Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(1): 373-377

Received: 24-11-2016
Accepted: 25-12-2016

Amitabh Shad
Department of Zoology and School of Entomology, St. John's College, Agra, Uttar Pradesh, India

J Andrew
Department of Zoology and School of Entomology, St. John's College, Agra, Uttar Pradesh, India

Correspondence
Amitabh Shad
Department of Zoology and School of Entomology, St. John's College, Agra, Uttar Pradesh, India

Larvicidal efficacy of ethanolic extracts of *Annona squamosa* (Annonaceae) over the filarial vector, *Culex quinquefasciatus* Say (Culicidae)

Amitabh Shad and J Andrew

Abstract
The mosquito *Culex quinquefasciatus* Say, is the major vector of filariasis, Japanese encephalitis and other plagues throughout the world, especially in the tropical and subtropical countries. For many decades chemical insecticides are widely used to control mosquitoes. Mosquitoes have developed resistance against these chemical insecticides. Excessive use of such insecticides has also adversely affected the environment. There is an imperative demand to search novel eco-friendly substitutes which are more effective, safe and economical. Plant extracts with proven insecticidal properties are an alternate to these insecticides. The present study describes the larvicidal activity of *Annona squamosa* plant over the mosquito, *C. quinquefasciatus*. The average larval mortality percentage after 24 hours of exposure to the ethanolic leaf extract of *A. squamosa* was recorded as 20%, 30%, 50%, 68% and 100% in respective concentrations of 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml. The results of the present study revealed that the ethanolic leaf extracts of *A. squamosa* plant can be used effectively as a potential, eco-friendly, biodegradable and economic larvicide in integrated mosquito control programme.

Keywords: *C. quinquefasciatus, A. squamosa*, larvicidal activity, mortality

1. Introduction
Mosquitoes are the most important single group of insects in term of public health because of their ability to transmit a number of outrageous diseases like Japanese encephalitis, filariasis, malaria, chikungunya and dengue, causing millions of deaths every year throughout the world [1, 2]. These diseases not only cause high levels of morbidity and mortality but also inflict great economic loss and social disruption on developing countries including India [2]. Among these communicable diseases, Lymphatic filariasis caused by *Wuchereria bancrofti* is a major problem of the tropics and subtropics. According to the expert committee on filariasis, 905 million people were at risk of filariasis with 90.2 million of victims worldwide in 1984 and the figures were 751.4 million and 78.6 million in 1992 respectively [3,4]. At present, 1.3 billion people are at risk of lymphatic filariasis infection and about 120 million people are affected in 83 countries amongst them about 45.5 million people are from Indian subcontinent [3]. Japanese encephalitis is another major mosquito borne disease endemic in at least 21 countries with nearly 30,000-50,000 cases annually from Asia [4]. In India, as estimated 378 million people are living at the risk of Japanese encephalitis in 12 states including Uttar Pradesh [5,6]. It is an alarming situation to limit such mosquito borne disease transmission through different means by reducing vector population by larvicides, adulticides and introduction of natural predators of mosquitoes in ecosystem.
The mosquito *C. quinquefasciatus* Say acts as a vector for filariasis, Japanese encephalitis and other such vector borne diseases in India. Lymphatic filariasis caused by *W. bancrofti* transmitted by *C. quinquefasciatus* is found to be more endemic in Indian subcontinent. It is reported that the mosquito *C. quinquefasciatus* infects more than 100 million individuals worldwide annually. The disease remains endemic in more than 100 developing tropical countries and its control is a major goal to achieve improved human health worldwide. *C. quinquefasciatus* are involved in transmitting viral, bacterial and protozoan diseases around the world. Medically the most important species, *C. quinquefasciatus* breeds in dirty waters polluted with organic debris, soak pits, drains, ditches, septic tanks and other such places. The adults of *C. quinquefasciatus* prefer to inhabit areas where there is human dense settlement; larvae dwell in polluted stagnant water.

Eliminating the source of infection is an essential step in control of mosquito-borne disease. Synthetic insecticides are being widely used as larvicides to control mosquitoes [8]. These insecticides are generally chlorinated hydrocarbon like DDT, dieldrin, endosulfan; organophosphates like diazinon, ben solice and carbamates like adizarb, carbofuron [8]. Mosquitoes develop genetic resistance to these synthetic insecticides and even to biopesticides such as *Bacillus sphaericus*. At the same time synthetic insecticides also adversely affect the environment by contaminating air, water, and soil. There is an urgent need to find alternatives to these synthetic insecticides which may be more potent, economic and eco-friendly. Traditionally in India and other parts of globe, plant based products have been used in human communities since many centuries for controlling insects [9]. Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target-insects and are eco-friendly [9]. About 2000 species of terrestrial plants have been reported for their insecticidal properties. Plant based products does not have any hazardous effect on ecosystem. Plant secondary metabolites and their synthetic derivatives provide alternative source in mosquito control. Several secondary metabolites present in plants serve as a defence mechanism against insect attacks. Recent research has proved that effectiveness of plant derived compounds, such as saponine, steroids, isoflavonoids, essential oils, alkaloids and tannins has potential mosquito larvicides. These bioactive chemical may act as larvicides, insecticides, antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones as well as attractants [9, 12]. Plant based pesticides are less toxic, delay the development of resistance because of its new structure and are easily biodegradable. Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities. It should also be noted that the larvicidal efficacy of a plant extracts differs depending upon the solvent. *Tagetes minuta* aqueous leaf extracts had less fatal effects against *C. quinquefasciatus* larvae. Similar trends were observed in previous studies on larvicidal activity, where ethanol and hexane leaf extracts of *Cassia occidentalis* and *Lantana camara* displayed better larvicidal activities toward *Anopheles stephensi* and *Aedes aegypti* larvae, respectively than aqueous extracts.

The family *Annonaceae* comprising of about 150 plant species is known for its insecticidal property. *Annona squamosa,* commonly known as 'Custard apple' is a native of West Indies and is cultivated throughout India, mainly for its edible fruit. It is a tropical fruit tree originating from Asia and America. This plant species is traditionally used in treatment of different disorders such as constipation, fever, ulcers, cancer and tumour [16]. It is also reported to exhibit antimicrobial, antioxidant, antibacterial and hepatoprotective properties [7, 16]. The leaves and roots of *A. squamosa* are also well known for their delayed insect growth regulating activity in the form of larval-pupal intermediates and half ecdysis adults. The plant is also effective against insects especially Lepidopterans and Coleopterans [17, 18].

Search for eco-safe, low cost and a highly potential insecticide for the control of mosquito needs the preliminary screening of plants to evaluate their insecticidal activities. The present investigation was carried out to explore the larvicidal potential of ethanolic extract of *A. squamosa* leaf against the fourth instar larvae of the mosquito, *C. quinquefasciatus* Say.

2. Materials and Methods

2.1 Screening and collection of plant material

The medicinal plant, *A. squamosa* (Figure-3a) was selected on the basis of ethnopharmacological information, aromatic smell and ethnobotanical literature surveyed. The leaves of selected plant were collected locally in and around Agra city (26° 44' N to 27° 25' N latitude; 77° 26' E to 78° 32' E longitude) during the year 2010-2011. The leaves were plucked early in the morning and brought to the laboratory in plastic sampling bags. The taxonomic identification was done with the help of experienced botanists at the Department of Botany, St. John's College, Agra. The voucher specimens were numbered and kept in our research laboratory for further reference.

2.2 Preparation of plant extracts

The leaves of the plant were washed using tap water; shade dried for 7–10 days at room temperature (27-37 °C) during day time and powdered individually using electrical blender (Bajaj, India). Powdered plant material was then sieved using strainer. The obtained dried powder (200 grams) of the plant was extracted with 600 ml of ethanol (Qualigens, Fine chemicals Mumbai, India) with a minimum of 8 hours up to 48 hours in Soxhlet apparatus at 55 °C to 60 °C [13]. The extracts thus obtained, was filtered using Whatmann Filter Paper (12.5 cm) and concentrated using rotatory evaporator to remove ethanol. The solidified plant extracts thus obtained was stored in sterilized amber coloured bottle and maintained at 4 °C in refrigerator for experimental use.

2.3 Mosquito culture

The larvae and adults of the mosquito, *C. quinquefasciatus* were collected from various mosquitogenic regions of Agra during the year 2010-2011. Identification was done using standard key [11]. Sampling of larvae and pupae were done using aquatic dipnet. Egg rafts, if any, were collected in the filter paper and kept in the specimen box.

The colony was maintained from the egg rafts kept in white enamelled breeding bowls 10 inches in diameter, 5 inches deep and half filled with de-chlorinated tap water. The water in the breeding bowls, on the onset of the first instars larvae was supplemented with yeast at an interval of every twenty-four hour, till the larvae transformed into pupae. Each bowl contained about 150- 200 larvae. The scum, if formed, was removed using a glass rod. Pupae were collected daily and transferred into empty 18″×18″×18″ mosquito breeding cages. The metamorphosed adults were fed with 10% sugar solution or honey soaked in a piece of cotton for three to four days. Each cage contained about 200♀ and 100♂ adults which were allowed to mate. The females were then given blood meals from a healthy but immobilized pigeon during nights on alternate days. For egg laying suitable containers half filled with water were introduced inside the breeding cages which contained fertilized females. Each container was lined with filter paper to prevent the egg rafts from adhering to the containers walls. The egg rafts were processed again to yield adults as described above. Deposited egg rafts were collected daily, numbered, marked and incubated for experimental usage.

2.4 Larvicidal bioassay

In the larvicidal bioassay, fourth instar larvae of *C. quinquefasciatus* were exposed to different concentrations of *A. squamosa* extract. The larvicidal activity was assessed by the standard WHO procedure with slight modification [14].

Five different concentrations of *A. squamosa* leaf extract were prepared in dechlorinated tap water i.e. 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml. 100 ml of each such concentration was taken in glass beakers (Borosil 250ml). Ten healthy fourth instar larvae of the mosquito *C. quinquefasciatus,* were then introduced in different test concentrations of plant extracts. Five replicates of each concentration were run simultaneously along with control at room temperature. The larvae were fed dried yeast powder on the water surface. The number of dead larvae was recorded at an interval of 24 hours, 48 hours and 72 hours of exposure. The dead larvae were removed soon after the mortality in order to prevent decomposition, which may cause rapid death of the remaining larvae. A total of three such trials were carried out. The corrected mortality was analysed using Abbott Formulae [15]. The behavioural changes, if any, were also observed and recorded.

2.5 Statistical analysis

The collected data pertaining to the toxicity assay were analysed statistically using SPSS, MS Excel 07 and Sigma plot 12.5 software.

3. Results and Discussion

A. squamosa extracts was taken in five different concentrations of 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml and the control was also maintained (Table-1, Fig-1). The average larval mortality percentage of *C. quinquefasciatus* was recorded as 20%, 30%, 50%, 68% and 100% in 24 hours of exposure to the given concentrations of plant extract respectively. After 48 hours of exposure the average larval mortality percentage was recorded as 52%, 70%, 70%, 100% and 100% for 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml, respectively. The average larval mortality after 72 hours of exposure was recorded as 60%, 90%, 100%, 100% and 100% in respective concentrations of 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml. The larval mortality was negligible in respective controls used during the experiment (Table-1, Fig-1). The larvae could not survive at these concentrations (Fig-2, 3b). However, the larvae treated with much lower concentrations of *A. squamosa* leaf extracts pupated but the emerging adults were morphologically deformed and could not survive while the larvae in the control transformed into normal adults.

Table 1: Larval mortality percentage of *C. quinquefasciatus* on exposure to different concentrations of *A. squamosa* leaf extract.

Concentration (Mg/Ml)	Larval Mortality %		
	24 hrs (M24)	48 hrs (M48)	76 hrs (M72)
2.00	20	52	60
4.00	30	70	90
6.00	50	70	100
8.00	68	100	100
10.00	100	100	100
Control	00	00	1

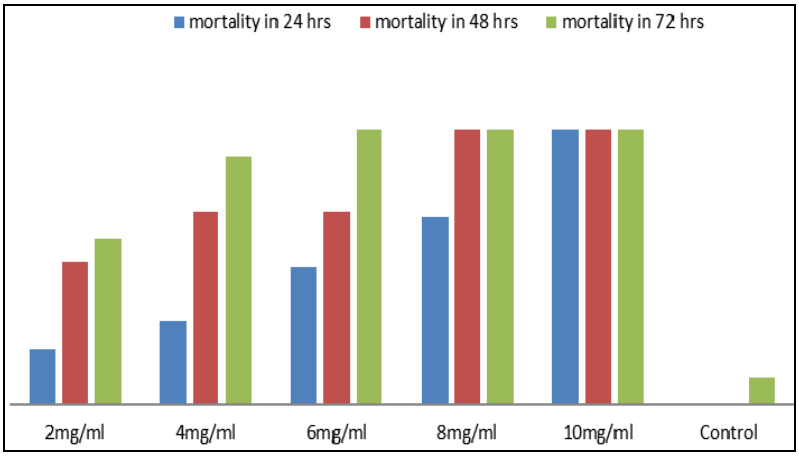

Fig 1: Larvicidal activity of *A. squamosa* extract over fourth instar larvae of *C.quinquefasciatus* Say.

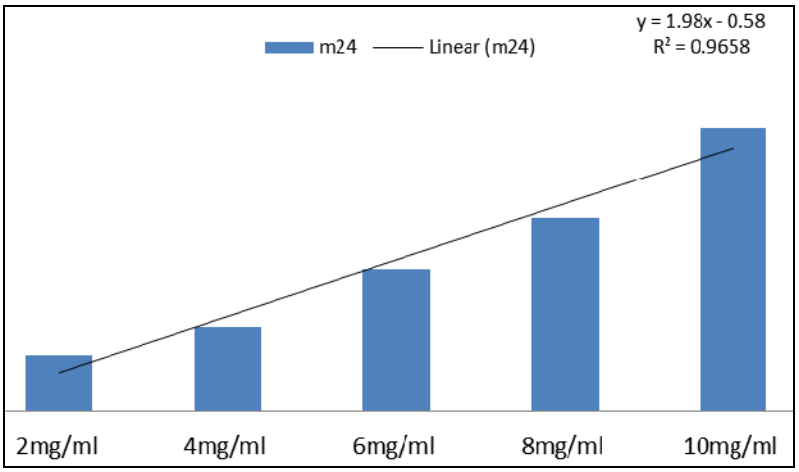

Fig 2: Larvicial activity of *A. squamosa* extracts over *C. quinquefasciatus* in 24 hours

Fig 3(a): *A. squamosa* Plant

Fig 3 (B): Dead *C. quinquefasciatus* larvae

The ethanolic rood wood extract of a Brazilian plant, *A. crassiflora* has been reported for its larvicidal activity against the mosquito *A. aegypti* with LC_{50} value of 0.71µg/ml [29]. Another Brazilian species, *A. glabra* was reported for its larvicidal activity of its seed extract in ethanol against *A. aegypti* with LC_{50} value of 0.06µg/ml [29]. The seed extract in ethanol of the plant *A. muricata* native to Thailand was reported to exhibit larvicidal efficacy of 20.26µg/ml against the mosquito *A. aegypti* [19]. In India the methanolic leaf extract of *A. squamosa* was reported for its larvicidal activity of 20. 26µg/ml against the larvae of the mosquito *A. aegypti* [20]. In this study also, the larvicidal activity of ethanolic leaf extracts of *Annona squamosa* plant in five different concentration of extract ranging from 2.00 - 10.00 mg/ml and the LC_{50} value in 24 hours (6.00) revealed that the *A. squamosa* extract was highly effective as larvicide against the fourth instar larvae of the mosquito, *C. quinquefasciatus*. The difference in the larvicidal toxicity of the different plants extract may be due to kind or concentration of the active compound present in the plant species which may be caused by differences of soil and vegetation type of habitat of plants. Previous studies that was done to validate the effect of geographical location on *Anogeissus leiocarpus*, *Ficus benghalensis* and *Harrisonia abyssinica* revealed that the toxicity of these plants differ in their activity form one location to other [21-23]. This may also be attributed to the differences in plant chemical composition between seasons of collection and the area where they are found. The potential of *Annona* as a larvicidal plant is further supported by the results of a study conducted in Thailand by Satoto (1993) which revealed that *Annona* seeds are one of the most effective larvicides against the mosquito *C. tritaeniorhynchus,* a rice field breeder [28]. In addition to insecticidal properties, the plant has been reported to serve as relief for sinusitis and dizziness and as cough expectorant.

During the experiment it was also noted that the treated mosquito larvae could not pupate and died as tanned fourth instar larvae (Fig-3b). However, a few fourth instar larvae treated with lower concentration of the *A. squamosa* extracts, moulted into pupae but could not transform into adults and remained entangled in the exuvium while the larvae in the control transformed into normal adults. This inability of *C. quinquefasciatus* mosquito larvae and puape to transform into normal adults, in the present study can be attributed to the imbalance of eclosion hormone in the haemolymph [26]. *A. squamosa* is also reported for its larvicidal growth regulator and chemosterilant activity against the mosquito *Anopheles stephensi* [24]. In the present study, it was also noted that the larvae at higher concentration showed extremely limited movements indicating decreased mobility prior to death of *C. quinquefasciatus* larvae. This may be due to the toxicity of annonaceous acetogenins or ubiquinone oxidoreductase the potent inhibitors present in *Annona squamosa* plant [27]. The sluggishness of the larvae at the surface may be due to the diminished availability of ATP molecules at the cellular level also [25].

4. Conclusion

In recent times, trend for combating mosquito borne diseases has shifted from relying solely on synthetic insecticides to the use of plant derived herbicides, biological control, source reduction and environmental management through community participation. The findings of present investigation has re-emphasised the need to explore the possibility of using herbal-based larvicides as supplementary and complimentary measures for mosquito control in order to reduce the chemical burden on the environment. The present study revealed that the ethanolic leaf extracts of *A. squamosa* plant can be used effectively as a potential, eco-friendly, biodegradable and economic larvicide in integrated mosquito control programme. Further investigations are needed to study the mode of action, its effect on non-target organisms and field evaluation as larvicide.

5. Acknowledgements

The authors are grateful to the University Grant Commission, New Delhi for providing the financial assistance through the Project F. No. 34-481/2008 (SR).

6. References

1. World Health Organisation. Vector resistance to pesticides. Fifteenth report of the WHO expert committee on Vector biology and control. Tech. Rep. Ser. 1992; 818:1-62.
2. World Health Organisation. Malaria prevention and control. WHO Report. Geneva, 1998.
3. Hag E, Nadi EAAH, Zaitoon AA. Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). Phyto. Res.1999; 13:388-392.
4. WHO. The World Health Report, Geneva, 1996.
5. Service MW. Management of vectors. In: Youdeowei, A. and M.W. Service (Eds.), Pest and Vec. Manag.in Trop, 1983; 265-280.
6. Gubler DJ. Resurgent vector-borne diseases as a global health problem. Emerg. Infect. Dis. 1998; 4(3):442-450.
7. Service MW. Medical entomology for students, 2nd edn. Cambridge University Press, Cambridge. 2000; 52-55.
8. Indian Council of Medical Research. Chemical insecticides in malaria vector control in India. ICMR Bull. 2002; 32:0377- 4910.
9. Indian Council of Medical Research. Prospects of using herbal products in the control of mosquito vectors. ICMR Bull. 2003; 33:1-12.
10. Perich MJ, Wells C, Bertsch W, Tredway KE. Toxicity of

extracts from three *Tagetes* against adults and larvae of yellow fever mosquito and *Anopheles stephensi* (Diptera: Culicidae). J Med. Entomol.1994; 31:833-7.
11. Barraud PJ. The fauna of British India: Diptera. Taylor and Francis London, 1934, V.
12. Rattan RS. Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Prot 2010; 29:913-920.
13. Vogel. Textbook of practical organic chemistry. The English Language Book Society and Longman, London, 1978.
14. World Health Organisation. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDC/WHOPES/GCDPP/2005.13.
15. Abbott WS. A method of computing the effectiveness of insecticides. J Econ. Entomol. 1925; 18:267-269.
16. Saleem MTS, Hema B, Ravi V, Bhupendra S, Verma NK, Patel SS *et al*. Phyto-pharmacological review of *Annona squamosa* Linn. Trade Science Inc. An Indian Journal. East Sikkim, India. 2009; 5(2):85- 88.
17. Babu PBS, Rao JM. Evaluation of some plant extracts as feeding deterrents against adult *Longitarsus nigripennis* (Coleoptera: Chrysomelidae). Entomon, 1996; 21(3-4): 291-294.
18. Kulkarni N, Joshi KC. Insecticidal action of some plant extracts against Albizia defoliator *Rhesala imparata* Walker (Lepidoptera; Noctuidae). Entomon, 1997; 22(2):75-79.
19. Promsiri S, Naksathit A, Kruatrachue MM, Thavara U. Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non-target fish. Insect. Sci. 2006; 13:179-188.
20. Das NG, Goswami D, Rabha B. Preliminary Evaluation of Mosquito Larvicidal Efficacy of Plant Extracts. J of Vect. Dis. 2007; 44(2):145.
21. Muraina I, Adaudi A, Mamman M, Kazeem H, Eloff J. Effects of geographical location on the yield and bioactivity of *Anogeissus leiocarpus*. J of Phar. Bior. 2008; 5:22-26.
22. Saifi A, Hemlata R, Patel NM. Evaluation of antimicrobial activity of stem bark of *Ficus bengalensis* Linn. Collected from different geographical regions. J of Phar. 2010; 2(7):178-180.
23. Innocent E, Magadula JJ. Variations of anti-mosquito larvicidal constituents in the *Harrisonia abyssinica* species of Tanzania. Inter. J of Bio and Chem. Sci. 2010; 4(1):218-225.
24. Saxena RC, Harshan V, Saxena A, Sukumaran P, Sharma MC, Larkshmana KM. Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi*. J Am. Mosq. Cont. Assoc. 1993; 9(1):84-87.
25. Gu Y, Hukriede NA, Fleming RJ. Serrate expression can functionally replace Delta activity during neuroblast segregation in the *Drosophila* embryo development. 1995; 121(3):855-865.
26. Mulye H, Gordon R. Effects of selected juvenile hormone analogs on sixth-instar larvae of the eastern spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae). Canad. Entomol. 1989; 121:1271-72.
27. Chang FR, Wu YC. Novel cytotoxic annonaceous acetogenins from *Annona muricata*. J Nat. Prod. 2001; 64:925-931.
28. Satoto TBT. A laboratory study of the biological effects of some medicinal plants on *Culex tritaeniorhynchus* sp. Fac. Trop. Med. 1993; 12:11.
29. Omena MC, Navarr O, Paula JE, Luna JS, Ferreira DLMR, Santana AEG. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. Bio. Techn. 2007; 98(13):2549-2556.
30. Goddard J. Infectious diseases and arthropods, 2nd edn. New Jersey: Humana Press, 2008, 31.
31. Vasudevan P, Kashyap S, Sharma S. *Tagetes*: a multipurpose plant. Biores. Tech. 1997; 62:29-35.