Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

JEZS

Journal of Entomology and Zoology Studies

ISSN 2320-7078
JEZS 2014; 2 (6): 246-248

Received: 05-10-2014
Accepted: 24-11-2014

Poonam Sethi
Assistant Professor, Guru Nanak College, Chennai, India.

Correspondence:
Poonam Sethi
Assistant Professor, Guru Nanak College, Chennai, India.

Larvicidal activity of a living fossil!

Poonam Sethi

Abstract
"Living fossils" refers to plants that are similar to extinct ones. One classic example –Cycads, prominent in the Jurassic and Cretaceous period but recently the cycads have started to disappear. *Cycas circinalis* L. commonly known as Queen Sago is an endemic restricted to the Western Ghats, was investigated for larvicidal activity. The fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* were exposed to test concentrations of 1000,750, 500,250 µg of hexane, chloroform:methanol [1:2] and ethanol extracts of leaf in dimethyl sulphoxide. The chloroform:methanol [1:2] extract showed 91% mortality after 48 hrs of incubation against the *Aedes*, LC50 of 0.302 followed by 0.219 and 0.214 for 750,500, and 250 µl concentrations respectively. Hexane extracts exhibited 95% mortality against *Culex* larvae. The formulations proved to be effective in inhibiting the metamorphosis. This research leads to possible utilization of new phytochemical compounds and their role in the near future as eco-friendly natural pesticides.

Keywords: *Aedes aegypti*, anti larvicidal, *Culex quinquefasciatus*, Cycas, living fossil, natural pesticides.

1. Introduction
Various essential oils such as citronella, eucalyptus, neem and pepper mint oil derived from these plants *Cymbopogon citratus* [1] *Eucalyptus globulus* [2] *Azadirachta indica* [3] and *Mentha piperita* [4] respectively are currently available in several commercially formulated repellents. The repellent potential of plant products to mosquitoes and other pest insects has been well known prior to and the advent of synthetic chemicals [5, 6]. However, their repellency is still lower in both efficacy and duration than that of chemicals Deet and A13-37220. Nevertheless, the possible side effects associated with use of these chemicals should be taken in to consideration. With this in mind, the present study was carried out to investigate the repellent activity of *Cycas circinalis* against the fourth instar larvae of mosquito *Aedes aegypti* and *Culex quinquefasciatus C. quinquefasciatus* transmits Filariasis while dengue fever are transmitted by the vector *A. aegypti* .
Current strategies based on the elimination of breeding sites and applications of chemical Insecticides for larval and adult mosquito control have resulted in development of resistance without eliminating the constant risk of epidemics. Thus new approaches are urgently needed.
Interest on possible use of ecofriendly natural products such as extracts of plants have increased to control vectors. Plant derived products have received increased attention from Scientists for more than 2000 plant species are known to have insecticidal properties [7, 8].
Hence the objective of the present study was to evaluate the larvicidal activity of plant extracts of Gymnospermae, *Cycas circinalis* is an ancient group of plants dating back to the age of the dinosaurs. This plant produces a symmetrical rosette of glossy, stiff fronds with recurved edges. As it grows, it slowly develops a thick, wooden trunk, which can produce basal shoots, later forming multiple crowns. According to Systemic position, Cycas plant occurs in Division, Cycadophyta; Class, Cycadopsida; Order, Cycadales and Family, Cycadaceae.

2. Materials and Method
2.1 Collection of plant material
The leaves of *Cycas circinalis* were collected from college campus during the month of March identified and authenticated by botanist of Regional Research Institute of Unani Medicine-RRIUM. The leaves were washed, shade dried and powdered.

2.2 Extraction
About 10 gm of dry leaves of the experimental plant were macerated in a shaker with 100 ml

of hexane, methanol and ethanol for 48 hrs separately. Then filtered through a Whatman no.1 filter paper by suction. Filtrate was evaporated under vacuum for 40 °C until completely dry.

2.3 Rearing of *Aedes aegypti* and *Culex quinquefciatus* larvae

The eggs of *Aedes aegypti* were procured from the Central Research Medical Entomology Institute at Madurai, Tamilnadu, India. The egg rafts of Aedes were kept in the tray containing tap water (culture medium) at laboratory condition 29±1 °C. After 24 hours of incubation, the eggs were observed to hatch out into first instar larvae. Appropriate amount of nutrient (sterilized yeast powder and dog biscuit in 1:1 ratio) were added to enhance the growth of larvae. The fourth instar larvae were used for further study. *Culex quinquefasciatus* were reared in laboratory, maintained at 27 ± 2 °C and 70 ± 5% relative humidity with a photoperiod of 12:12 hours (Light:Dark) with 90 minutes down and dusk simulation periods. Adult mosquitoes were provided with 10% sucrose. The fourth instar larvae were used for further study.

2.4 Larvicidal bioassay

The plant extracts were dissolved in 10 µl of DMSO for its solubility, in water. Larvicidal activity was determined according to WHO protocol [9]. The larvae were treated with the plant extracts of 1000 µg/ml concentration in a conical flask. A corresponding control was maintained. The larval mortality of fourth instar of Aedes and Culex was observed. The number of larvae surviving at the end of 24 and 48 hours were recorded and the percent mortality was calculated [10].

Percentage of mortality = (Number of dead larvae/Total number of larvae) x 100.

2.5 Lethal Concentration

The LC50 is the plant extract that showed nearly 50% mortality was determined by a similar procedure as mentioned above 750, 500, and 250 µg/ml concentration were tested and the observation was recorded after 24 hrs of incubation. The LC50 was determined by a Probit analysis, Percentage Mortality = mean ±SD [11].

2.6 Phytochemical analysis

The plant extracts of hexane, ethanol and methanol: chloroform that showed 100 % mortality of Aedes and Culex larvae were screened for the phytochemicals [12, 13, 14]. Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars, cardiac glycosides and anthraquinones.

2.7 Statistical analysis

Data was analysed for statistical significance using SPSS (Statistical Package for Scientific Studies) software.

3. Results and Discussion

The chloroform: methanol extract at 1000 µg concentration of Cycas was highly toxic to the 4th instar larvae, *Aedes aegypti* and showed 91% of mortality rate but against Culex the hexane extract exhibited 95% mortality. All other plant extracts had no significant effect on the larvae. (Table 1). All the treatments were replicated thrice, mortality calculated by the formula of Abbott (1925). Table 2 shows the activity of active extracts only *Aedes aegypti* showed maximum mortality in 750 µg/ ml concentrations when compared to 500 and 250 concentration. It was observed that the LC50 of 750 µg/ ml is 0.302 against *Aedes aegypti* and hexane extracts at 750 µg/ ml showed LC50 of 0.363 against *Culex quinquefasciatus*. It was concluded that the mosquito larvae exposed to plant extracts showed behavioural changes morphologically. The most obvious sign of behavioural changes observed in Aedes was restlessness, loss of equilibrium which finally led to death while in Culex it showed only repellent activity. The phytochemical test of plants extract which showed nearly 100% mortality revealed that extract contained mainly alkaloid, tannin and resin which might have or enhance the larvicidal properties (Table 3). This is a first report of a gymnosperm being used as a larvicidal agent.

Table 1: Larvicidal activity of extracts at different time intervals

Mosquito Larvae (Fourth Instar Larvae)	**TREATMENT**	**% mortality at 1000 µg/ml concentration**	
		24 HRS	**48 HRS**
Aedes aegypti	Control	0	0
	Hexane	10.11	21.30
	Ethanol	33.30	51.75
	Chloroform:Methanol	65.11	91.18
Culex quinquefasciatus	Control	0	0
	Hexane	55.52	95.11
	Ethanol	32.18	51.23
	Chloroform:Methanol	28.22	44.21

Table 2: Lethal Concentration of Plants Extracts after 24 Hrs

Mosquito Larvae	**Treatment**	**Concentration (µg/mL)**	**% Mortality**	**LC_{50}**
Aedes aegypti	Methanol: Chloroform	750	73.3±2.88	0.302
		500	78.3±2.88	0.219
		250	83.8±1.88	0.241
Culex quinquefasciatus	Hexane	750	70±5.11	0.363
		500	81.6±2.88	0.288
		250	83.3±2.88	0.251

Table 3: Phytochemical Analysis of Plant Extracts

Phytochemical	Hexane extract	Ethanol extract	Methanol:Chloroform[1:2] Extract
Alkaloid	+	-	+
Phenol	-	+	-
Flavonoid	+	-	+
Tannin	+	-	+
Terpenoid	+	-	+
Gum -	-	-	-
Anthraquinone	-	+	-
Phlobatannin	+	-	+
Resin	+	-	+
Sugar	-	+	+
Saponin	-	+	-

Earlier it was suggested that Neem Azal is a promising candidate for the use in integrated management programme to replace chemical insecticides [15] as the effect of Azadirachtin, from *Azadirachta indica* [16] against larvae and pupae of *Culex pipiens* mosquito in the Republic of Algeria was found effective but only in controlling mosquito larvae in different breeding sites under natural field conditions. When compared to the study of Sharma, the protective effect of hexane extracted of *Cyperus rotundus* against *Culex quinquefasciatus*, seem to be higher than that of neem oil (37.5%). Selection of a repellent for further development cannot be based on the results of any one test against a single insect because mosquito responses to repellents vary within and among species [17] as suggested by other researchers, oil yielding plants are effective in curtailing mosquito population. Cycas a gymnosperm is also rich in resin and oils which might be a promising candidate against the larvae of mosquitoes. Cycas is known to possess other medicinal uses the folia has been reported for inhibition of cytochrome P-450 aromatase used in the treatment of estrogen-dependent tumors [18]. Terminal shoots are considered as astringent and diuretic. Seeds are considered emmenagogue, expectorant. Cycas stems and seeds are known to be used for high blood pressure, headaches, congestion, rheumatism and bone pain. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities as observed by many researchers [19].

4. References

1. Thorsell W, Mikiver A, Malander I, Tunón H. Efficacy of plant extracts and oils as mosquito repellents. Phytomedicine 1998; 5(4):311–323.
2. Collins DA, Brady JN, Curtis CF. Assessment of the efficacy of Quwenling as a mosquito repellent. Phytotherapy Research 1993; 7(1):17–20.
3. Sharma VP, Ansari MA, Razdan RK. Mosquito repellent action of neem (*Azadirachta indica*) oil. American Mosquito Control Association Journal 1993; 9:359–360.
4. Ansari MA, Vasudevan P, Tandon M, Razdan RK. Larvicidal and mosquito repellent action of peppermint (Mentha piperita) oil. Bioresource Technology 2000; 71(3):267–271.
5. Granett P. Studies of mosquito repellents II Relative performance of certain chemicals and commercially available mixtures as mosquito repellent. Journal of Economic Entomology 1940; 33:566–572.
6. Rutledge LC, Collister DM, Meixsell VE, Eisenberg GHC. Comparative sensitivity of representative mosquitoes (Diptera: Culicidae) to repellents. Journal of Medical Entomology 1983; 20:506–510.
7. Pankaj T, Anita S. Assessment of larvicidal properties of aqueous extracts of four plants Against Culex quinquefasciatus larvae. Jordan Journal of Biological Sciences 2010; 3(1):1-6.
8. Larvicidal Activity of Edible Plant-Derived Essential Oils Against the Pyrethroid Susceptible and -Resistant Strains of Aedes aegypti (Diptera: Culicidae). Journal of Vector Ecology 2010; 35(1):106-115.
9. World Health Organization. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC 1981; 81:807-962.
10. Abbott WS. A method for computing the effectiveness of an insecticide. J Econ Entomol 1925; 18:265-7
11. Finney DJ. Probit analysis. Cambridge: Cambridge University Press, 1971.
12. Trease GE, Evans WC. Pharmacognosy, Edn 14th, Brown Publication, 1989.
13. Harborne JB. Phytochemical method, Edn 3rd. Chapman and Hall, London, 1983, 135- 203.
14. Sofowora AO. Traditional Medicine and Medicinal Plants in Africa. Edn 2, University of Life Press, 1993, 320.
15. Rutledge LC, Collister DM, Meixsell VE, Eisenberg GHC. Comparative sensitivity of representative mosquitoes (Diptera: Culicidae) to repellents. Journal of Medical Entomology 1983; 20:506–510.
16. Sharma VP, Ansari MA, Razdan RK. Mosquito repellent action of neem (Azadirachta indica) oil. American Mosquito Control Association Journal 1993; 9:359–360.
17. Singh N, Kulshrestha VK, Gupta MB, Bhargava KP. A pharmacological study of Cyperus rotundus. Indian Journal of Medical Research 1970; 58:103–109.
18. Sobha RM, Rao VC, Gunasekhar AB, Bodo B. A Bioflavonoid from *Cycas beddomei*. Phytochemistry 1998; 47(2):319-321.
19. Mourya MK, Archana P, Swami A, Singh GK, Mathur A. Leaves of *Cycas revoluta*: Potent Antimicrobial and Antioxidant Agent. World Journal of Science and Technology 2011; 1(10):11-20.