period of three days. The adult female mosquitoes were allowed to feed on the blood of a rabbit (exposed on the dorsal side) for two days to ensure adequate blood feeding for 5 days. After blood feeding, the enamel trays with water from the culture trays was kept in the cage for the adults to lay eggs.

A laboratory colony of *Culex quinquefasciatus* larvae were used for the larvicidal activity. Twenty-five numbers of first, second, third and fourth instar larvae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1ml of desired concentration of plant

extracts were added. Larval food was given for the test larvae. At each tested concentration 2 to 5 trials were made and each trial consists of three replicates. The control was setup by mixing 1ml of acetone with 249 ml of dechlorinated water. The larvae exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula.

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

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

A laboratory colony of, *Culex quinquefasciatus* pupae were used for pupicidal activity. Twenty numbers of freshly emerged pupae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1ml of desired plant extract concentrations was added. Five replicates were setup for each concentration and control was setup by mixing 1ml of acetone with 249ml of dechlorinated water. The control mortality was corrected by Abbott's formula

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

$$\text{Percentage mortality} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \times 100$$

**Repellent assay**

The repellent dose - protection time response method was used. Approximately 1 h prior to the start of a test, 100, 3-4 day-old blood-starved female *Culex quinquefasciatus* (100) were placed into a net cage (45 cm W x 45 cm H x 45 cm L). Then, both arms of a human test subject were washed with ethanol and allowed to air dry. Three doses of MM, MB, MC and MN were tested (1, 2.5, and 5 mg/cm$^2$). A single dose was applied to the forearm skin of a test subject in each test. The other forearm was used as a negative control. At the beginning of a test, the control and treated arms were introduced simultaneously into the cage. The number of mosquitoes that landed on the exposed skin on each arm in 3 minutes was recorded at 30 minute intervals between 18.00 h and 06.00 h. Each dose of *plant extract* was tested for repellency 5 times. The effectiveness of the extract was assessed by determining the percent protection against mosquito landing that it provided on the treated arm compared with the untreated arm.

The average larval mortality data were subjected $LC_{50}$, $LC_{90}$, regression equation and 95% confidence limit of lower confidence of limit (LCL) and Upper confidence limit (UCL) were calculated from toxicity data by using probit analysis [12].

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## RESULT AND DISCUSSION

*Culex quinquefasciatus*, the potential vector of bancroftian filariasis is the most widely distributed mosquito in India. It is responsible for major public health problem in India with around 31 million microfilaraemics, 23 million cases of symptomatic filariasis, and about 473 million individuals potentially at risk of infection [13].

The results of the larval and pupal susceptibility of *Culex quinquefasciatus* using the ethnolic extract of MM, MB, MC, MN are presented in figure 1, 2, and 3. There is no mortality was observed in the control and MC extract. The ethanol extract of MM, MB and MN were effective against larvae and pupae mosquito. The effect of larval and pupal mortality was concentration dependent.

From the figure 1 showed that the larval mortality of the MM extract did not exceed 50% at the concentration 50 ppm, except first instar (52). This indicating no significant toxicity to *Culex quinquefasciatus*. While at the concentration 150 ppm the extract caused 95% larval mortality. In the pupal mortality of MM extract did not exceed 50% at the concentration of 150 ppm indicating significant toxicity. Exposure time also has crucial role in causing toxicity. It is observed that as the concentration and exposure time increases, progressively mortality also increases in severity.

From the figure 2 showed that the larvicidal mortality of second, third, fourth instar and pupae of MB extract did not exceed 50% at the concentration of 150 ppm. First instar indicates moderate toxicity because this will exceed 50% mortality. From the figure 3 the fourth instar and pupael mortality of MN extract did not exceed 50% but other instars showed moderate significant toxicity.

The $LC_{50}$, $LC_{90}$, 95% confidence limit and chi square value of MM, MB and MN against I, II, III, $IV^{th}$ and pupaee are presented in Table 1, 2 and 3. MM exhibited different larvicidal activities on different instars and pupicidal activities. The $LC_{50}$ and $LC_{90}$ value of first instar larvae were 59.52 ppm and 136.13ppm, second instar 68.74 ppm and 167.34 ppm, third instar 96.46 ppm and 202.32 ppm, Fourth instar 123.17 ppm and 237.48 ppm and pupae were 153.25 ppm and 282.68 ppm. Among the different larval stage first instar were more susceptible than other instar. The chi square value were significant at $p<0.005$ level

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In MB, the $LC_{50}$ and $LC_{90}$ value of first instar larvae were 140.31 ppm and 251.29 ppm, second instar 178.15 ppm and 331.64 ppm, third instar 202.34 ppm and 366.19 ppm, Fourth instar 257.26 ppm and 468.81 ppm and pupae were 153.25 ppm and 282.68 ppm. The chi square value were significant at $p<0.005$ level. From the $LC_{50}$ it was evident that higher concentration is required for 2nd, 3rd and 4th instars. In MN, the $LC_{50}$ and $LC_{90}$ value were represented as follows: first instar larvae were 70.53 ppm and 184.38 ppm, second instar 116.75 ppm and 259.63 ppm, third instar 158.37 ppm and 280.59 ppm, Fourth instar 182.26 ppm and 348.82 ppm and pupae were 199.35 ppm and 322.41 ppm. first instar were more susceptible than other instar and the higher concentration is required for 3rd and 4th instars. The chi square value was significant at $p<0.005$ level.

**Fig 1: Effect of variation in concentration of MM on the larvae and pupae of *Culex quinquefasciatus***

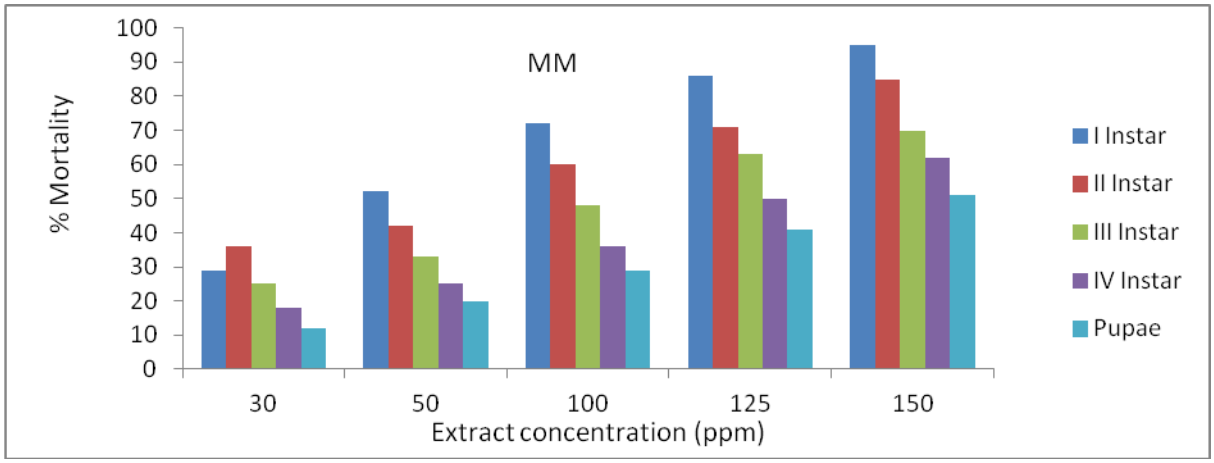


**Fig 2: Effect of variation in concentration of MB on the larvae and pupae of *Culex quinquefasciatus***

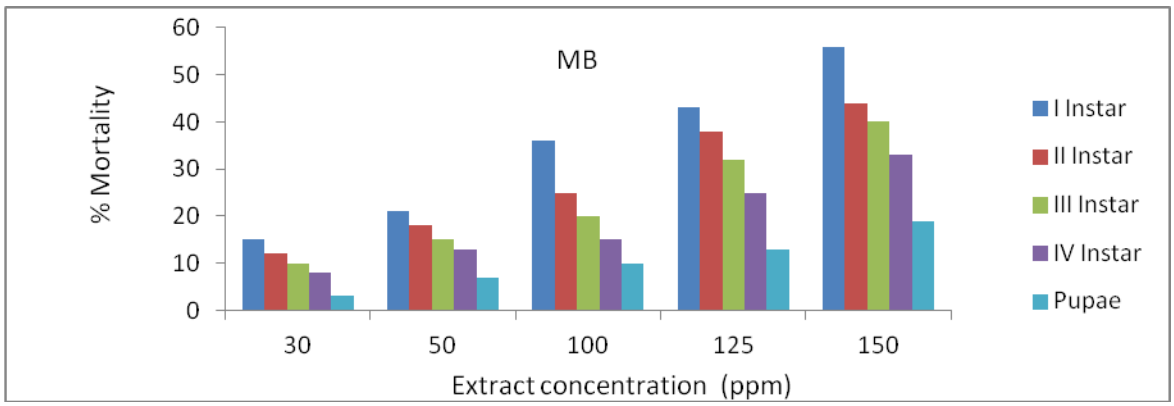

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**Fig 3: Effect of variation in concentration of MC on the larvae and pupae of *Culex quinquefasciatus***

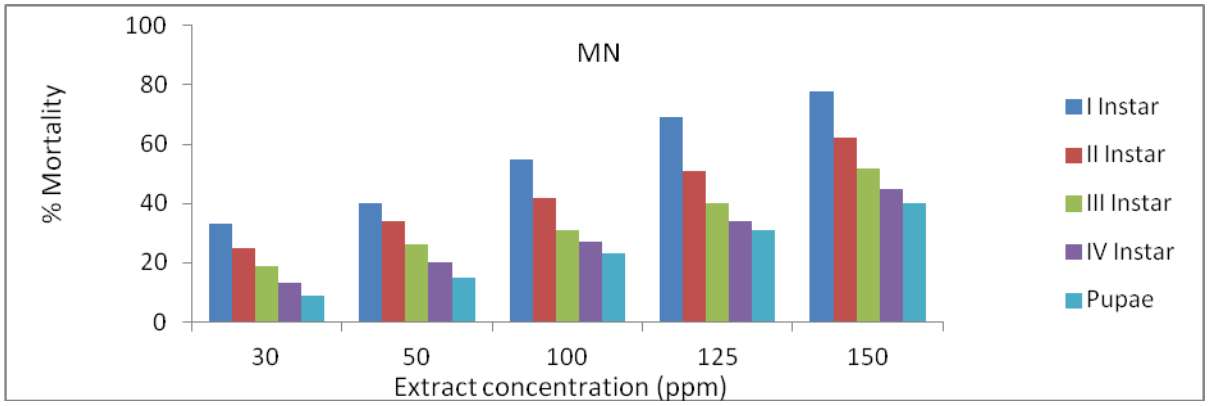


**Table 1 - Larvicidal and pupicidal activity (LC50, $LC_{90}$ and χ2-values ) of MM against *Culex quinquefasciatus***

| Larval in star | $LC_{50}$ | $LC_{90}$ | Reg. Equation | 95% Confidence limit | | | | Chi square value |
|---|---|---|---|---|---|---|---|---|
| | | | | LL | | UL | | |
| | | | | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | |
| I | 59.52 | 136.13 | y = 31.5x - 3.5 | 55.56 | 120.51 | 63.47 | 149.74 | 2.246* |
| II | 68.74 | 167.34 | y = 0.4003x + 22.373 | 73.61 | 154.60 | 73.61 | 180.07 | 2.297* |
| III | 96.46 | 202.32 | y = 0.3781x + 13.396 | 88.21 | 189.08 | 101.10 | 215.52 | 5.42* |
| IV | 123.17 | 237.48 | y = 0.3517x + 6.1971 | 115.85 | 223.73 | 130.48 | 253.22 | 7.27* |
| Pupae | 153.25 | 282.68 | y = 0.3075x + 2.6166 | 131.92 | 247.41 | 164.57 | 302.94 | 1.009* |

* - Significant at p<0.05

**Table 2 - Larvicidal and pupicidal activity (LC50, $LC_{90}$ and χ2-values ) of MB against *Culex quinquefasciatus***

| Larval in star | $LC_{50}$ | $LC_{90}$ | Reg. Equation | 95% Confidence limit | | | | Chi square value |
|---|---|---|---|---|---|---|---|---|
| | | | | LL | | UL | | |
| | | | | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | |
| I | 140.31 | 251.29 | y = 0.3275x + 4.4002 | 126.27 | 235.16 | 154.34 | 287.41 | 3.207* |
| II | 178.15 | 331.64 | y = 0.2612x + 3.6339 | 169.3 | 308.76 | 191.96 | 354.44 | 4.263* |
| III | 202.34 | 366.19 | y = 0.2375x + 1.792 | 187.10 | 339.57 | 215.48 | 392.80 | 6.378* |
| IV | 257.26 | 468.81 | y = 0.1888x + 1.6161 | 241.53 | 435.92 | 278.98 | 485.69 | 0.583* |
| Pupae | 153.25 | 282.68 | y = 0.1169x - 0.2376 | 137.92 | 254.41 | 168.57 | 310.94 | $0.096^{ns}$ |

* - Significant at p<0.05

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**Table 3 - Larvicidal and pupicidal activity (LC50, $LC_{90}$ and χ2-values) of MC against** *Culex quinquefasciatus*

| Larval in star | $LC_{50}$ | $LC_{90}$ | Reg. Equation | 95% Confidence limit | | | | Chi square value |
|---|---|---|---|---|---|---|---|---|
| | | | | LL | | UL | | |
| | | | | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | |
| I | 78.53 | 184.38 | y = 0.3745x + 20.92 | 71.67 | 170.67 | 82.38 | 196.84 | 5.702* |
| II | 116.75 | 259.63 | y = 0.2817x + 17.164 | 110.07 | 241.66 | 123.42 | 163.59 | 3.27* |
| III | 158.37 | 280.59 | y = 0.2453x + 11.282 | 146.54 | 257.53 | 298.63 | 392.80 | 3.352* |
| IV | 182.26 | 348.82 | y = 0.2412x + 5.8503 | 169.03 | 323.93 | 194.48 | 369.70 | 1.072* |
| Pupae | 199.35 | 322.41 | y = 0.1169x - 0.2376 | 185.41 | 297.18. | 211.28 | 242.57 | 5.722* |

* - Significant at p<0

Plant derivatives are highly toxic to many insect species without being phytotoxic [14]. Roark (1947) described approximately 1200 plant species listed and discussed 344 plant species that exhibited mosquitocidal activity. The current state of knowledge on larvicidal plant species and listed the growth and reproduction inhibiting phytochemical, botanical ovicides, synergistic, additive and antagonistic joint action effects in nontorget organisims and appearance of resistance. Usually it has been found that secondary metabolites produced by plant are responsible for their chemical defense and toxicity to other animals.

Crude extract or isolated bioactive phytochemicals from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. However, further studies on the identification of the active principals involved and their mode of action and field trials are usually needed to recommend any of these plant materials as an anti-mosquito product used to combat and protect from mosquitoes in a control program. The secondary metabolite of plant orgins makes up a vast respository compounds with a wide range of biological activities.

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, and repellent and ovipositor attractant and have different activities. The secondary metabolites of plants (such as steroids, alkaloids, terpenoids, saponins, phenolics, essential oil, etc.) are associated with a wide range of biological activities. The bio-control potentiality of the ethanolic extract of aerial roots of *Rhaphidophora aurea* intertwined over *Lawsonia inermis, Areca catechu*, *Cocos nucifera* and *Azadirachta Indica* have been well established in the laboratory condition. The highest mortality was recorded in aerial roots of *Rhaphidophora aurea* intertwined over *Lawsonia inermis.* The phytochemical analysis of the plant extract reveled the presence of alkaloids, flavonoids, saponins, phenols, anthraquinone, anthocyanin and glycosides combinations may be responciple for higher activity. Plant alkaloids resulted in a significant loss in fecundity and fertility in the adult species of mosquitoes [15].

The moderate mortality was recorded in ethanolic extract of aerial roots of *Rhaphidophora aurea* intertwined over *Areca catechu*, and *Azadirachta Indica*. These extract revel the presence of secondary metabolites like alkaloids, flavonoids, saponins, phenols, anthraquinone, anthocyanin and alkaloids, flavonoids, carbohydrates, reducing sugars, phenols, anthraquinone, betacyanin. These constituents are responsible for moderate activity. Higher concentrations may yield higher mortality ratio.

The skin repellent activity MM, MB, MC and MN extracts does not exhibit any repellent activity. There are many factors that affect the efficacy of repellent against mosquitoes, such as species and density of mosquito [16], age of person, sex and biochemical attractiveness to biting mosquitoes [17], ambient temperature, humidity, and wind speed [18]. The absence of essential oils, may be one of the reasons for failure to show repellent activity. Plant essential oils in general have been recognized as an important natural source of repellents [19]. Also mosquito repellent may be exhibited at higher concentration of plant extract, since concentration is one of the depending factors.

The percentage reduction (fig 1, 2 and 3) of larval mortality also showed the variations among the different breeding habitats of mosquito vectors. This may due to the impact of geographical distribution of *Culex quinquefasciatus* at the breeding sites. The lethal effect on mosquito larvae may be due to the active plant compounds on the gut lining of the mosquito larvae. The larval density was decreased after the treatment of MM, MB and MN extracts at the breeding sites (drinking water and ditches water).

In conclusion natural products are generally preferred in vector control measures due to their less deleterious effect on non-target organisms and their innate biodegradability. In the context of resistance developed by the mosquito larvae against chemical insecticides, it is worthwhile to identify new active compounds from natural products against mosquitoes. The findings of the present investigation revealed that the ethanolic extract of aerial roots of *Rhaphidophora aurea* intertwined over *Lawsonia inermis, Areca catechu*, and *Azadirachta Indica* has good larvicidal and pubicidal properties against *Culex quinquefasciatus*. Ethanol extract of aerial roots of *Rhaphidophora aurea* intertwined over *Lawsonia inermis* were more efficient than compared to other extracts. These extract show good effective mosquito control properties and also can act as an eco-friendly, bio-pesticide for further vector control programs.

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