Mosquitocidal effect of **Euphorbia heterophylla** Linn. against the **Bancroftian filariasis** vector, **Culex quinquefasciatus** say (Diptera: Culicidae)

Kuppusamy C’, Murugan K

Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India

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**Abstract**

An investigation has been made on the whole plant ethanolic extracts and petroleum ether seed extracts of **Euphorbia heterophylla** Linn. were evaluated for larvicidal, pupicidal and adult repellency and ovicidal properties against the bancroftian filariasis vector, **Culex quinquefasciatus** Say. The bioassays were carried out according to the recommendation of World Health Organisation. The whole plant ethanolic extracts and petroleum ether seed extracts were effective against the larvae of all instars. The effect on larval mortality was concentration dependent. The highest concentration 53ppm of the ethanolic extract produced almost 98 to 93% mortality in first to third instars and the petroleum ether seed extract produced 96, 94, 90% for first, second, third instars respectively. The fourth instar larvae of **Culex quinquefasciatus** were less susceptible to the samples than the first instar larvae with 90 and 89 percent mortality for ethanolic and petroleum ether seed extracts respectively. The treatment of ethanolic whole plant extracts on pupae showed 77-87 % mortality and the petroleum ether seed extracts on pupae showed slightly weaker pattern of mortality with 76-83% when compared to whole plant ethanolic extract treatment. In repellent bioassays the petroleum ether seed extracts showed more effective than the whole plants ethanolic extracts with 86 and 88% protection upto 6 hrs for petroleum ether seed extracts and whole plant ethanolic extracts respectively. The treatment of the eggs of various age groups of **Culex quinquefasciatus** with whole plant ethanolic extracts and petroleum ether seed extracts of **Euphorbia heterophylla** Linn. caused only moderate ovicidal activity per se but inflicted delayed effects such as high larval and low pupal and adult mortality. Whole plant ethanolic extracts proved more effective than the petroleum ether seed extracts against eggs of **Culex quinquefasciatus**. The age of the eggs and the duration of the treatment also influenced the ovicidal activity.

**Keywords:** **Euphorbia heterophylla**; **Culex quinquefasciatus**; Larvicide; Pupicide; Adult repellency; Ovicide.

**INTRODUCTION**

Vector control is a serious concern in developing countries, every year a large part of the population in the world is affected by one or more vector borne diseases. Mosquitoes alone transmit diseases to more than 700 million people annually (Taubles, 1997). Mosquito-borne diseases usually represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk (Fradin and Day, 2002) which indirectly impediment for the economic development not only for India but also for the entire world. Control of mosquito borne diseases is becoming difficult because of increasing resistance of mosquitoes to pesticides (Ranson et al., 2001) and public health concern over environmental pollution necessitate a continued search for alternatives, cheaper vector control methods which require little or not sophisticated technology but gives excellent results (Minijas and Sarda,1986) In view of this study there has been recent interest in plant derived compounds as alternatives to the synthetic insecticides (Grainge and Ahamed,1988) as these are biodegradable and safe to natural enemies and higher organisms (Sharma and Ansari et al., 1994).

A wide range of plants are toxic, larvicidal or repellent to insects or have antifeedant properties (Sukumar et al.,1991), therefore biologically active plant extracts are being studied by several authors for their potential efficacy to minimize the extent of vector borne diseases (Selvaraj pandian et al., 1994). It is estimated that over 40,000 bioactive compounds so far been isolated but only about 10,000 of them have been characterized...
chemically (Swain, 1977) many plant extracts of terrestrial origin have been reported to suppress mosquito larval populations (Chavan and Nikan, 1982; Saxena and Yadav, 1993) and suggested to be advantageous for field use in mosquito control programmes (Kalyanasundram and Das, 1985).

Lymphatic filariasis represents a major, vector borne, public health and puts at risk more than a billion people in more than 80 countries. It is estimated that 1.2 billion (20% of the world’s population) are at risk of acquiring infection, one third of these infected live in India, one third in Africa and most of the remainder in Asia, the Pacific and the Americas. Ninty percent of these infections are caused by *Wuchereria bancrofti*, the major vectors for *Wuchereria bancrofti* are Culex mosquitoes i.e *Culex quinquefasciatus* (WHO-file eliminating lymphatic filariasis).

The *Euphorbia heterophylla* Linn commonly known as Mexican fire plant can be referenced under Symbol EUHET 4 (http://pubs.cas.uga.edu/caespubs/pubmed/). It is also called wild poinsettia, and is invasive to India, but its native is Southern United States to Argentina and West Indies (Wagner et al., 1991) and classified within the group Dicot of the Euphorbiaceae family. It is a weed of road side, waste land, young plantations, wildly spread at low altitudes but seldom common (Henty and Pritchad, 1975). It can be distinguished by being erect, annual herb can grow up to 24 - 58 inches tall, milky sap that oozes from damaged stems, upper most leaves never pink or red at base, flowers male or female in terminal clusters, seeds are brown or grey mottled, ovoid 2.5-3 mm long and produce an average of 520 seeds per plant (http://www.org.au/cgi-bin/weedident; Parsons and Cuthbertson, 1992) and euphorbia is considered by many in South Africa as synonymous with poison (http://www.theamateursdigest.com/epoisons.htm)

It has been used as insecticide and tuberculocide (Wing et al., 1998), being co-carcinogenic, causing toxicosis and its diterpene polyesters showed molluscidal activity (Abdolguleil et al., 2005) Hence in the present study an attempt has been made to assess the efficacy of mosquitocidal properties of *Euphorbia heterophylla* Linn against *Culex quinquefasciatus*.

**MATERIALS AND METHODS**

**Preparation of plant extract**

The fresh plants of *Euphorbia heterophylla* Linn were collected locally from the foothills of Western Ghats area of Coimbatore city, Tamil Nadu state, India. The leaves, seeds, roots and stems were washed with double distilled water and shade dried at room temperatures, the seeds were collected and powdered separately and other parts, i.e. leaves, roots, stems were put together and powdered with the help of electric blender. The dried whole plant powder excluding seeds was then subjected to extraction in ethanol and the powdered seeds were subjected to extraction in petroleum ether individually. From each of the sample 100 g was taken and run with ethanol and petroleum ether separately for 8 hrs with the help of Soxlet apparatus. After removing the solvents from the plant extracts in a vacuum rotary evaporator, stock solution of 1% was prepared with 200 mg residue in 20 mL ethanol and was kept in a screw-cap vial with aluminum foil over its mouth. The stock solution was then serially diluted ten-fold in ethanol (2 mL solution to 18 mL solvent) and test concentrations were obtained by adding 0.1-1.0 mL of the appropriate dilution to 100 mL distilled water (WHO 2005).

**Mosquito culture**

*Culex quinquefasciatus* were maintained in the laboratory from the lines obtained from National Institute of Communicable Diseases-Field station Cunnoor. The colonies of mosquitoes were maintained at conditions 27±2°C and 80%±5 relative humidity under 12 L:12 D cycles. The larvae were reared in enamel trays and fed finely ground dog biscuits and Yeast at 60:40 ratios. Water in rearing pans was replaced every other day, and pupae were transferred from the trays to a cup filled with dechlorinated tap water and placed in screened cages where adults emerged. The adult mosquitoes were maintained in a net cage (90×90×90 cms) and were continuously 10% sucrose solution provided in a jar with a cotton wick. For continuous culture selected numbers of Mosquitoes were allowed to feed chicken blood and every third day, thereafter moist filter paper was kept in beaker in the cages for mosquitoes to lay their eggs on. Eggs laid on the filter paper were immersed in larval basins containing water for the maintenance of the colony.

**Larvicidal Bioassay (WHO 2005)**

The larvicidal bioassay was assessed by using standard WHO Protocols (WHO 2005) For experimental treatment, one ml of test concentration prepared from the stock of whole plant ethanolic or petroleum ether extracts and were added to 100 ml of distilled water in a 250 ml of enamel bowel which was shaken lightly to ensure a homogenous test solution, Then 25 numbers of each instars of larvae and pupae of *Culex quinquefasciatus* were transferred using fine mesh strainers to separate test bowel for each instar and each experiment was performed in 4 replicates with a final total of 100 larvae for each concentration and the equal number of controls were set up simultaneously with distilled water to which 1 ml of ethanol was added, experiments were conducted at 27±1°C, 85% RH with photoperiod of 12L:12D. Symptoms of the treated larvae were observed and recorded immediately and at timed intervals and no food was offered to the larvae,
Mortality and survival was registered after 24 and 48 h of the exposure period. The moribund and dead larvae in four replicates were combined and expressed as a percentage of larval mortality of each concentration, dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region, moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. The larvae showed discoloration, unnatural positions, tremors, uncoordination or rigor were also counted.

Ovicidal Bioassay

In our preliminary studies we noted that the hatching rates of eggs laid over 24 h by gravid females in ethanolic extract of *Euphorbia heterophylla* Linn was lower than those of eggs laid in water. On this basis we initiated further studies to determine the non-larvicidal effects of ethanolic extract in a multiple concentration test of ovicidal activity. Freshly laid eggs were collected by providing ovitraps in mosquito cages from 10.00 to 16.00 hours for collecting 0–6 h old eggs, and 16.00 to 10.00 the next morning for collecting 0–18 h old eggs. Ovitraps were kept in the cages 2 days after the female mosquitoes were given a blood meal. The eggs were laid on filter paper lining provided in the ovitraps. After scoring, the filter papers containing the eggs were exposed to graded doses of ethanolic extract (23, 30, 36, 42 and 48 p.p.m.) or control solution. A minimum of 100 eggs was used for each treatment, and the experiment was replicated four times. Eggs of the age group 0–6 h were exposed to the extract for 18 h, while eggs of the age group 0–18 h were exposed for 6 and 18 h. After exposure, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in enamel bowls filled with dechlorinated water for hatching. The hatching rate of eggs was assessed 3 days later. The percent egg mortality was calculated on the basis of non-hatchability of eggs with unopened opercula.

Repellent Bioassay

The repellent bioassay was studied by adopting the standard procedure of WHO (1996). The repellent bioassay carried out separately with whole plant ethanolic extracts and petroleum ether seed extracts of *Euphorbia heterophylla* Linn according to *Culex quinquefasciatus* with human volunteers, Twenty 3-4 days old blood starved female *Culex quinquefasciatus* were released into a net cage. The arms of test person were cleaned with isopropanol. After air-drying the arm only 25 cm² of the dorsal side of the skin was exposed. The remaining area was covered with rubber gloves. The exposed area was treated with plant extracts of different concentrations i.e 50, 100, 250, 500, 1000, 2000 ppm and the treated arms were exposed for 5 min at different time intervals i.e 0- ½, 1, 2, 3 and 4 hr. one untreated arm was exposed as control simultaneously into the cage. Then the numbers of mosquitoes landing on the treated surface were recorded. The percentage repellency was calculated as follows:

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\text{% Repellency} = \left(\frac{C-T}{C}\right) \times 100
\]

C - Number mosquitoes collected from the control areas
T - Number of mosquitoes collected from the treated areas.

Statistical analysis

In larvicidal, pupidal bioassays, percent control mortality were corrected using Abbott’s transformation (Abbott, 1925). LC50 and LC90 (lethal concentrations causing 50% and 90% mortality) was calculated using probit analysis (Finney, 1971). Data from larval and pupal mortality, adult repellency and ovicidal activity were subjected to analysis of variance (ANOVA). Statistical software SPSS were used for data analysis.

RESULTS

The results of the larval, and pupal susceptibility of *Culex quinquefasciatus* to the whole plant ethanolic extracts and petroleum ether seed extracts of *Euphorbia heterophylla* Linn are presented in Figure 1 and 2. The ethanolic extract and seed extracts were effective against larvae of all instars. The effect on larval mortality was concentration dependent (Fig 1 [Supplementary data] and Fig. 2 [Supplementary data]). LC50 and LC90 values of ethanolic extracts against the all the instars of larvae and pupae are presented in the Table 3 [Supplementary data]. The LC50 and LC90 values for first instars were 14.98, 40.84 ppm ; 19.55, 44.75 ppm for second instars ; 22.53, 50.02 ppm for third instars ; 24.19, 52.07 ppm for fourth instars ; and 25.72, 56.65 ppm for pupae respectively. The highest concentration 53 ppm of the ethanolic extract produced almost 98 to 93 % mortality in first to 3rd instars, percent mortality in the 4th instar at 53 ppm was 87%. With treatment of petroleum ether seed extract produced 96, 94, 87 % mortality in first, second and third instars respectively, percent mortality of the 4th instar was 86 % at 53 ppm. The LC50 and LC90 values of petroleum ether seed extracts for first instars were 12.12, 43.73ppm ; 23.65, 47.17 ppm for second instars ; 23.65, 52.55 ppm for third instars ; 25.30, 53.98 for fourth instars ; and 29.48, 57.04 ppm for pupae respectively. On the other hand, treatment of petroleum ether seed extracts at 53 ppm on pupae showed slightly weaker pattern of mortality when compared to whole plant ethanolic extract.

Tables 1 [Supplementary data] and Table 2 [Supplementary data] show the repellent activity of whole plant ethanolic extract and petroleum ether seed extracts *Euphorbia heterophylla* Linn. The whole plant ethanolic extract provided 86 % protection up to 6 hrs
but petroleum ether seed extract provided 88% protection up to 6 hrs. Repellent Dose 50 and 90 of whole plant ethanolic extract for 6 hrs was 453.13, 1726.89 ppm respectively. The petroleum ether seed extracts showed more effective than the whole plant ethanolic extract with Repellent Dose 50 and Repellent Dose 90 were 374.63, 1715.77 ppm respectively.

The treatment of eggs of *Culex quinquefasciatus* with whole plant ethanolic extract and petroleum seed extract of *Euphorbia heterophylla* caused embryonic death resulting in failure to hatch the eggs. In addition, the treatments had deleterious delayed effect in causing high larval mortality and moderate pupal or adult mortalities in the larvae hatched out of the treated eggs. The mortality data of the egg treatments is presented in tables 3 [Supplementary data] and Table 4 [Supplementary data].

In general egg mortality per se in any of the treatments did not go beyond 50% even with the highest concentration of whole plant ethanolic extract or petroleum ether seed extract of *Euphorbia heterophylla*. However, the larvae, which hatched out from the treated eggs, showed much higher levels of mortality in all the treatments. Also, the treatments produced low to moderate levels of pupal mortality or adult mortality at the time of adult emergence.

Treatment of eggs of 0-6h was more effective in inducing higher rates of mortality as compared to eggs of 0-18h treated for 6 or 18 h. Shorter duration of treatment was decisively inferior to longer exposure to insecticides at the egg stage. The eggs of *Culex quinquefasciatus* were more susceptible to whole plant ethanolic extract than the petroleum ether seed extracts. Exposure to 48 ppm of whole plant ethanolic extracts for 18h of eggs of 0-6 h caused 90-95% cumulative mortality in different life stages of *Culex quinquefasciatus*. Almost identical results were obtained in similar treatments with petroleum ether seed extracts at a dose 48 ppm (Table 4 [Supplementary data]). Exposure to 48 ppm of petroleum ether seed extracts for 18 hr of eggs of 0-6 h elicited about 95% cumulative mortality, though egg mortality per se did not increase 36%. The results showed similar trend with regard to treatments of 0-18 h old eggs exposed for 6 or 18 h to whole plant ethanolic extracts or petroleum ether seed extracts (Tables3, 4). Treatments for 6 h were inferior to 18 h treatment in all the cases. Also 18 h exposure of older eggs was less effective as compared to similar treatment of eggs of lower age.

**DISCUSSION**

In the present study, the increased larval and pupal mortality, decreased egg hatchability and decreased adult emergence of treated individuals reduce the overall performance of filarial vector *Culex quinquefasciatus*. Our data support this hypothesis and conclude that whole plant ethanolic extracts of *Euphorbia heterophylla* Linn possess various activities such as larvicidal, pupicidal, ovicidal, and adult repellency effects and reduce larval, pupal and adult survival.

**Effect of Euphorbia heterophylla extracts on larval and pupal mortality**

The larval and pupal toxicity has been evident after the treatment with whole plant ethanolic extracts and petroleum ether seed extracts of *Euphorbia heterophylla* on all the four instars of larvae and pupae of *Culex quinquefasciatus*. The results showed that the mortality of larvae increases, as the doses of the samples were increased. The same trends were also observed in cause time elapse mortality. The ethanolic extract treatments were superior to seed extract with increased larval and pupal mortality. The fourth instar larvae of *Culex quinquefasciatus* were less susceptible to the samples than 1st instar larvae. The LC50 and LC90 values were age dependent. This may clearly support the ideas of others that insect age plays an important role in influencing the susceptibility (Kumar and Dutta, 1987). The sluggish movement and peculiar coiling of treated larvae seem to suggest some neural or muscular disturbance by some active compounds which might be caused acute lethal effect. This observed biological activity may be due to the Jolkinolide- B(II) and 3-acetyl alpha-amysin(I) diterpenoids from roots of *Euphorbia heterophylla* Linn has been used as insecticide and tuberculoside(Wang and Ding, 1998).The delayed lethal effects of the extracts and the compounds however are more likely to be caused by a disturbance of the endocrine mechanisms that regulate the molting and metamorphosis. This mechanism of action and observation is similar to Liu et al.,(2002), reported that Jolkinolide -B exhibited the most potent anti-proliferative activity and inhibiting DNA synthesis, arrest neuro-endocrine differentiation and caused DNA fragmentation when exposed to higher concentration than 25 microg/ml,moreover euphorbon-a poison from *Euphorbia heterophylla* Linn caused severe inflammation of the walls of the gut system and in some times wall of the gut has been perforated and act as a contact and gut poison (http://www.the amatteur.digest.com/epoisons.htm). This toxic effect can be comparable to molluscidal activity of *Euphorbia splendens* Var.hislopii (Schall et al., 2001) and its toxic effect can be comparable to previously screened plants in our laboratory such as neem extracts and its exposure caused prolonged larval development, reduced pupal weight (Murugan et al., 1996) and *Albizzia amara* and *Ocimum sanctum* showed larvicidal and repellent properties against *Aedes aegypti* (Murugan et al., 2006) and neem seed kernel extracts
on *Aedes aegypti* which showed higher larvicidal activity at 2000 ppm (Shigeo kondô *et al.* 2004).

**Effect of Euphorbia heterophylla on adult repellency**

The adult repellency in higher concentrations of 2000 ppm of whole plant ethanolic extract is inferior to the petroleum ether seed extracts of *Euphorbia heterophylla*, which provided 88% repellency at 6 hrs. This differential responses are influenced by the differences in distribution of toxic chemicals in different parts of the plant (Sukumar *et al.*, 1991). The repellent effect may be due to the presence of various compounds such as physiologically active volatile oils, complex esters, diterpenoids or euphorbia factors (Bichi *et al.*, 2001), bioactive steroids (Tanaka *et al.*, 2000) and other compounds in *Euphorbia heterophylla* Linn. The repellent activity can be comparable to *Litsea salicifolia* recorded higher repellent activity at 2000 ppm against *Culex quinquefasciatus* where protection time lasted 4-5 hrs (Phukan and Kalita, 2005). The insect repellent that is widely available in DEET, continuous application of DEET causes infolding of the epidermis with fever, hairs and a thickened dermis with more vascularity (AI. Sagaff *et al.*, 2001). In our study the leaf extract did not cause any such discomfort or skin irritation to the volunteers.

**Effect of Euphorbia heterophylla Linn on egg hatchability and survival**

It is evident from the present date that exposure of eggs of mosquitoes to whole plant ethanolic extract and petroleum ether seed extracts of *Euphorbia heterophylla* elicit not only egg mortality but also delayed effects resulting in mortality at larval, pupal and adult stages. Though ovicidal activity per se is only moderate, an important finding is that the larvae, which hatch out of the treated eggs immediately, succumbed to death. The pupal or adult mortality was significantly less as compared to larval or egg mortality.

Exposure of freshly laid eggs was more effective than the older eggs. The age of the embryos at the time of treatment played a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin to *Culex quinquefasciatus*. Exposure time also has a crucial role in causing toxicity (Miura *et al.*, 1976). According to Smith and Salkeld (1966), differences in susceptibility to ovicides are due to differential rates of uptake, penetration through the chorion, conversion to active inhibitor, detoxication and failure of the toxicant to reach the target. Grosscut (1977) observed that the efficiency to act on the embryo inside the eggshell depends on an efficient penetration of the insecticide, which in turn is influenced by the exposure period. Total inhibition of egg eclosion when eggs were directly exposed to high concentrations of the compounds indicated more entry of the chemical inside the eggshell, which affected the embryogenesis (Broad bent and Pree, 1984). Similarly, longer exposure periods also facilitated the increased penetration of the compounds into the shells, thus increasing their effectiveness.

The eggs of mosquitoes are found to be much more tolerance to the action of insecticides compared to larval stages. Insect eggs are covered with a shell, which differs biochemically from the integument of the larvae, and the difference in penetration of the insecticide through the eggshell, and the larval integument is reflected in the observed toxicity differences. It may be concluded that whole plant ethanolic extract and petroleum ether seed extracts of *Euphorbia heterophylla* even when treated at egg stage can inflict high cumulative mortality in *Culex quinquefasciatus* due to their ovicidal activity and delayed effects at other life stages of mosquitoes.

The phytochemical constituents of *Euphorbia heterophylla* Linn. is appreciable, contains euphorban as a special toxic substance and diterpenoids such as Jolkinolide -B (II), amyrin (I) (Wang and Ding, 1998), triterpenoids, active diterpene polyesters, seed oil contains series of euphorbia factors or L-factors (Bicchi *et al.*, 2000), bioactive steroids latex contains series of triterpene alcohols and various alkaloids, tannins, flavonoids, phlobatannins, cardiac glycoside etc. (Tanaka *et al.*, 2000), and other phytochemical compounds.

These compounds may jointly or independently contribute to cause larvicidal, pucidal and repellent and ovicidal activity against *Culex quinquefasciatus*. Further investigation is needed to identify the active volatile compounds of the extract responsible for its activity. The present study revealed that *Euphorbia heterophylla* Linn can be further developed as a potential eco-friendly phytocidal and as a mosquito repellent and may be beneficial for future vector control program. Further work in this direction is necessary since biopesticides from plant origin contributing in an effective vector control tools. So these new agents can preferentially be applied in integrated vector control strategies.

**References**


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