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ORIGINAL ARTICLE

MOSQUITO LARVICIDAL AND PUPICIDAL ACTIVITY OF *Pongamia Pinnata L.* Pierre (Papilionaceae) PLANT FLOWER EXTRACT AGAINST *Culex quinquefasciatus* Say, *Aedes aegypti* Linn. and *Anopheles stephensi Liston(Diptera:Culicidae).*

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ABSTRACT

Among the various groups of insects, the order Dipterans act as vectors and play a role in spreading disease among man. Among the thirteen genera of the family Culicidae individuals of genus *Culex*, *Aedes* and *Anopheles* are considered dangerous because they cause significant public health threat all over the world. Mosquito – borne diseases have an economic impact, including loss in commercial and labor outputs, particularly countries with tropical and subtropical climates; however, no part of the world is free from vector – borne diseases. The aim of the present study, to evaluate the larvicidal and pupicidal activities of methanol crude extracts of *Pongamia pinnata* (*P. pinnata*) flower extract (PPFME) were assayed for their toxicity against three important vector mosquitoes, viz., *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera; Culicidae). The fresh flowers of *P. pinnata* were washed thoroughly in tap water and shade dried at room temperature (27±2°C) for 7-9 days. The air dried materials were powdered separately using commercial electrical blender. The powder (500g) of the flower was extracted with 1.5 litre of organic solvent of methanol using a soxhlet apparatus at 60-80°C for 8 hours, the extract was concentrated under reduced pressure at 45°C and the residue obtained was stored at 4°C. The larval, pupal mortality was observed after 24h of exposure; no mortality was observed in the control group. The first – to fourth instar larvae and pupae of *Cu. quinquefasciatus* had values of LC_{50} =8.285% 1st instar, 9.047% 2nd instar, 9.986% 3rd instar, 10.973% 4th instar and 12.880% pupa respectively. PPFME against *Ae. aegypti* first to fourth instar larvae and pupa with LC_{50} value 1st instar was 7.666%, 2nd instar 8.235%, 3rd instar 9.227%, 4th instar 10.662% and pupa was 12.229% respectively. Moreover PPFME against *An. stephensi* values of LC_{50} of 1st instar was 6.684%, 2nd instar was 7.384%, 3rd instar was 8.114%, 4th instar was 9.383% and pupa was 11.586% respectively. 100 percent mortality was observed with *Ae. aegypti* and *An. stephensi*. No mortality was observed in the control. The results of the larvicidal pupicidal activity of plant flower extract (PPFME) are candidate for controlling lymphatic filarial vector, *Cu. quinquefasciatus*, danque vector *Ae. aegypti* and malarial vector *An. stephensi*. This results suggests that the flower extract have the potential to be used as an ideal eco- friendly approach for the control of vector mosquitoes as target species.

Keywords: *Pongamia pinnata*, *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi*, larvicidal, pupicidal

1.INTRODUCTION

Mosquitoes are the most indisputable medicinal significant arthropod vectors of diseases. The vector-borne diseases caused by mosquitoes are one of the major health problems in most of the countries. It is affecting the socio economical status of many nations and it is an important pest against human causing allergy too. *Culex quinquefasciatus* is one of the most annoying vectors which transmit lymphatic filariasis and Japanese encephalitis in India (Mourya *et al.*, 1989; Das *et al.*, 2002; Vasudevan et al., 2009; Govindarajan et al., 2013). Pandian *et al.*,(1989) observed the repellent activity of herbal smoke on the biting activity of *C. quinquefasciatus*. Thangam and Kathiresan (1992a) stated that smoke from burning various dry materials has been used since early times to deter insects especially mosquitoes. *C. quinquefasciatus* and many other *Culex* species bite their hosts at night. *C. quinquefasciatus* commonly rest indoors both before and after feeding, but also shelter in outdoor resting places (Service, 2000).

Aedes aegypti (L.) is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas, and also for yellow fever in Central and South America and West Africa. In the past decade, Chikungunya - a virus transmitted by *Aedes* spp mosquitoes - has re-

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emerged in Africa, southern and southeastern Asia, and the Indian Ocean Islands as the cause of large outbreaks of human disease (Burt et al., 2012). Malaria is a protozoan infection of erythrocytes caused in human beings by five species of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*). In most cases, malaria is transmitted via the bite of an infected female *Anopheline* mosquito, but congenital malaria and acquisition through infected blood transfusion are well described (Falade et al., 2007). More than 40 per cent of the world's population - approximately 3 billion people - are exposed to malaria in 108 endemic countries (WHO, 2009). About one million cases of malaria are reported in India every year. In 2010 an estimated 219 million (range 154-289 million) cases occurred worldwide and 660,000 people died (range 610 000-971 000), mostly in children under five years of age. Presently, organochlorine, organophosphate, and synthetic pyrethroid insecticides are being used for mosquito control. Successive changes in the insecticides result in multiple insecticide resistant malarial vectors. Malaria vectors in India are resistant to several insecticides (DDT, HCH, Malathion, and Deltamethrin). (Ragavendra and Subbarao, 2002) There has been increased interest in anti mosquitoes products derived from natural origin because of its environmental safety. The toxicity problem due to the continued applications of synthetic compounds leads to wide spread development of insecticide resistance(Raghavendra and Subbarao,2002).The toxicity problem, together with growing incidence of insect resistance, has called attention to the need for more detailed studies of naturally occurring insecticides (Macedo et al., 1997) it is therefore, necessary to develop new materials for controlling mosquitoes in an environmentally safe way, using biodegradable and target specific insecticides against them (Rattan, 2010, Lame et al., 2015).

The larvicidal activity of different solvent extracts of the flower *Centella asiatica*, *Datura metal*, *Mukia seabrella* extracts of whole plant of *citrullus colocynthis* and *Sphaeranthus indicus* (Rahuman et al., 2008) extracts of dried fruits of *P. nigrum* (Vasudevan et al., 2009). Different extracts of *Acalypha indica* (Govindarajan et al., 2008). The methanol extracts of leaves of *Dysoxylum mallabaricum* have been tested against mature and immature stages of *An. stephensi* under laboratory conditions (Senthilnathan et al., 2006). Larvicidal and ovicidal efficacy of *Pithicellobium dulce* against *An. stephensi* and *Ae. aegypti* (Govindarajan et al. 2013). Larvicidal, pupicidal and ovicidal properties of acetone leaf extract against *Ae. aegypti* (Pravin et al., 2015).

Root bark extracts of *Turraea wakefieldii* and *Turaea floribunda* against third instar larvae (Ndung'u et al., 2006) and extracts of *Pelargonium citrosa* flower have been tested for their biological, larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency, and biting deterrency aginast *An. stephensi* (Jayabalan et al., 2003). Hexane extract obtained from leaves of *Eucalypitus citriodora* was tested against larvae of *An. stephensi*, *Cu. quiguefasciatus* and *Ae. aegypti* to assess its toxicity and growh-inhibiting activity (Singh et al., 2007). The high larvicidal activity of *Tinospora rumphii* flower is supported the abundance of phytochemicals which show synergestic effects in terms of larvicidal action against larvae of *Ae. aegypti* (Gutierrez Jr. et al., 2014). Mosquitocidal properties of *oxystelma esculentum* against *Aedes aegypti* (Elumalai and Krishnappa, 2015).

Pongamia pinnata (L) Pierre (Papilionaceae) is a medium sized tree with grayish – green or brown, smooth or tuberculate bark, leaves imparipinnate; flowerlets 5 to 7, ovate or elliptic, ever green tree up to 15m height, found throughout the plains of India. The presence of furanoflavone, glycoside, saponins, lipids, phospholipids, poneaglaborone has been reported from leaves and flower (Koyama). The plant have been shown to have potential in treating a number of ailments in ayurveda used for treating oedema, poisoning, worm infestation, leprosy and stomach disorder; in Siddha used to treat venereal diseases, pain, poisoning and skin eruptions. However, no information was available on the larvicidal and pupicidal activities of this plant species against *Cu. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, therefore, the aim of this study to investigate the mosquito larvicidal and pupicidal activities of the methanol solvent extract of *P. pinnata* flowers.

2.MATERIALS AND METHODS

Collection and maintenance of insect:

The eggs/egg rafts of *Cu. Quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were collected from the Vector Control Research Centre (ICMR) Pondicherry and ICMR Medical Entomology Field Research Station of Vridhachalam, Tamil Nadu, India. Using an "O"-type brush. These eggs/egg rafts were brought to the laboratory and transferred to 18×13×4-cm enamel trays individually containing 500 ml of water for hatching. The mosquito larvae were fed with ground mice pellet feed and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500 ml of water with the help of a dipper. The plastic jars were kept in a 90×90×90-cm mosquito cage individually for adult emergence. Mosquito larvae were maintained at 27°C±2°C, 75–85% of relative humidity, under a photoperiod of 14:10 hrs light:dark. A 10% sugar solution was provided for a period of 3 days before blood feeding.

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Collection and preparation of plant extract:

P. pinnata were collected from in and around the Annamalai University campus, Annamalai Nagar, Tamilnadu, India. The plants were identified at the Department of Botany, Faulty of Science, Annamalai University, and the plants were deposited at Botany Department, Annamalai University, Annamalai Nagar, Tamil Nadu, India. *P. pinnata* flowers was washed with tap water and shade-dried at room temperature. The dried plant materials (flowers). The powder (500 g) of the flowers was extracted with 1.5 litre of organic solvents of methanol using a Soxhlet apparatus at 60–80°C for 8 hrs (Vogel, 1978). The

extract was concentrated under reduced pressure 22–26 mm Hg at 45°C and the residue obtained was stored at 4°C. The extracts were filtered through a Buchner funnel with Whatman No. 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 8 to 18%, respectively.

Larval/pupal toxicity test

Laboratory colonies of mosquitos' larvae/pupae were used for the larvicidal/pupicidal activity. Twenty-five numbers of 1^{st} to 4^{th} instars larvae and pupae were introduced into 500 ml glass beaker containing 249 mL of dechlorinated water and 1 mL of desired concentrations of plant extract were added. Larval food was given for the test larvae. Each tested concentration, was thrice replicated. The control was set up by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae and pupae which were exposed to dechlorinated water without acetone served as control (Kovendan et al.,2012). The control mortalities were corrected by using Abbott's formula (Abbott's 1925). The LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis (Finney, 1971).

Statistical analysis

All data were subjected to analysis of variance; the means were separated using Duncan's Multiple Range Tests by Alder and Rossler (1977). SPSS (Statistical software package) 9.0 version was used. Results with $P<0.05$ were considered to be statistically significant.

3.RESULTS

Larval and pupal mortality of *Cu. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* after the treatment of methanolic extract of *P. pinnata* flower (PPFME) was evaluated. The results of the larvicidal and pupicidal activity PPFME against the larvae of three important vector mosquitoes are presented in table 1,2 and 3. Thirty six percent mortality was noted at 1^{st} instar larvae by the treatment of *P. pinnata* at (PPFME) 8%. Where as it has been increased to 18% of *P. pinnata* flower extract treatment. Similar treatments has been adopted for II, III, IV instar larvae and pupa of *Cu. quinquefasciatus* at different concentration of PPFME treatment (Table 1). *Cu. quinquifasciatus*. LC_{50} and LC_{90} values were 8.285%; (LCL 6.836 – UCL 9.194) and 14.213% (LCL 13.090-UCL 16.285) respectively against 1^{st} instar larvae. Similar trend has been noted for all the instar and pupa of the vector mosquito. *Cu. quinguefasciatus* at different concentrations of PPFME treatment (Table 1).

The results of larvicidal and pupicidal activity of PPFME against larval and pupal instars of *Ae. aegypti* and *An. stephensi* are presented in Table 2 and 3. It was found that PPFME was highly toxic to all the larval instars and Pupa of *Ae. aegypti* and *An. stephensi*. The mosquito larval instars exposed to plant extract showed, early instars are more susceptible than the late instars and pupa of all three vector mosquitoes studied. PPFME treated mosquito larval instars showed significant behavioural changes. The most obvious sign of behavioural changes observed in *Cu. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* was restlessness, loss of equilibrium which finally led to death. PPFME 18% extract produced 100% mortality in 1^{st} instar larvae of *An. stephensi* (Table 3) within 24 hours. Among the vector mosquitoes tested larvae and pupa of *An stephensi* was more susceptible, LC_{50} and LC_{90} of pupa was 11.596% (LCL 10.748 - UCL 12.370) and 19.149% (LCL 17.781-UCL 21.214) respectively, which is comparatively more susceptible than *Cu. quinguefasciatus* and *Ae. aegypti*. The LC_{50} and LC_{90} values were dose and time dependent one.

4.DISCUSSION

Overall larvicidal and pubicidal activity of methanol flower extract of *P. pinnata* were demonstrated on *Cu. quinquefasciatus*, *Ae. agypti* and *An. stephensi*. Indeed, Plants are extensively reported to possess toxic effect and used plant origin products and secondary metabolites in pest control since early historical times. Phytochemicals are botanicals which are naturally occurring insecticides may serve as suitable alternative to synthetic insecticides in future as these are relatively safe, inexpensive, and are readily available in many areas of the world. Different plant species contains a complex of chemicals with unique biological activity which is thought to be due to toxins and secondary metabolites, which act as mosquitocidal agents. Further more, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors (Govindarajan et al.2013). Our results showed that crude methanol extract of *P.pinnata* flower were effective against the larvae and pupa of three important vector mosquitoes, viz. *Cu.quinquefasciatus*. *Ae aegypti* and *An.stephensi*. Chowdhury et al. (2009) have reported that the chloroform and methanol extracts of mature leaves of *Solanum villosum* showed the LC_{50} values for all instars between 24.20 and 33.73 mg/l after 24 h of exposure period against *An.subticus*. Similarly, Govindarajan *et al* (2013) reported that flower and seed extracts of *P.dulce* with the LC_{50} and LC_{90} were values 145.43, 155.78 mg/l and 251.23, 279.73 mg/l respectively against larvae of *An.stephensi* and *Ae aegypti*. The Methanolic extracts of *Solanum suratence*, *Agadiracta indica* and *Hydrocotyl javanina* exhibited larvicidal activity against *Cu.quinquefasciatus* (Venkatachalam and Jabanesan, 2001), *Annonaceae senegalensis* leaf extracts against malarial and filarial vector mosquitoes larvae (Lame et al., 2015)

The larvicidal activity of various plant extracts such as *Pedalium murax, Cleome icosondrta* and *Dictyosa dictotoma* have been found to the promising against larva of *Cu.quinquefasciaus* and *An.stephensi* (Kalyanasundram and Das, 1985). Larvicidal activity of dried ripened fruits crude extracts of *Piper nigram* against *Cu. quinguefasciatus* larval instars (Vasudevan et al., 2009). Naturally occurring insecticides may play a vital role in mosquito control programs in future (Wandscheer et al., 2004)

Mullai and Jabenesan (2007) have reported that the ethyl acetate, petroleum ether and methanol flower extracts of *C.colocynthis* and *Cucurbita maxima* had LC_{50} values 47.58, 66.92 and 118.74 mg/l and 75.91, 117.73 and 171.14 mg/l respectively, against *Cu.quinquefasciatus* larvae. The crude extract (PPFME) had strong larvicidal and pupicidal action

Table 1 Larval and pupae toxicity effect of *P. pinnata* plant flower extracts against lymphatic filarial vector, *Cu. quinquefasciatus*

Mosquito larval instar and pupa	% of larval and pupal mortality Concentration of the extract (%)						*95% confidence limit* LC_{50} (LCL-UCL)	LC_{90} (LCl-UCL)	χ^2 df=4
	8	10	12	14	16	18			
1st Instar	36^{a}	49^{a}	57^{a}	68^{a}	76^{a}	90^{a}	8.285 (6.836 – 9.194)	14.213 (13.090-16.285)	1.586*
2nd Instar	31^{b}	45^{b}	52^{b}	62^{b}	69^{b}	82^{b}	9.047 (7.844-9.878)	15.641 (14.522-17.437)	0.514*
3rd Instar	26^{b}	41^{b}	47^{c}	58^{c}	63^{c}	76^{c}	9.896 (8.742-10.729)	17.373 (15.915-19.868)	0.923*
4th Instar	22^{bc}	36^{c}	42^{a}	51^{d}	56^{d}	71^{d}	10.973 (10.078-11.706)	18.267 (17.025-20.117)	5.360*
Pupa	14^{d}	25^{d}	33^{cd}	40^{e}	52^{e}	64^{e}	12.880 (12.170-13.582)	20.024 (18.679-22.294)	1.672*

Control – Nil mortality, LCL - Lower confidence limit, UCL - Upper confidence limit, x^2- Chi-square, df – degrees of freedom, within a column means followed by the same letter (S) are not significantly different at 5% level by DMRT. *significant at P<0.05 level.

Table 2 Larval and pupae toxicity effect of *P. pinnata* plant flower extracts *against* dengue vector *Ae. aegypti*

Mosquito larval instar and pupa	% of larval and pupal mortality Concentration of the extract (%)						*95% confidence limit* LC_{50} (LCL-UCL)	LC_{90} (LCl-UCL)	χ^2 df=4
	8	10	12	14	16	18			
1st Instar	41^{a}	53^{a}	59^{a}	68^{a}	74^{a}	93^{a}	7.666 (6.165-8.615)	13.528 (12.662-14.851)	2.149*
2nd Instar	36^{a}	48^{b}	53^{ab}	63^{ab}	68^{b}	82^{b}	8.235 (6.452-9.299)	15.877 (14.584-18.131)	0.550*
3rd Instar	32^{ac}	42^{bc}	47^{b}	58^{c}	62^{c}	78^{abc}	9.227 (7.528-10.281)	18.211 (16.360-21.779)	0.531*
4th Instar	27^{cd}	36^{c}	42^{c}	51^{bc}	54^{c}	71^{d}	10.662 (8.174-12.157)	18.859 (16.497-24.634)	6.982*
Pupa	16^{cd}	29^{bc}	36^{d}	49^{d}	51^{c}	64^{d}	12.229 (11.433-12.961)	19.843 (18.394-22.038)	2.496*

Control – Nil mortality, LCL - Lower confidence limit, UCL - Upper confidence limit, x^2- Chi-square, df – degrees of freedom, within a column means followed by the same letter (S) are not significantly different at 5% level by DMRT. *significant at P<0.05 level.

Table 3 Larval and pupae toxicity effect of *P. pinnata* plant flower extracts against malarial vector *An. stephensi*

Mosquito larval instar and pupa	% of larval and pupal mortality Concentration of the extract (%)						*95% confidence limit* LC_{50} (LCL-UCL)	LC_{90} (LCl-UCL)	χ^2 df=4
	8	10	12	14	16	18			
1st Instar	46^{a}	57^{a}	64^{a}	71^{a}	78^{a}	100^{a}	6.684 (4.223-7.904)	12.769 (11.791-14.583)	0.251*
2nd Instar	41^{ab}	52^{b}	58^{ab}	66^{b}	71^{b}	93^{b}	7.384 (5.403-8.904)	14.540 (13.450-16.859)	0.381*
3rd Instar	37^{b}	46^{c}	52^{b}	61^{ab}	65^{b}	86^{bc}	8.114 (5.918-9.348)	17.085 (15.420-20.304)	0.281*
4th Instar	34^{c}	39^{d}	44^{c}	55^{c}	59^{bc}	79^{c}	9.383 (7.286-10.514)	20.122 (17.573-25.790)	0.760*
Pupa	21^{d}	31^{a}	38^{ac}	49^{c}	54^{d}	68^{d}	11.596 (10.748-12.370)	19.149 (17.781-21.214)	2.761*

Control – Nil mortality, LCL - Lower confidence limit, UCL - Upper confidence limit, x^2- Chi-square, df – degrees of freedom, within a column means followed by the same letter (S) are not significantly different at 5% level by DMRT. *significant at P<0.05 level.

against the three species of mosquitoes, the LC_{50} values were ranging from 8.258-10.973 (%) against 1^{st} - 4^{th} instar larvae and 12.80 (%) pupa for *Cu.quinquefasciatus* was observed. Larval and pupal toxicity effect against *Ae.aegypti* and *An.stephensi* LC_{50} concentrations were 7.666-12.299 (%) and 6.684-11.596 (%) respectively,significant pupicidal activity was recorded with PPFME. Our results showed that crude methanol solvent extract of flower of *P.pinnata* were effective against the larvae and pupa of three important vector mosquitoes viz., *Cu.quinquefascitaus*, *Ae.aegypti* and *An.stephensi*.

In conclusion, our findings showed that the PPFME of *P.pinnata* exhibits larvicidal and pupicidal activity against three important vector mosquitoes. These results could encourage the search for new active natural compound & other medicinal offering an alternative to synthetic insecticides from other medicinal plants. The results of this study also demonstrate the potential of new alternative sources for biopesticides due to their safety and free of adverse effects. Further analysis is required to isolate the active principles and purification and evaluation of these compounds will be needed to identify the active compounds.

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