



Efficacy of Botanical Extracts from *Tagetes erecta* and *Cymbopogon citratus* Against the Yellow Fever Mosquito, *Aedes aegypti*

M. Manimegalai and S. Binu Kumari

P. G. and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore-641 029, T. N., India

Nat. Env. & Poll. Tech.

Website: www.neptjournal.com

Key Words:

Botanical extracts

Tagetes erecta

Cymbopogon citratus

Aedes aegypti

Ovicidal effects, Repellent

ABSTRACT

The plant extract of *Tagetes erecta* and *Cymbopogon citratus* were evaluated for ovicidal and repellent activities against *Aedes aegypti*. The egg hatchability was observed after 24 hour exposure. 100% ovicidal activity was observed at 5.5% and 3.0% respectively. Skin repellent test at 0.25, 0.5, 0.75 and 1.0 mg/cm² concentration gave 100% protection upto 4.0, 4.5, 5.0, 5.5 and 6.0 hrs respectively. From the present study it was revealed that extracts from *Tagetes erecta* and *Cymbopogon erecta* can be effectively used in the control of *Aedes aegypti*.

INTRODUCTION

Mosquitoes are the most important single group of insects well-known for their public health importance as they transmit serious diseases to man. They not only cause nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue fever and Japanese encephalitis, and contribute significantly to poverty and social debility in tropical countries (Surendran et al. 2008). Of late Chikungunya, a serious mosquito-borne epidemic has gained momentum in India. In every year hundreds of millions of people suffer from various diseases, mosquitoes alone transmit diseases to more than 700 million people annually (Ana Claudia melo et al. 2004).

Among the different species of mosquitoes, individuals of genus *Aedes* are considered highly dangerous because these show more dependency on human blood and breed in artificial containers like discarded automobile tires, flower vases, tin cans, jars, unused water closets, cisterns and around human habitations (Barrera 1996).

The limitations of conventional insecticides are most concern because of their broad spectrum activity affecting the non-target organisms including human and their higher persistence. Use of commercially available conventional synthetic insecticides has raised serious ecological and economical problems due to their high cost as environmental effects. Increasing the use of synthetic pesticides in agriculture leads to serious problems like environmental pollution health hazards and insect resistance to insecticides.

Botanical insecticides may serve as suitable alternatives to synthetic ones in future, as they are relatively environmentally safe, biodegradable, eco-friendly, target specific and

non-toxic to non-target organisms including human (Govindarajan et al. 2008).

Delonix regia and *Syzigium cumini* were proved to have excellent toxic effect on larval population of different mosquito species. The biological activity of these plant extracts might be due to various compounds including phenols, terpenoids and alkaloids present in plants. The essential oil of *Cymbopogon citratus* was found to be effective against mosquitoes. The study on the acetone extract of *Tagetes erecta* was found as an effective growth regulator against *C. quinquefasciatus* (Pathak et al. 2000).

MATERIALS AND METHODS

For the present study the ethanolic extract of *Cymbopogon citratus* and acetonic extract of *Tagetes erecta* were used to test the efficiency and insecticidal effects against different developmental stages of the mosquitoes *Aedes aegypti*.

Test organisms: To satisfy the number of mosquitoes needed for the day to day bioassays, a colony was essential. The eggs of *Aedes aegypti* were procured from National Institute of Communicable Disease (NICD) at Mettupalayam, India. The mosquito colony was maintained at 7.0-8.5 pH, 28 ± 2°C temperature and 14 : 10 light and dark photo period cycle. The larvae were fed on powdered mixture of dog biscuits and yeast tablets in 3:1 ratio. The pigeon blood meal was given to the female adult mosquitoes and 10% glucose solution was given to the male adult mosquitoes.

Preparation of phytochemical extract: The plants of *Tagetes erecta* and *Cymbopogon citratus* were collected from fields and brought to the laboratory. They were washed with tap water followed by distilled water. The plants were dried

under the shade, and the dried leaves of (100mL) *Tagetes erecta* and *Cymbopogon citratus* were extracted with acetone (300mL) and ethanol (300mL) respectively by using soxhlet apparatus for 8 hours (Vogel 1978). The extract was concentrated in a vacuum evaporator to yield a dark, greenish and gummy extract. The residue was then made into 2:1 stock solutions with respective solvent and taken for further bioassay test.

Phytochemical screening methods: The extracts were used to find out the presence of alkaloids, flavanoids, phenols, steroids, carbohydrates, glycosides tannins and proteins.

Ovicidal activity of *Aedes aegypti* was assessed using the standard method. The *Tagetes erecta* and *Cymbopogon citratus* plant extract were diluted in acetone and ethanol to achieve different concentrations of 1% to 5.5% for *Aedes aegypti* and the eggs were exposed to each dose of plant extract until died. Eggs exposed to acetone and water served as control. After treatment, the eggs from each concentration were transferred to distilled water cups for hatching assessment after counting them under microscope. The test was replicated five times. The hatch rate was calculated 48 h post treatment by the following formula.

$$\frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100$$

Repellent activity: The repellent activity of the *Tagetes erecta* and *Cymbopogon citratus* was determined using the techniques of Fradin & Day (2002) with modification. Three to four days old blood starved 25 adult females of *Aedes aegypti* mosquito were kept in a different net cage (42 × 30 × 30 cm³). The arms of the test person were cleaned with isopropanol. After air drying the arm of the test person, only 25 cm² dorsal side of the skin on each arm was exposed and the remaining area was covered with rubber gloves. The plant extract was dissolved in isopropanol which served as control. The *Tagetes erecta* plant extract of 0.25, 5.0, 7.5 and 10 mg/cm² concentration was applied. The control and treated arms were introduced simultaneously into the cage for a few minutes for every five minutes from 8 to 18 hour for *Aedes aegypti*. The experiment was conducted for five times. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula.

$$\frac{\text{No. of bites received by control} - \text{No. of bites received by treated}}{\text{No. of bites received by control}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical screening of *Tagetes erecta* and *Cymbopogon citratus* was made and the results are presented in Table 1.

The results revealed that the mortality rate and unhatchability of eggs were increased as the exposure concentration was increased and the larvae also undergo melanization slowly. The data were subjected to Finney's method (1964).

Although mosquito-borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk. In attempt to overcome these problems, greater emphasis has been recently placed on the research and development for forms of pest control using natural plant products as larvicides, which have indicated that they could provide possible alternatives to synthetic chemical insecticides. According to Bowers et al. (1995) the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.

Plants contain many chemicals, which are important in their defence against insects. These chemicals fall into several categories including repellents, feeding, deterrents, toxins and growth regulators. Plant extracts in insects cause suppression, sterilization, ovipositional changes and change in biological fitness in them.

The results show that in the acetonic extract of *Tagetes erecta*, alkaloids, flavonoids, proteins and glycosides are present in small quantity, steroids and carbohydrates moderately, and phenols and tannins in high quantity. In the ethanolic extract of *Cymbopogon citratus*, phenols, protein, carbohydrates and tannins are present in very low quantity, flavanoids and glycosides in moderate quantity, and alkaloids and steroids in high quantity.

The eggs of *Aedes aegypti*, exposed to the extract of *Cymbopogon citratus*, show 100% unhatchability at 3% concentration and 21% unhatchability at 1% concentration. The eggs of *Aedes aegypti*, exposed to *Tagetes erecta* plant extract, show 100% unhatchability at 5.5% concentration and 52% unhatchability at 3.5% concentration (Table 2). The results showed that the leaf extracts of *Tagetes erecta* and *cymbopogon citratus* have a log dose based effect of the toxicant on the egg hatchabilities of mosquito *Aedes aegypti*.

The reduction in hatchability may be due to an alteration in morphogenetic hormone due to neuroendocrinological and physiological impairments. Mohanraj et al. (2000) observed a considerable dose based reduction in the hatchability of eggs treated with neem formulation 'econem' with one ppm of azadirachtin found to produce 100% unhatchability in *Aedes aegypti* eggs exposed for 44 hours. Numerous studies on the effect of neem products of hatching of eggs in variety of insects other than mosquitoes are available. Penetration of the active component of insecticides into shells and their effects on embryogenesis may be the reason for the unhatchability.

Table 1: Results of phytochemical screening of *Tagetes erecta* using acetonic extract and *Cymbopogon citratus* using ethanolic extract.

	Alkaloids	Flavanoids	Phenols	Steroids	Proteins	Carbohydrates	Glycosides	Tannins
<i>Tagetes erecta</i>	+	+	+++	++	+	++	+	+++
<i>Cymbopogon citratus</i>	+++	++	+	+++	+	+	++	+

Table 2: Ovicidal effect of *Tagetes erecta* and *Cymbopogon citratus* extract against eggs of *Aedes aegypti*.

Name of the Plant	Extraction	Control	Concentration (%)				
			3.5	4.0	4.5	5.0	5.5
<i>Tagetes erecta</i>	Acetone	100	96	66	12	10	-
			1.0	1.5	2.0	2.5	3.0
<i>Cymbopogon citratus</i>	Ethanol	100	79	57	31	20	-

values are mean of 5 replicates.

Table 3: Laboratory repellent activity of *Tagetes erecta* and *Cymbopogon citratus* extract against *Aedes aegypti*.

Plant species	Concentration of plant extract (mg/cm ²)	Mean number of bites received in control	Mean number of bites received in treated	% of repellency after application				
				4	4.5	5	5.5	6
<i>Tagetes erecta</i>	0.25	8 ± 1	5.67 ± 0.58	100	93	80	70	55
	0.5	8 ± 1	4.33 ± 0.58	100	92	80	71	63
	0.75	6.67 ± 0.58	2.67 ± 0.58	100	100	94	86	78
	1.0	6 ± 1	1.33 ± 0.58	100	100	100	100	92
<i>Cymbopogon citratus</i>	0.25	8 ± 1	4.67 ± 0.58	100	94	81	72	60
	0.5	7.67 ± 0.58	2.67 ± 0.58	100	100	92	80	72
	0.75	7.33 ± 0.58	2.33 ± 0.58	100	100	100	97	90
	1.0	5.67 ± 0.58	0	100	100	100	100	100

This study revealed that *Tagetes erecta* and *Cymbopogon citratus* have repellency activity against the adult mosquito *Aedes aegypti*, and the results of mean protection in relation to dose of these 2 plant extracts are presented in Table 3. 100% protection time was obtained at the concentration of 1.0/cm². The results show that percentage of repellency is directly proportional to the concentration of extracts.

The finding of the present investigation revealed that the leaf extracts of *Tagetes erecta* and *Cymbopogon citratus* possess oviposition deterrent and skin repellent activity against *Aedes aegypti*. The biological activity of the plant extract is due to a variety of compounds in this plant including alkaloids, flavanoids, phenol, protein, carbohydrates and tannins. These compounds may jointly or independently contribute to cause oviposition deterrent against *Aedes aegypti*. Among the two plant extracts, *Cymbopogon citratus* plant extract was found to be more lethal than the other extract.

Cymbopogon citratus is also traditionally used as mosquito repellent in India. Field test in Bolivia showed that 25% *Cymbopogon citratus* in ethanol provided 77.39% and 90.67% protection for three hours against *An. darlingi* respectively.

The present finding showed that the acetone and ethanol leaf extracts of *Tagetes erecta* and *Cymbopogon citratus* were effective for ovicidal control of *Ae. aegypti*. These new agents should preferentially be applied in integrated control strategies to gain maximum impact on adult mosquito population; the feasibility of their use in field, however, needs extensive field trials.

REFERENCES

- Ana Claudia Melo, Michael Rutzelf, R., Jason Pitts and Laurence, J. Zwiebel 2004. Identification of a chemosensory receptor from the yellow fever mosquito, *Aedes aegypti*, that is highly conserved and expressed in factory and gustatory organs. *Chem. Senses*, 29: 403-410.
- Berrera, R. 1996. Completion and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. *Ecol. Entomol.*, 21: 117-127.
- Bowers, W. S., Senser, B., Evans, P., Bingol, F. and Eradogon 1995. Activity of Turkish medicinal plant against mosquitoes *Aedes aegypti* and *Anopheles gambiae*. *Insect Sci. Appl.*, 16: 339-342.
- Finney, O.J. 1971. *Probit Analysis*. Cambridge University Press, London, pp. 68-72.
- Fradin, M.S. and Day, J.F. 2002. Comparative efficiency of insect repellents against mosquito bite. *New England Journal of Medicine*, 347(1): 13-18.
- Govindarajan, M., Jebanesan, A. and Pushpanathan, T. 2008a. Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasitol. Res.*, 102: 289-292.

- Mohanraj, R.S., Saraswathy, S., Thangavel, K. Logaswamy, S. and Dhanakkodi, B. 2000. Effect of a neem formulation on pre-adult stages and egg hatchability of *Aedes aegypti*. *Neem News Letter*, 17: 4-6.
- Pathak N., Mittal, P.K., Singh, O.P., Sagar, V. and Vasudevan, P. 2000. Larvicidal action of essential oils from plants against the vector mosquitoes *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L). *Int. Pest Control*, 42: 53-55.
- Surendran, A.R. and Selvaraj Pandian 2008. Evaluation of larvicidal effect of *Pseudomonas fluorescens* isolates against the filariasis vector mosquito, *Culex quinquefasciatus* Say. *Asian Journal of Microbial Biotech. Env. Sc.*, 10(1): 65-68.
- Vogel 1978. *Textbook of Practical Organic Chemistry*. The English Language Book Society and Longman, London, pp. 1368.