Evaluation of the larvicidal activity of the leaf extracts of *Duranta erecta* Linn. (Verbenaceae) on the larvae of *Culex quinquefascitatus* (Say) (Culicidae)

McConnell Marie Serena[1*], M. Balasubramani[1], K. Rajan[1] and I. A. J. Gerald[2]

ABSTRACT

There is an urgent need to explore and utilize naturally occurring products for combating harmful agricultural and public health pests. The present study was carried out to evaluate the insecticidal property of the methanol (ME) and water (AqE) extracts of *Duranta erecta* Linn. leaves against larvae of *Culex quinquefascitatus* (Say)**.** The extraction was done by using methanol (ME) and water (AqE) as solvents. The preliminary phytochemical screening of the extracts showed the presence of sugars, tannins, saponins, steroids, alkaloids, phenols, flavanoids, glycosides, triterepens and carboxylic acid. Both extracts of *D. erecta* have larvicidal activity. Between the extracts, ME has more than AqE.

Key words : *Duranta erecta,* crude extracts, larvicidal activity, *Culex quinquefascitatus.*

INTRODUCTION

Mosquitoes which are responsible for the transmission of more diseases than any other group of arthropods play an important role as etiologic agents of malaria, filariasis, dengue, yellow fever, Japanese encephalitis and other viral diseases (James, 1992). The management of larvae through the use of larvicides is an ideal method for controlling mosquitoes by reducing mosquito breeding (Gluber, 1998). Since "adulticides" may only reduce the adult population temporarily, most mosquito control programmes target the larval stage in their breeding sites with larvicides (El Hag *et al.,* 1999, 2001). It is easier to control delicate mosquito larvae that have not yet left their aquatic habitat than to control adult mosquitoes. This method reduces the overall application of pesticides needed to control the mosquito population (Dharmagadda *et al.,* 2005; Mohan *et al.,* 2010).

Natural products of plant origin with insecticidal properties have been tried in the recent past in order to control a variety of insect pests and vectors. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability. Several plant species like marine plant extracts (Thangam *et al.,* 1991), *Ageratum conyzoides* (Saxena *et al.,* 1994), *Solanum xanthocarpum* (Mohan *et al.,* 2006, 2010), neem (El Hag *et al.,* 2001), *Tagetes patula* (Dharmagadda *et al.,* 2005), *Annona squamosa* Linn. And *Pongamia glabra* Vent. to *Azadirachta indica* A. Juss (George *et al.,* 2005), *Adhatoda vasica*, *Azadirachta indica* and *Ocimum sanctum* (Pandian *et al.,* 1995), *Toddalia asiatica* (Borah *et al.,* 2010) and their commercial products (Caraballo, 2000). The present study was done to evaluate the insecticidal property of the crude leaf extracts of *Duranta erecta* Linn. In the Indian sub - continent the genus is represented by two species *D. erecta* and *D. stenostachya*. In the present investigation, the joint action of *Duranta erecta* crude methanol (ME) and water (AqE) were evaluated against the larvae of the filarial vector, *Culex quinquefasciatus*.

MATERIALS AND METHODS

Plant collection and extraction

The healthy, fresh leaves of *Duranta erecta* Linn. were collected during the month of December. They were washed and died in the shade, these were used for the preparation of methanol extract, and fresh leaves were used for the aqueous extract. After collection, the plant was washed three times in tap water and the leaves were thoroughly shade dried for two weeks and powdered in a domestic grinder and stored in a refrigerator for further use. From the stock 10 gm of powder was used for the extract by the cold method. The powder was dissolved in 100 ml of methanol/DW in an air tight separating funnel for about 7 days. After this period the final volume was measured and the concentration assumed as 100%. From this stock, different concentrations were prepared in water

medium and used for the LC_{50} tests. The same were used for all the phytochemical analysis.

Phytochemical analysis

Preliminary phytochemical analysis of the extracts was done following the methods of Harborne (1973). Quantitative analysis was done for alkaloids (Buzarbarbua, 2000), tannins (Folin - Denis method), total phenols (McDonald *et al.*, 2001) and total flavonoids (Buzarbarbua, 2000).

Insect collection and maintenance

The larvae were collected from our college campus, Tiruchirappalli District and they were identified by the Vector Control Institute, Pondicherry. They were reared and the adult colony were fed by 10% sucrose solution and the females on rat blood and maintained in the laboratory conditions (30°C ± 3°C) for further experiments. Larvae were fed a diet of brewer's yeast, dog biscuits, and algae collected from ponds in a ratio of 3:1:1, respectively.

Bioassay

The treatments were carried out taking 0.01% (10ìL); 0.02% (20 ìL); 0.03% (30 ìL); 0.04% (40 ìL); 0.05% (50 ìL); 0.06% (60 ìL); 0.07 % (70 ìL); 0.08 % (80 ìL); 0.09 % (90 ìL); 1.0% (1000 ìL); 1.5% (1500 ìL); 3 % (3000 ìL) from both ME and AqE and made up to 50 ml by adding with dechlorinated tap water. The control was prepared without any extract. In each concentration 10 larvae of third instar of *Culex quinquefasciatus* were introduced and observed. All experiments were done in triplicate and the mean values were tabulated. Mortality observations were carried out at 24 and 48 hours post-treatment. Alive larvae were maintained without the plant extracts and recorded hatching per cent of the adults.

RESULTS AND DISCUSSION

Nowadays, mosquito control is mostly directed against larvae and only against adults when necessary. This is because the fight against adult is temporary, unsatisfactory and polluting for the environment, while larval treatment is more localized in time and space resulting in less dangerous outcomes. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified (Howard and Zhou, 2007). The methanol extract of *D. erecta* was found to contain sugars, tannins, alkaloids, phenols, flavonoids, saponins, triterpenes and carboxylic acid. The aqueous extract was found to contain tannins, alkaloids, phenols, flavonoids, saponins, catechins and glycosides (Table 1).

Table 1. Qualitative (+ present, - absent) and quantitative (µg/ml) estimation of chosen secondary metabolites of *D.erecta*

Metabolites	Extracts	
	Methanol	Water
Quantitative		
Tannins	83	79
Saponins	890	293
Alkaloids	114	150
phenolic compounds	163	129
Flavonoids (%/ml)	7.0	3.0
Qualitative		
Steroids	-	-
Catechins	-	+
Glycosides	-	+
Xanthoprotein	-	-
Triterpenoids	+	-
Anthrocyanins	-	-
Carboxylic acid	+	-

Qualitative - + present, - absent
Quantitative - µg/ml

The secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities. Most studies report active compounds as steroidal saponins. Saponins are freely soluble in both organic solvents and water, and they work by interacting with the cuticle membrane of the larvae, ultimately disarranging the membrane, which is the most probable reason for larval death (Hostettmann and Marston, 1995). The plant *D. erecta*, shows high content of saponins and tannins which are also toxic, and these compounds could be an effective method of control of the mosquito larvae.

When the mosquito larvae were treated with aqueous extract (AqE) of *D. erecta*, a significant mortality ($p < 0.05$) of the larvae was noticed. In the concentrations, 0.01 to 0.05, no larval mortality was noticed. However, when the concentration increases from 0.06 onwards, larval mortality was significantly increased. Among this concentration a less mortality rate was noticed in 0.06 (10±0%). When the mosquito larvae were treated with aqueous ME extract of *D. erecta*, a significant mortality ($p < 0.05$) of the larvae was noticed. In the concentration of 0.01 no larval mortality were noticed in sewage water. But when the concentration increases from 0.03 onwards larval mortality was significantly increased. Among this concentration a less mortality rate was noticed in 0.02 and 0.03 (10 ± 0%) (Table 2).

Table 2. Effect of aqueous extract of *D. erecta* on the larval mortality and adult emergence of *Cx. quinquefasciatus*

Conc. %	Larval mortality (%)	Mean larval mortality	Mean larval mortality in minutes	Adult Emergence (%)	Mean adult emergence (%)	Mean adult emergence in days
Control	-			100 ± 0	-	-
0.01	-	-	-	100 ± 0	100 ± 0	5 ± 0.58
0.02	-	-	-	100 ± 0	100 ± 0	8 ± 0.58
0.03	-	-	-	100 ± 0	100 ± 0	9 ± 0.58
0.04	-	-	-	100 ± 0	100 ± 0	9 ± 0.58
0.05	-	-	-	100 ± 0	100 ± 0	10 ± 0.58
0.06	10 ± 0	-	-	90 ± 0	100 ± 0	11 ± 0.58
0.07	13.33 ± 3.33	-	-	86.67 ± 3.33	100 ± 0	12 ± 0.58
0.08	16.67 ± 3.33	10 ± 0	120 ± 1.15	83.33 ± 3.33	100 ± 0	13 ± 0.58
0.09	23.33 ± 6.67	13.33±3.33	105±1.15	76.67 ± 6.67	83.33 ± 3.33	15 ± .58
1.0	26.67 ± 8.82	16.66 ± 3.33	85 ± 1.15	73.33 ± 8.82	50 ± 0	16 ± 0.58
1.5	33.33 ± 13. 33	23.33 ± 3.33	70 ± 1.15	-	-	-
3.0	33.33 ± 13.33	100 ± 0	65 ± 1.15	-	-	-

The larval mortality occurred from 0.07% concentration onwards in AqE and from 0.05% concentration in ME. Hundred per cent mortality was recorded at the concentrations of 1.5 % and 3% of AqE and 0.09 % and 1.0% of ME. Comparison between both the extracts reveals that the ME at higher concentrations of the extract took longer to act (Table 3) than the AqE at lower concentrations. This might be due to the extraction of more bioactive principles in the extracts as observed by Mohan *et al*. (2010) in *Cx*. *quinquefasciatus*. All adults emerged at 0.01 and 0.02% concentrations of ME and 0.01% to 0.05% concentrations of AqE. At 1.5% and 3% concentrations of AqE there was complete inhibition of adult emergence. The concentration of plant extracts was found to be directly proportionate to the mean adult emergence in days i.e. as the concentrations increased the mean adult emergence in days increased from 5 to 11 days for ME extracts and 5 to 16 days incase of AqE. It is, therefore, safe to conclude that the extracts are acting on the rate of metamorphosing stages (time to change from larvae to pupa and pupa to adult).

It is already known that mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat. The use of conventional chemical pesticides has resulted in the development of resistance undesirable effects on non-target organisms and fostered environmental and human health concerns. In the present study we found that both the extracts showed complete inhibition of adult emergence from the larvae at low concentrations (0.09% and 1.5% for ME and AqE, respectively). The present study thereby proves that the extracts of the leaves of *Duranta erecta* Linn both have larvicidal activity on the larvae of *Cx*. *quinquefasciatus*. They are less toxic than the existing insecticides and may even replace them one day if they can be shown to be less polluting to the environment.

Table 3. Effect of methanol extract of *D. erecta* on the larval mortality and adult emergence of *Cx. quinquefasciatus*

Conc. %	Larval mortality %	Mean larval mortality	Mean larval mortality in minutes	Adult Emergence %	Mean adult emergence %	Mean adult emergence in days
Control	-	-	-	-	100 ± 0	9 ± 0.58
0.01	-	-	-	100 ± 0	100 ± 0	10 ± 0.58
0.02	10 ± 0	-	-	100 ± 0	100 ± 0	11 ± 0.58
0.03	10 ± 0	-	-	90 ± 0	100 ± 0	12 ± 0.58
0.04	13.33 ± 3.33	-	-	90 ± 0	83.33 ± 3.33	15 ± 0.58
0.05	16.67 ± 3.33	10 ± 0	120 ± 1.15	86.67 ± 3.33	50 ± 0	16 ± 0.58
0.06	23.33 ± 6.67	6.66 ± 1.57	115 ± 1.15	83.33 ± 3.33	43.33 ± 0.33	17 ± 0.58
0.07	26.67 ± 8.82	13.33 ± 3.33	105 ± 1.15	76.67 ± 6.67	43.33 ± 0.33	17 ± 0.58
0.08	26.67 ± 8.82	16.67 ± 3.33	95 ± 1.15	73.33 ± 8.82	30 ± 0.33	19 ± 0.58
0.09	33.33 ± 13.33	100 ± 0	80 ± 1.15	66.67 ± 13.33	-	-
1.0	33.33 ± 13.33	100 ± 0	70 ± 1.15	66.67 ± 13.33	-	-

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McConnell Marie Serena[1*], M. Balasubramani[1], K. Rajan[1] and I. A. J. Gerald[2]

1. Department of Plant Biology and Plant Biotechnology, St. Joseph's College (Autonomous) Tiruchirappalli - 620 002, Tamil Nadu, South India.
2. Department of Zoology, Jamal Mohamed College, Tiruchirappalli - 620 004, Tamil Nadu, South India

*Correspondence author E-mail : m_s_mcconnell @ yahoo.com

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