ORIGINAL ARTICLE

Evaluation of mosquito larvicidal effect of Nagarmotha (*Cyperus rotundus*) extracts against *Aedes aegypti* L. larvae

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Background: Mosquitoes transmit serious human diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, dengue haemorrhagic fever and yellow fever causing millions of deaths every year. Extensive use of chemical insecticides for control of vector borne diseases has created problems related to physiological resistance to vectors, adverse environmental effects, high operational cost and community acceptance. Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals and biodegradable. In this study, mosquito larvicidal activity of *Cyperus rotundus* against *Aedes aegypti* larvae was assessed in laboratory according to World Health Organisation guidelines 2005 with slight modification. **Materials and Methods:** Five concentrations of petroleum ether (PE) and ethyl alcohol (EA) extracts of *C. rotundus* in the range of 200-1000 ppm were used in bioassay against late 3rd and 4th instar larvae of *A. aegypti.* Observation of mortality response was assessed after 24 h. The mortality data were subjected to probit regression analysis to determine the median lethal concentration LC_{50} and LC_{90}. *C. rotundus.* **Results:** PE extract and EA extract produce 98% and 97% mortality at 1,000 ppm, respectively. PE extracts exhibits LC_{50} 443.80 ppm and LC_{90} 882.98 ppm whereas EA extract exhibits LC_{50} 594.22 ppm and LC_{90} 936.25 ppm. **Conclusion:** PE and EA extracts of *C. rotundus* showed good mosquito larvicidal potential.

Key words: Alternative insecticides, extracts, LC_{50}, LC_{90}, World Health Organisation guidelines

INTRODUCTION

Interest in *Aedes* mosquito lies in the fact that it acts as a vector for dengue fever and dengue haemorrhagic fever (DHF) which is endemic in Southeast Asia, the Pacific Islands area, Africa and the Americas.[1] Today, about two-fifths of the world's population is at risk for dengue, with cases reported in more than 100 countries. In 2007 alone, there were more than 890,000 reported cases of dengue in the Americas, of which 26,000 cases were of DHF.[2,3] Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throwaway society and to the increasing resistance of mosquitoes to current commercial insecticides.[4] Years and millions of money has been spent on researches on the dengue vaccine but nothing much is produced. Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, active against specific target-insects and are biodegradable.[5] Mosquitoes develop genetic resistance to synthetic insecticides,[6] and even to biopesticides such as *Bacillus sphaericus*.[7] The present paper studied the therapeutic and pesticide properties of Nagarmotha (*C. rotundus* L.) because this plant is abundant in India. It is attributed with many medicinal uses for properties such as central nervous system depressant, anti-helminthic, insecticidal, mosquito larvicidal and insect repellent. Pesticides and drugs that will be made out from this plant are environment friendly and cheap.[8]

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MATERIALS AND METHODS

Study was conducted after obtaining the ethical clearance by the Institutional Animal Ethics Committee of National Institute of Unani Medicine, Bangalore, India (Reg. No. IAEC/VII/04/TST).

Plant Material

Fresh rhizome of *C. rotundus* was collected from National Institute of Unani Medicine (NIUM) herbal garden and identified by Botanist Dr. Ravi Kumar at Foundation for Revitalisation of Local Health Traditions, Bangalore, and voucher specimen was deposited in the herbarium of NIUM, Bangalore.

Preparation of Extract

The rhizomes of *C. rotundus* was carefully washed and rinsed with tap water for at least 30 min. Dead

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rhizomes were removed. Roots were separated from the rhizomes, and shade dried at room temperature of 28°C ± 1°C for 15 days. Dried rhizomes of *C. rotundus* were pulverized in electric grinder in the form of coarse powder at pharmacy of NIUM. Two hundred and fifty grams of coarse powder were extracted in Soxhlet extractor with 1,000 ml petroleum ether (PE) and then with ethyl alcohol (EA) (99.99% analytical grade) at the temperature of 50°C till discolouration. The liquid extract of each type was cooled and filtered by Whatman filter paper 40. The filtrates were evaporated under reduced pressure at 45°C to dryness with the help of rotary vacuum evaporator. The resultant brownish black crude petroleum and EA extracts were kept in Petri dish and stored in vacuum desiccators. The solvents used are the most feasible ones for extracting the whole constituents to the maximum concentrations according to their polarity.

Rearing of Larvae

The *Aedes aegypti* larvae were reared at NIMR (National institute of Malaria research), Bangalore, an egg strip of F12 generation was obtained from a maintained colony. Eggs strip was dipped into a plastic tray (20 × 15 × 5 cm) containing de-chlorinated tap water for hatching. To reduce variation in adult size at emergence, larvae were reared at a fixed density of 800-1,000 larvae per tray. Larvae were fed once a day initially and twice during the later stages of development with a diet of finely ground brewer yeast and dog biscuits (3:1).[9] Adults were fed with 10% sucrose solution. Five days after emergence, female mosquitoes were allowed to blood feed on albino mice for 2-3 h. A few days after having a blood meal, the gravid mosquitoes laid their eggs. Small plastic bowl having 250 ml of tap water lined with filter paper was kept inside the cage for oviposition. The laboratory colony was maintained at 25-30°C and 80-97% relative humidity under a photoperiod of 14:10 h light and dark as per the procedure of Sharma and Saxena.[10,11] Under these conditions, the full development from egg to adult lasted about 3 weeks.

Preparation of Stock Solutions and Test Concentrations

Dried extracts of *C. rotundus* were dissolved separately in Dimethyl sulphoxide (DMSO) to prepare dilute solutions. Homogeneous suspensions were obtained by gentle shaking or stirring. The 20 ml volume of stock solution of 1% was obtained by weighing 200 mg of the technical material and adding 20 ml solvent to it. It should be kept in a screw-cap vial, with aluminium foil over the mouth of the vial. The mixture was shaked vigorously to dissolve the material in the solvent. Test concentrations ranging from 400 ppm to 1,000 ppm were obtained by adding appropriate dilution to 250 ml chlorine free or distilled water. The plain control solution was made with 1 ml of DMSO with 249 ml of de-chlorinated water. For other volumes of test water, aliquots of dilutions added were adjusted. While making a series of concentrations, the lowest concentration was prepared first. Small volumes of dilutions were transferred to test beakers by pipettes with disposable tips.

Larvicidal Testing

Bioassay was performed according to World Health Organisation guidelines.[12] After making test concentration, 25, 3rd and 4th instar larvae were introduced into each plastic bowel (sterilised plastic bowel of 500 ml capacity) after removing small, unhealthy or damaged larvae. Each experiment was performed in four replicates with a final total of 100 larvae for each concentration. Each batch of replicates contained one plain control. The number of dead larvae at the end of 24 h was recorded in the data record form. During the treatment, no food was offered to larvae. Moribund larvae were counted and added to dead larvae for calculating mortality percentage. Initially, the mosquito larvae were exposed to a wide range of test concentrations. After determining the mortality of larvae in this wide range of concentrations, a narrow range of 4-5 concentrations yielding between 10% and 99% mortality in 24 h were used to determine LC_{50} and LC_{90} values using statistical package for the social sciences (SPSS) software.

Statistical Analysis

Data from all replicates were pooled for analysis. LC_{50} and LC_{90} values were calculated using SPSS software (IBM SPSS Statistics, Version 20) by probit analysis. The 95% confidence interval values, and degrees of freedom, χ^2 goodness-of-fit tests and regression equations were recorded. Whenever χ^2 value was found significant ($P < 0.05$) a heterogeneity correction factor was used in the calculation of confidence limits. The control mortality between 5% and 20% necessitated that the mortalities of treated groups to be corrected according to Abbott's formula.[13]

$$\% \text{ Corrected mortality} = \frac{\%\text{Kill in treated} - \%\text{Kill in control}}{100 - \%\text{ Kill in control}} \times 100$$

RESULTS

Dose-dependent mortality was observed. After 24 h exposure, five different concentrations of 200, 400, 600, 800 and 1,000 ppm were tested for *C. rotundus* (PE and EA extracts). The PE and EA extract of *C. rotundus* produced 11, 35, 68, 86, 98 percent and 01, 14, 44, 79, 97 percent larval mortality, respectively [Table 1, Figure 1].

Probit analysis revealed LC_{50}, LC_{90} (lower and upper confidence limit), χ^2, degree of freedom and slope of PE extract 443.80 ppm, 882.98 ppm (342.91-542.44,697.06-1395.91), 8.730, 3, 4.290 respectively. For EA extract 594.22 ppm, 936.25 ppm (476.04-711.51, 768.40-1520.28), 12.119, 3, and 6.491, respectively [Table 2]. The obtained results revealed the LC_{50} of PE is less than the LC_{50} of EA, so PE extract is

consider more potent than EA extract. The probit regression line is plotted in Figure 2. From this probit regression line, different parameters were calculated.

DISCUSSION

The extensive use of synthetic organic chemical insecticides results in environmental hazards and resistance in major species and this has necessitated the need to develop more potent and environmentally safe insecticides. This study was carried out to examine the larvicidal activity of *C. rotundus* against *A. aegypti* larvae. The results from the study showed that this plant exhibited larvicidal activity. PE extract showed the highest larvicidal effect against the 3rd and 4th instar larvae with LC_{50} value 443.80 ppm and LC_{90} value 882.98 ppm. EA extract showed LC_{50} value 594.22 ppm and LC_{90} value 936.25 ppm [Table 2]. When the dose effect curves of PE and EA extracts are compared, they were found similar [Figure 2] indicating that the mortality increased with increased concentration ($P < 0.05$). This confirms the report of Shadia *et al.*, that there is a positive correlation between concentration and the percentage of mortality.[14]

Kempraj studied the ovicidal and larvicidal efficacy of essential oils extracted from the tubers of *Cyperus giganteus* and *C. rotundus* Linn. on eggs and 4th instar larvae of *Aedes albopictus*. The eggs and larvae were exposed to serial concentration of the oils ranging from 5 ppm to 150 ppm and kept under observation for 24 h. Both the oils showed remarkable ovicidal and larvicidal activities indicated by EC_{50} values <5 ppm and LC_{50} and LC_{90} values <20 ppm.[15] Larvicidal activity of *C. rotundus* may be attributed to the 4, 11-Selinnadien-3-one and 1, 8-cineole. Toxicity against the Diamondback Moth (*Plutella xylostella* L.) was observed using different concentrations of the active compound, 4, 11-selinnadien-3-one. The LC_{50} against 2nd and 3rd instar larvae of diamondback moth was 7-12 ppm. Larvicidal activity of 4, 11-selinnadien-3-one is due to the inhibition of detoxification enzymes: Mono oxygenase, esterases and glutathione-S-transferase.[16] 1, 8-Cineole isolated from *Artemisia annua* was tested against *Tribolium castaneum* (Herbst) for contact toxicity, fumigant toxicity and anti-feedant activity. The adults of *T. castaneum* were more susceptible than larvae to both contact and fumigant toxicity of 1, 8-cineole, and LD_{50} value 108.4 micro gram per mg body weight of adult insect was found.[17] 1, 8-cineole, one of the components of the essential oil of *Artemisia annua*, was evaluated for toxicity against three stored product coleopterans: *Callosobruchus maculates* F. (Coleoptera:

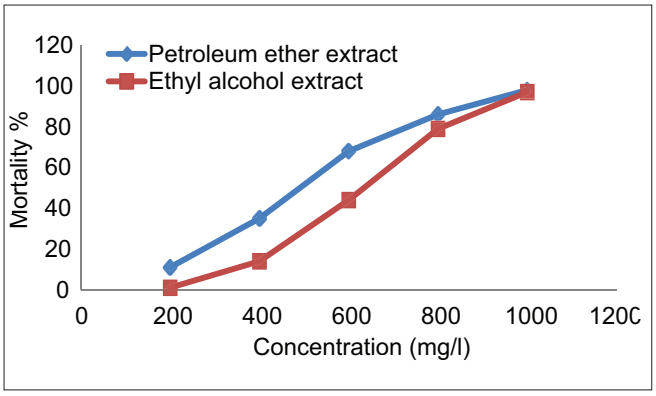

Figure 1: Dose response relationship for petroleum ether and ethyl alcohol extract of *Cyperus rotundus* applied for 24 hours on *Aedes aegypti* L. larvae

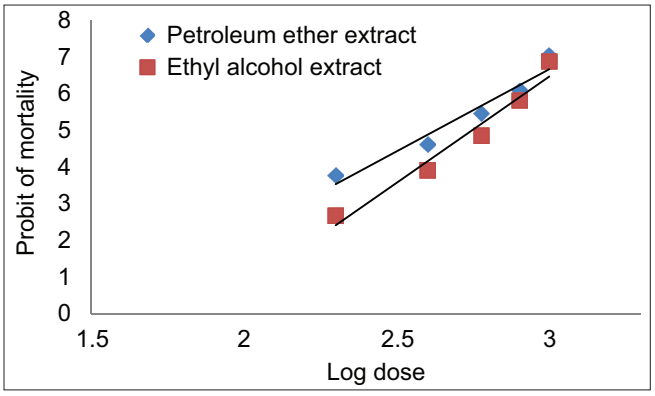

Figure 2: Larvicidal effects of petroleum ether and ethyl alcohol extract of *Cyperus rotundus* applied against 3rd and 4th instars larvae of *Aedes aegypti* L. expressed as linear regressions

Table 1: Larvicidal activity of *Cyperus rotundus* extracts on the 3rd and 4th instar larvae of *Aedes aegypti* L.

Plant extracts	Observed mortality in percentage after 24 h						
	Conc. (ppm)	200 (%)	400 (%)	600 (%)	800 (%)	1000 (%)	Control (%)
Cyperus rotundus (PE extract)	Mortality	11	35	68	86	98	0
Cyperus rotundus (EA extract)	Mortality	1	14	44	79	97	0

Conc.- Concentration; ppm – Parts per million; PE – Petroleum ether; EA – Ethyl alcohol; Note – 0% mortality was recorded in control

Table 2: LC_{50} and LC_{90} with fiducial limits (95%) of tested plant extracts against larvae of *Aedes aegypti* L.

Plant material	LC_{50} (95% CL)	LC_{90} (95% CL)	χ^2 value	df	Slope±SE	Regression equation	*P* value
Cyperus rotundus (PE extract)	43.80 (342.91-542.44)	882.98 (697.06-1395.91)	8.730	3	4.290±0.324	y=4.4816x-6.7776	0.033*
Cyperus rotundus (EA extract)	594.22 (476.04-711.51)	936.25 (768.40-1520.28)	12.119	3	6.491±0.523	y=5.7989x-10.929	0.007**

χ^2 – Chi square; df – Degree of freedom; PE – Petoleum ether; EA – Ethyl alcohol; CL – Confidence limit; SE – Standard error; *P<0.05, **P<0.01

Bruchidae), *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae). A contact toxicity assay revealed LD_{50} values 0.03, 0.04 and 0.04 ml/insect against *C. maculatus*, *R. dominica* and *S.oryzae*, respectively, in the topical application assay whereas the LC_{50} in the fumigant assay was 0.28, 0.33 and 0.46 µl/l against *C. maculatus, R. Dominica* and, *S. oryzae*, respectively.[18]

CONCLUSION

On the basis of above results, we concluded that the extract of 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 constituents involved and their mode of action and field trials are needed to recommend Nagarmotha (*C. rotundus*) as an anti-mosquito product used to combat and protect from mosquitoes in a control programme.

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REFERENCES

1. Park K. Environment and health. Parks Textbook of Preventive and Social Medicine. 20th ed. Jabalpur: Banarsidas Bhanot Publishers; 2009. p. 674-6.
2. WHO. Action Against Dengue: Dengue Day Campaigns Across Asia. Geneva: World Health Organization; 1998. p. 9, 87.
3. Pérez-Guerra CL, Halasa YA, Rivera R, Peña M, Ramírez V, Cano MP, *et al*. Economic cost of dengue public prevention activities in Puerto Rico. Dengue Bulletin 2010;34:13-23.
4. Ciccia G, Coussio J, Mongelli E. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. J Ethnopharmacol 2000;72:185-9.
5. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: A review. J Am Mosq Control Assoc 1991;7:210-37.
6. Wattal BL, Joshi GC, Das M. Role of agricultural insecticides in precipitating vector resistance. J Commun Dis 1981;13:71-4.
7. Tabashnik BE. Evolution of resistance to Bacillus thuringiensis. Annu Rev Entomol 1994;39:47-79.
8. Bañez SE, Castor L. Phytochemical and pesticidal properties of barsanga (*Cyperus rotundus* Linn.) JPAIR Multidiscip J 2011;6:197-214.
9. Asahina S. Food material and feeding procedures for mosquito larvae. Bull World Health Organ 1964;31:465-6.
10. Sharma M, Saxena RC. Phytotoxicological evaluation of *Tagetes erectes* on aquatic stages of *Anopheles stephensi*. Indian J Malariol 1994;31:21-6.
11. Gerberg EJ. Manual for Mosquito Rearing and Experimental Technique. AMCA Bulletin 05. California: American Mosquito Control Association; 1970 p. 53-5.
12. WHO. Guidelines for Laboratory and Field Testing of Mosquito Larvicides 2005, Document WHO/CDS/WHOPES/GCDPP/2005.13. Geneva**:** World Health Organisation; 2005. p. 10-2.
13. Abbott WS. A method for computing the effectiveness of an insecticide. J Econ Entomol 1925;18:265-7.
14. Shadia E, El-Aziz A, Omer AE, Sabra SA. Chemical composition of ocimum americanum. Essential oil and its biological effects against, agrotis ipsilon, (Lepidoptera: Noctuidae). Res J Agric Biol Sci 2007;6:740-7.
15. Kempraj V, Bhat SK. Ovicidal and larvicidal activities of *Cyperus giganteus* Vahl and *Cyperus rotundus Linn*. Essential oils against Aedes albopictus (Skuse). Nat Prod Rad 2008;7:416-19.
16. Visetson S, Milne M, Milne J. Toxicity of 4, 11-Selinnadien-3-one from Nutsedge (*Cyperus rotundus L.*) Tuber Extracts to Diamondback Moth Larvae (Plutella xylostella *L.*), Detoxification Mechanisms and Toxicity to Non Target Species. Kasetsart J (Nat Sci) 2001;35:284-92.
17. Tripathi AK, Prajapati V, Aggarwal KK, Kumar S. Toxicity, feeding deterrence, and effect of activity of 1,8-cineole from Artemisia annua on progeny production of *Tribolium castanaeum* (Coleoptera: Tenebrionidae). J Econ Entomol 2001;94:979-83.
18. Aggarwal KK, Tripathi AK, Prajapati V, Kumar S. Toxicity of 1, 8-Cineole towards three species of stored product Coleopterans. Int J Trop Insect Sci 2001;21:155-60.

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