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# Efficacy of *Rauwolfia tetraphylla* Leaf extracts against the Vector of Lymphatic *Filariasis culex quinquefasciatus*

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## ABSTRACT

The *Rauwolfia tetraphylla* leaf extract with different solvents n-hexane, chloroform, acetone and methanol were tested for larvicidal, ovicidal, oviposition attractant/deterrent activities. Their effect on larval nutritional reserve against *Culex quinquefasciatus* was also studied. Larval mortality was observed after 24 hours exposure. The LC<sub>50</sub> values for n-hexane, chloroform, acetone, methanol extracts are 16.80, 91.56, 214.85, 44.68 ppm, respectively. The mean percentage hatchability of the ovicidal activity was observed 48 hours post treatment. For *R. tetraphylla*, methanolic extract exhibited 56 % egg hatchability at 125 ppm. The effective oviposition repellency was 100 % at 320 ppm and the oviposition activity index value -1 was shown by n-hexane extract of *R. tetraphylla*. The fourth instar of *C. quinquefasciatus* where exposed to LC<sub>50</sub> concentration of extracts and its on larval nutritional reserves was checked. All extracts showed moderate effect on larval nutritional reserves, i.e. changes in total concentration of sugar, glycogen, lipid and protein. The average values of total sugar, glycogen, lipid and protein are 9.47, 32.32, 54.50, 183.35 µg/larvae was found in fourth instar larvae of *C. quinquefasciatus*. From the all-leaf extracts, the methanol extract affects the total sugar, lipid; n-hexane extract to glycogen and acetone extract to protein of fourth instar larvae of the mosquito and decreases up to 3.39, 20.71, 11.18, 91.05 µg/larvae, respectively. These results reveal that the *R. tetraphylla* leaf extracts have the control potential against *C. quinquefasciatus*.

**Key words:** *Rauwolfia tetraphylla*, *Culex quinquefasciatus*, Larvicidal Activity, Ovicidal activity, Ovipositional activity.

## Introduction

Mosquitoes transmit diseases like malaria, Japanese encephalitis, yellow fever, dengue and filariasis that are major causes of human mortality world wide. No part of the world is free from vector-borne diseases and mosquito-borne diseases currently represent a significant health problem in tropical and sub-tropical countries (Fradin and Day, 2002). *Culex quinquefasciatus* mosquito is generally known as the Southern House mosquito and a vector for many an

arbovirus responsible for lymphatic filariasis, West Nile virus, St. Louis encephalitis (Udonsi, 1986; Rajasekariah *et al.*, 1991). Lymphatic filariasis, endemic to India, caused by *Wuchereria bancrofti* is transmitted by mosquito *C. quinquefasciatus*. *W. bancrofti* is the predominant filarial nematode, causing filariasis usually characterized by progressive debilitating swelling at the extremities (Myung *et al.* 1998). The most common approach to control insect vectors is the use of chemical pesticides and insecticides. Their repeated use has disrupted natural bio-

logical control systems, led to development of resistance to the chemicals, with undesirable effects on non-target organisms, raising concern about the environment and human health (Brown, 1986; Thomas *et al.*, 2004). The drawbacks of the synthetic chemicals have renewed interest in the search of eco-friendly, safer and cheap plant derivatives. The plant extracts and phytochemicals are usually pest specific, easily biodegradable, generally risk free, and an effective mosquito control agent (Bowers, 1992). Many plant products mean of potential mosquito control agent have been reported that are larvicidal, repellents, oviposition deterrent and growth inhibitors (Rajkumar and Jebanesan, 2005; Pushpanathan *et al.*, 2006; Isman, 2000). The present work systematically investigates the larvicidal, ovicidal, oviposition attractant/deterrent and nutritional reserves effect of *R. tetraphylla* leaf solvents extracts against *C. quinquefasciatus*.

*R. tetraphylla* L. (Apocynaceae) is commonly known as devil-pepper or be still tree. In Sanskrit, it is known as Vanasarpagandha and Sarpanasini. It is a small erect woody shrub. It is native to West Indies that was introduced in India. It has become naturalized in many localities. Medicinally, *R. tetraphylla* plant extract mixed with castor oil is used for chronic refractory skin diseases and destroys parasites. The root is also used to stimulate uterine contraction and is recommended for use in difficult child birth (Villar *et al.*, 1998; Parrotta, 2001). Pharmacologically the roots of *R. tetraphylla* contain alkaloids, like Reserpine, Ajmalicine and Tetraphyllicinine which possess hypotensive property (Faisal *et al.*, 2005). Shariff *et al.* (2006) reported the antimicrobial activity of the chloroform extract of *R. tetraphylla*.

## Materials and Methods

### Rearing of Mosquito colony

*C. quinquefasciatus* larvae were obtained from National Chemical Laboratory, Pashan, Pune. The larvae were maintained at 75 – 85% relative humidity &  $27 \pm 2$  °C temperature. The larvae were fed dry yeast powder and dog biscuit in 1:3 proportions. Pupae were kept in 60×60×60 cm wire mesh cage for adult emergence. The 10% sucrose cotton swab was provided as food for adult mosquitoes. The adult female mosquitoes of *C. quinquefasciatus* were fed on the blood meal of hen.

### Plant material collection and extraction

Fully developed leaves of the *R. tetraphylla* were collected from in and around Pune University campus, Pune, Maharashtra, India in September 2010. It was authenticated by Botanical Survey of India (BSI), Western regional office, Pune, India. Voucher specimen was deposited at the BSI, Pune. The leaves were collected, washed with tap water and shade-dried at room temperature. The dried material was finely ground and exposed to Soxhlet apparatus for extraction. The finely ground plant leaf powder was extracted with four different solvents, namely, n-Hexane, Chloroform, Acetone and Methanol, individually (Vogel, 1978). The rotary vacuum evaporator was used for removing solvents from extracts and to collect the crude extract. The standard stock solution (1 %) was made by dissolving the residues in acetone. Test concentrations were prepared and used for larvicidal, ovicidal, ovipositional attractant/deterrent properties and its effects on nutritional reserve of larvae.

### Larvicidal bioassay

The larvicidal bioassay was carried out by Standard method recommended by World Health Organization (WHO) (2005). Batches of 25 fourth instar larvae were transferred to disposable test cups, each containing 150 ml of water in the cups to obtain desired target concentrations, starting with the lowest concentration. Doses ranged from 10 – 200 ppm. For each concentration, five replicates were taken; controls were set using tap water with acetone. A pinch of larval food was provided to test larvae and mortality was recorded after 24 h. Data obtained was subjected to probit analysis (Finney, 1971) and  $LC_{50}$  and  $LC_{90}$  values were calculated.

### Ovicidal bioassay

Ovicidal bioassay was performed by the procedure of Govindarajan *et al.* (2011). The egg rafts of *C. quinquefasciatus* were exposed to various concentrations ranging from 25 – 125 ppm of leaf extracts. The egg rafts which have around 100 eggs are used for treatment for four hours. After treatment, the egg rafts were individually transferred to distilled water cups for hatching assessment. The total number of eggs and hatched eggs were counted under dissecting microscope (Olympus, Magnus MS×24). Each experiment was replicated five times along with appropriate control. The hatch rates were assessed

48 h post treatment by following formula.

$$\% \text{ of egg hatchability} = \frac{\text{No. of unhatched eggs}}{\text{Total no. eggs}} \times 100$$

### Oviposition Attractant/Deterrent assay

For this assay, the double choice method was used. For this assay, 30 males and 30 females were separated in the pupal stage and were introduced into screen cages (60×60×60 cm). The pupae were allowed to emerge into adults in the test cages. Adults were fed on 10 % sucrose solution in plastic cup with a cotton wick. They were blood fed (from hen) five days after emergence. In the double choice method, two cups each contains 100 ml distilled water were kept in cage (one treated with test material and other with solvent as control). During replication, position of cups was alternated so as to nullify any effect of position on oviposition. After 24 hours, the no of eggs laid in treated and control cups were counted under dissecting microscope (Olympus Magnus, MS×24). The five replicates for each concentration were run. The percentage effective repellency for each concentration was calculated by,

$$\text{ER \%} = \frac{\text{NC} - \text{NT}}{\text{NC}} \times 100$$

Where, ER = effective repellency, NC = total no. of eggs in control, NT = total no. of eggs in treated cups (Rajkumar and Jebanesan, 2009). The oviposition experiments were expressed as mean number of eggs and Oviposition Activity Index (OAI) which was calculated using the following formula.

$$\text{OAI} = \frac{\text{NT} - \text{NS}}{\text{NT} + \text{NS}}$$

Where, NT = total no. of eggs in test solution, NS = total number of eggs in the control solution. Oviposition activity index of + 0.3 and above are considered as attractants while those with - 0.3 and below are considered as repellents (Kramer and Mulla, 1979).

### Quantification of Nutritional Reserves

The nutritional reserves consist of sugar, glycogen, lipid and proteins are more important in larval and adult development are required for survival and energy purpose (Foster, 1995). This quantification of nutritional reserves was done to check that whether

the *R. tetraphylla* extracts affects the nutritional reserves of larval or not. To quantify nutrient reserves, firstly the larvae were exposed to LC<sub>50</sub> value of *R. tetraphylla* leaf extracts and also to control with solvent. After the treatment of leaf extracts, each larva was separated and immobilized on ice. The mosquito larva was homogenized in 0.1 ml sodium sulphate, 0.8 ml methanol and chloroform (1:1, v:v) added in it. It was centrifuged at 3000 rpm for 2 minutes. Sugar, glycogen and lipids were measured in the samples using modified version of Van Handel and Day (1988). The methanol precipitated the glycogen and remaining sample was transferred to another clean micro centrifuge tube. In that, 3 ml of distilled water were added and again centrifuged for 3000 rpm. Two layers are formed, the upper layer of sugar analysis and lower layer of lipid analysis. Glycogen in the precipitated and sugar in the aqueous fraction were measured with the Anthrone reaction (3 ml of Anthrone/tube) with glucose standard (0.1%). Lipid was quantified by a Vanillin-phosphoric acid reaction (5 ml Vanillin/tube) with 0.1% soybean oil in Chloroform as a standard. The total protein of larva was measured in the sample by using modified version of Van Handel (1988). The mosquito larva was homogenized in 0.3 ml phosphate buffer (pH 7.6) and centrifuged at 8000 rpm for 20 min. The supernatant was transferred in another centrifuge tube and again 0.3 ml of phosphate buffer was added in pellet centrifuge tube. The tube was centrifuged at 8000 rpm for 20 min. Supernatant collected in pervious tube to it 0.6 ml of 20 % Trichloroacetic acid (TCA) was added with shaking and centrifuged the same way.

The pellet washed with acetone and centrifuges it. The pellet dissolved in 1 ml of 0.1 N Sodium Hydroxide and analyzed for total protein. The protein was quantified by Folin's reagent reaction (0.5 ml/tube) with 0.1 % Bovine Serum Albumin (BSA) as a standard. Absorbance value for sample and standard were measured by spectrophotometer (Jasco, V-630 at  $\lambda = 625$  nm for sugar, glycogen and lipid, protein at  $\lambda = 660$  nm). All values converted to microgram ( $\mu\text{g}$ ) per larval body, based on a regression line equation derived from the standard curve. The all nutritional reserves quantification was ten times replicated. Results were analyzed by One Way Analysis of Variance (ANOVA), followed by Tukey's test using trial version of XL-STAT 2011.4.04.

## Results

Larvicidal activity of *R. tetraphylla* leaf extracts against *C. quinquefasciatus* was observed (Table 1). Among the leaf extract tested, the highest larvicidal activity was observed in n-hexane extract against the tested mosquito with LC<sub>50</sub> and LC<sub>90</sub> values were 16.80 ppm, 125.65 ppm respectively. It is followed by methanol, chloroform, acetone extract by giving LC<sub>50</sub> value 44.68, 91.56, 214.85 ppm for tested mosquito species respectively.

The chi-square values are significant at  $p < 0.05$  level. The chi-square values in the bioassays indicated probably the heterogeneity of the population. The 95 % confidence limits (LC<sub>50</sub> (LCL - UCL)) were also calculated (Table 1). The percentage of egg hatchability of *C. quinquefasciatus* were tested with four different solvents at different concentrations of *R. tetraphylla* plant leaves extracts (Table 2).

The methanol extract of the *R. tetraphylla* leaf shows highest 56 % egg hatchability at 125 ppm for tested mosquito. It is followed by chloroform (62 %), n-hexane (63 %) and acetone (69 %) extracts at 125 ppm concentration respectively for *C. quinquefasciatus*.

The present results showed that in the oviposition attractant/deterrent assay, gravid female of *C. quinquefasciatus* preferred to lay eggs in the control cups than in the treated cups with solvents extracts of *R. tetraphylla* (Table 3). All test concentrations under investigation show negative OAI indicating ovipositional deterrent activity.

The present result showed that at 320 ppm concentration of n-hexane extract, ovipositional repellent activity is 100% and -1 OAI. It is followed by the methanol extract and chloroform extracts that show 96.37 %, 93.79 % effective oviposition repellency and -0.92, -0.88 OAI, respectively at 320 ppm

**Table 1.** Larvicidal activity of *Rauvolfia tetraphylla* leaf extracts against *Culex quinquefasciatus*

Extracts	LC <sub>50</sub> (ppm)	95 % confidential limit		Regression equation	LC <sub>90</sub> (ppm)	Chi-square (df)
		LCL	UCL			
n-Hexane	16.80	4	28.07	Y=1.46X+3	125.65	3.57* (7)
Chloroform	91.56	79.05	104	Y=3.69X-2.25	203.41	10.93* (7)
Acetone	214.85	164.86	370.24	Y=2.07X+0.15	889.38	0.58* (7)
Methanol	44.68	29.85	57.23	Y=2.14X+1.46	176.93	5.07* (7)

LC<sub>50</sub> and LC<sub>90</sub> are lethal concentration at which 50% and 90% population dies respectively, LCL lower confidential limit, UCL upper confidential limit, df degree of freedom, \*Significant at  $p < 0.05$

**Table 2.** Ovicidal activity of *Rauvolfia tetraphylla* leaf extracts against *Culex quinquefasciatus*

Extracts	Percentage of egg hatchability ± SE					
	Control	25 ppm	50 ppm	75 ppm	100 ppm	125 ppm
n-Hexane	100	91±3.08	86±2.95	79±1.04	73±2.77	63±1.79
Chloroform	100	95±0.26	88±0.30	79±0.49	75±3.15	62±1.36
Acetone	100	96±0.35	90±0.39	85±0.53	78±0.11	69±0.21
Methanol	100	93±0.23	84±0.93	76±0.34	67±0.22	56±0.31

Each value represents the mean of five replicates ± SE

**Table 3.** Oviposition Attractant/Deterrent assay of *R. tetraphylla* leaf extracts against *C. quinquefasciatus*

Concentrations (ppm)	n-Hexane		Chloroform		Acetone		Methanol	
	ER %	OAI	ER %	OAI	ER %	OAI	ER %	OAI
20	69.54	-0.05	61.86	-0.45	48.88	-0.32	52.79	-0.35
40	81.21	-0.68	68.53	-0.51	53.78	-0.36	58.10	-0.40
80	86.34	-0.75	75.85	-0.61	59.39	-0.42	66.40	-0.49
160	94.68	-0.89	81.57	-0.68	70.40	-0.54	73.38	-0.57
320	100	-1	93.79	-0.88	79.82	-0.66	96.37	-0.92

All values are mean of five replicates, ER effective repellency, OAI oviposition activity index

concentration. A positive value indicates the more egg was deposited in test cup than in control cup and that test solution were attractive. It was seen that more eggs were deposited in control cup than in test cup and that test solution were intended to deter (Elango *et al.*, 2009 and Elango *et al.*, 2010).

All nutritional reserves quantification of control and exposed to different LC<sub>50</sub> concentration of *R. tetraphylla* leaf extracts are shown in Figure 1. The levels of all nutritional reserves were compared between controls and exposed to different *R. tetraphylla* leaf extracts found to be significantly different by One-Way Analysis of Variances (ANOVA), followed by Tukey's test ( $p < 0.05$ ,  $p < 0.001$ ).

The solvent extracts of *R. tetraphylla* leaf treatment shows an over all effect on nutritional reserves of fourth instar larvae. After comparing all the extracts, the methanol extracts of *R. tetraphylla* leaf most affect the total sugars and lipid of fourth instar of mosquito and it decreases the sugars, lipid (Figure 1a and 1c). The acetone extracts of experimental plant affects mainly protein content of mosquito larvae. Protein level of larvae was decreased up to 91.05 µg (Figure 1d).

## Discussion

In tropical and subtropical countries, the main reason of mortality is vector borne diseases mostly. The repellent and insecticide which are derived from the plants are used traditionally against insects. Plant-derived natural products have traditionally been used as repellents and insecticides against insects. The tested *R. tetraphylla* leaf extracts have exhibited promising larvicidal, ovicidal and oviposition deterrent activities against *C. quinquefasciatus*. The n-hexane extract of *R. tetraphylla* leaf shows LC<sub>50</sub> at 16.80 ppm (Table 1). This result is also comparable to earlier reports. Singh *et al.* (2003) observed the larvicidal activity of *Ocimum canum* oil against vector mosquitoes, namely, *C. quinquefasciatus* (LC<sub>50</sub> 301 ppm) and *A. aegypti* (LC<sub>50</sub> 363.07 ppm). Larvicidal efficacy of leaf methanol extracts of *Pavonia zeylanica* and *Acacia ferruginea* were tested against the late third instar larvae of *Culex quinquefasciatus* with LC<sub>50</sub> values of 2,214.7 and 5,362.6 ppm, respectively (Vahitha *et al.*, 2002). The emodin compound was isolated from seeds and showed the LC<sub>50</sub> values of 1.4, 1.9, and 2.2 mg/l against *Culex pipiens*, *Aedes aegypti*, and *Aedes togoi*, respectively (Yang *et al.*,

2003). Bagavan *et al.* (2008) have reported that peel chloroform extract of *Citrus sinensis*, leaf ethyl acetate extracts of *O. canum* and *O. sanctum*, and leaf chloroform extract of *Rhinacanthus nasutus* against the larvae of *Anopheles subpictus* (LC<sub>50</sub>=58.25, 88.15, 21.67, and 40.46 ppm; LC<sub>90</sub>=298.31, 528.70, 98.34, and 267.20 ppm) and peel methanol extract of *Citrus sinensis*, leaf methanol extract of *O. canum*, and ethyl acetate extracts of *O. sanctum* and *R. nasutus* against the larvae of *Culex tritaeniorhynchus* (LC<sub>50</sub>=38.15, 72.40, 109.12, and 39.32 ppm; LC<sub>90</sub>=184.67, 268.93, 646.62, and 176.39 ppm), respectively. Ansari *et al.* (2005) observed the larvicidal activity of *Pinus longifolia* oil against three vector mosquitoes namely *Aedes aegypti* (LC<sub>50</sub>, 82.1 ppm), *Culex quinquefasciatus* (LC<sub>50</sub>, 85.7 ppm), and *Anopheles stephensi* (LC<sub>50</sub>, 112.6 ppm). Khandagale *et al.* (2011) had shown that among the essential oil tested, the essential oil of *Zingiber officinalis* was highly effective against *Aedes aegypti* (LC<sub>50</sub> 154 ppm) and *Culex quinquefasciatus* (LC<sub>50</sub> 197 ppm). The highest activity of *Zingiber officinalis* oil was followed by *Achyranthes aspera* stem oil which showed LC<sub>50</sub> 668 ppm and LC<sub>50</sub> 355 ppm values against *Aedes aegypti* and *Culex quinquefasciatus*. The findings of our results is in corroboration with important findings of Sumroiphon *et al.* (2006) who have reported that the effect of water extract of citrus seed extract showed LC<sub>50</sub> values of 135,319.40 and 127,411.88 ppm against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*.

The results of ovicidal activity of *Rauvolfia tetraphylla* leaf extracts were also comparable with earlier reports. According to Su and Mulla (1998), early stage of *C. quinquefasciatus* and *Culex tarsalis* eggs were more susceptible to neem products (Azadirachtin) because the egg shell is very thin at early stage, which facilitate the entry of neem products into the egg and interrupt the embryogenesis process. The bioactive compound Azadirachtin (*Azadirachta indica*) showed complete ovicidal activity in eggs of *C. tarsalis* and *C. quinquefasciatus* exposed to 10 ppm concentration (Su and Mulla, 1998). The younger age groups of egg rafts showed poor hatchability rate when exposed to higher concentrations of extract and that older age groups of egg rafts or eggs showed high hatchability rate when exposed to lower concentrations of extract and it was reported by Govindarajan *et al.* (2008a). Rajkumar and Jebanesan (2004) studied ovicidal activity of *Moschosma polystachyum* leaf extract against *Culex quinquefasciatus* and observed 100% egg mortality at

100 ml/l. The completer ovicidal activity was observed when *Culex quinquefasciatus* eggs were exposed to seed extract of *Atriplex canescens* at 1,000 ppm concentration (Ouda *et al.*, 1998). According to Mullai *et al.* (2008), *Citrullus vulgaris* benzene extracts shows zero hatchability at 250 ppm and 11.80% low hatchability at 200 ppm, complete ovicidal activity at 300 ppm. The fraction I at 80 ppm exerted a very low hatchability rate of 3.2% followed by fraction II (6.9%), and fractions III and IV afforded 4.9% and 5.3% hatchability recorded against *Anopheles stephensi* and *Aedes aegypti*, respectively (Mullai *et al.*, 2008). The benzene extract of *Ervatamia coronaria* exerted 100% mortality (zero hatchability) at 300, 250, and 200 ppm, respectively. The leaf extract of *Ervatamia coronaria* was found to be most effective than *Caesalpinia pulcherrima* against eggs of three vector mosquitoes (Govindarajan *et al.* 2011).

Results are mean  $\pm$  SE (Standard error) of three different set of observations. (n=10), All Nutritional reserves quantification are expressed as  $\mu\text{g}$  / larvae,

Data analyzed by One-Way ANOVA, followed by Tukey’s test and Significance is based on \*p < 0.05, \*\*p < 0.001 compared with control value, Different letters (a, b and c) indicate significant difference between respective extracts treatments (p < 0.05)

The present results indicated that the oviposition deterrence activity was dependent on concentration, as 320 ppm, of n- hexane, chloroform, acetone, and methanol leaf extracts of experimental plant exhibits strong deterrent effect when compared with 20 ppm against oviposition. The present study was also supported by previously reported result. Rajkumar and Jebanesan (2009) pointed out that the oviposition deterrence activity of ethanolic leaf extract of *Cassia obtusifolia* at higher concentration (400 mg/l) showed 92.5% effective repellency against oviposition, followed by 300, 200, and 100 mg/l showed 87.2 %, 83.0 %, and 75.5 %, respectively. According to Xue *et al.* (2006) the oviposition deterrent effectiveness (76 – 100 % repellency) tested against *Aedes albopictus* of 21 commercial insect repellent products

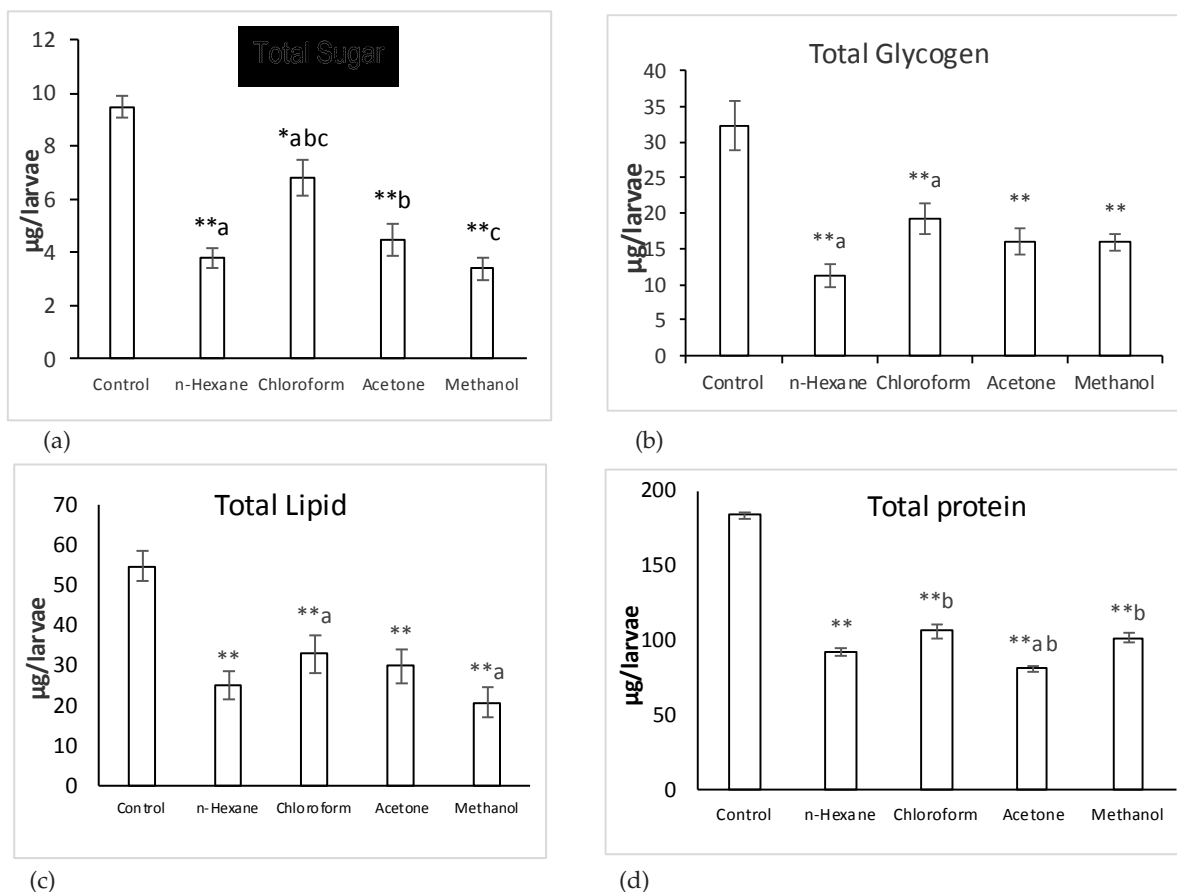


Fig. 1. The *Culex quinquefasciatus* fourth instar Nutritional Reserves comparison of control and *Rauwolfia tetraphylla* leaf extracts treated (a) Total sugar, (b) Total glycogen, (c) Total lipid and (d) Total protein

(at 0.1 % concentration), including 12 botanical six deet-based and three synthetic organics. The *Solanum trilobatum* leaf extract was reduced egg laying by gravid females of *Anopheles stephensi*, from 18 % to 99 % compared with ethanol treated controls at 0.01 %, 0.025 %, 0.05 %, 0.075 % and 0.1 % (Rajkumar and Jebanesan, 2005). The crude extract of *Cuscuta hyaline* was an effective oviposition deterrent against *Culex quinquefasciatus* at a concentration of 80 ppm which was reported by Mehra and Hiradhar (2002). The full oviposition deterrence was obtained with *Melia Azedarach* leaf extract at 1 g/l against *Aedes aegypti* (Coria *et al.*, 2008). The *Zingiber officinalis* Rhizome oil exhibited 91.21 % and 83.33 % oviposition deterrence while *Achyranthes aspera* stem oil caused 100 % and 85.71 % oviposition against *Aedes aegypti* and *Culex quinquefasciatus* respectively at 0.1 % concentration (Khandagale *et al.*, 2011).

The essential oil from leaves and stem of *Piper marginatum* exhibited an oviposition deterrent effect against *Aedes aegypti* at 50 ppm and 100 ppm in that significantly lower numbers of eggs (< 50 %) were laid in glass vessels containing the test solutions compared with the control solution which was reported by Autran *et al.* (2009). Essential oils of *Curcuma longa* (94.7%), *Schefflera leucantha* (91.6%), and *Zingiber officinale* (90.1%) has relatively high oviposition deterrence, while *Piper nigrum* (82%), *Litsea cubeba* (80.6%), and *Eleutherococcus trifoliatum* (80.2%) shows moderate degrees of deterrence (Tawatsin *et al.*, 2006). Among the 10 essential oils screened for oviposition deterrent activity, essential oil of *Cinnamomum zeylanicum* was found to be highly effective in preventing egg laying by three mosquito species with an order of deterrence, *Anopheles stephensi* > *Aedes aegypti* > *Culex quinquefasciatus* (OD<sub>50</sub> : 32.4, 33.5 and 50.1 µg/ml, respectively) (Prajapati *et al.*, 2005).

The *R. tetraphylla* affect all nutritional reserves but mainly they affect the sugar and glycogen of mosquito larvae (Figure 1). Van Handel (1988) and Van Handel and Day (1988) shows the nutritional requirement for larval development in mosquito and our result of control larvae comparatively match with them. When adult mosquito encloses nutritional reserves consist of lipid and glycogen accumulated during larval development (Foster, 1995). About 60 % of glycogen and sugar reserves and 35 % lipid reserves are metabolized during the females first gonotrophic cycle, acting as sources of energy

and as provision for developing eggs (Zhou *et al.* 2004). As result shows, there is decreased in the nutritional reserves after treatment of *R. tetraphylla* leaf extracts which may lead to deformities in adult mosquito like low host seeking ability, shortening of wings, low flight ability. There are many previous reports which showed that larval nutrient deficiency lead to above mention deformities in adult stage of mosquito. Alves *et al.* (2004) reported that the Ivermectin insecticides treated larvae of *C. quinquefasciatus* shows damage in protein, fat body and the females arisen from ivermectin exposed larvae shows decreasing number of egg laid in the adult stage. Klowden *et al.* (1988) reported that adult female's *Aedes aegypti* which was reared on suboptimal diet as larvae were less likely to engage in host seeking behaviour than the adults derived from larvae reared on an optimal diet. The relationship of larval nutrition and adult body size to the susceptibility of *Aedes aegypti* to Ross River Virus infection was examined by Nasci and Mitchell (1994). It was shown that large adult produced by feeding larvae a high-level diet consumed significantly more virus particles than did smaller mosquitoes.

Since ancient times, plants have been used by several communities to treat a large number of diseases and their vectors. Certain plant essential oils or their extracts have a broad spectrum of activity against insect. As such, they have considerable potential for pest management. Like other alternative pest management products, plant extracts and essential oil based pesticides will be a panacea for pest management. The present study revealed the Larvicidal, ovipositional, ovicidal activities of *Rauvolfia tetraphylla* at low concentration with reducing their larval nutritional reserves. The plant essential oils and solvent extracts have potential for the development of new and safe control products for pest management. The results suggest that the n-Hexane and methanol extracts of *Rauvolfia tetraphylla* shows promising in mosquito control. Consequent upon the results obtained the test plant extracts deserve to be explored to assess their efficacy in field trials as well as to study their economic and residual viability to be consider for mosquito control program. These extracts can be also considered for their use in larvicidal, ovicidal products against mosquito either as individuals or in combination with natural or synthetic insecticides products.

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