Plant stem infused water on mosquitocidal activity to *Culex quinquefasciatus* Say

Kitherian Sahayaraj*, Iyyappan, S., Manickam Jothimuthu., Ajitha, C., Nisha Juliet, I and Ganeshan Petchidurai

ABSTRACT

Locally available plants, *Azadirachta indica, Citrus aurantifolia, Citrus aurantium* and *Tamarindus indica* stems were infused in water and tested efficiency against *Culex quinquefasciatus* Say (Diptera: Culicidae) life stages. Preliminary phytochemical profiling of the plant infused water reveals the presence of phenolic compounds, tannins, flavonoid, cardiac glycosides and terpenoids. Tannins quantification revealed that *C. aurantifolia* had significantly more amounts than other plants. *Citrus aurantium* stem infused water (CASIW) caused more mortality to *C. quinquefasciatus* larvae and pupae. Furthermore, CASIW increased larval and pupal developmental period and reduced adult longevity. However, tested plants did not affect the morphology of the insect. Results suggested utilize this indigenous technique to minimize *C. quinquefasciatus* population.

Keywords: *Azadiracta indica, Tamrindus indica, Citrus aurantium, Citrus aurantifolia,* Tannins, Mortality, Life Traits, Mosquitoes

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INTRODUCTION

Culex quinquefasciatus Say (Diptera: Culicidae) an urban vector of St. Louis Encephalitis virus (SLEV) (Tsai *et al.*, 1989), Alfuy, Almpiwar, Corriparta, Sindbis, Ross River virus, Japanese Encephalitis virus (JEV) (Reuben *et al.*, 1994), Murray Valley encephalitis (Weinstein *et al.*, 1997), Reticuloendotheliosis virus (Holder *et al.*, 1999), Edge Hill, Eubenangee, Getah, Kokobera, Koongol, Kowanyama, Kunjin, Mapputta, Stratford, Trubanaman, Wongal, Reovirus type 3 and Chikungunya virus (Holder *et al.*, 1999), West Nile virus (WNV) (Goddard *et al.*, 2002), *Wuchereria bancrofti* Cobbold (WHO, 2011), Zika virus (ZIKV) (Tanise *et al.*, 2019) etc.

Culex quinquefasciatus is controlled by various methods including breeding site management (Okiwelu and Noutcha 2012), using various pesticides which cause detrimental impacts against fauna, flora and also our mother earth. Asteraceae, Lamiaceae, Meliaceae, Piperaceae, Rutaceae etc. plants crude extracts or their fractions or bioactive compounds to manage *C. quinquefasciatus.* These plants displays different effects including, toxicity, antifeedant, insect growth regulation (IGR), ovicidal, deterrent, suppression of calling behaviour and reduction of fecundity and fertility.

Previously, whole plant of *Azadirachta indica* A. Juss *(Meliaceae) (*Benelli *et al.*, 2017*)*, essential oil of *Citrus aurentium* (Rutaceae) (Michaelakis *et al.*, 2009; Sanei-Dehkordi *et al.*, 2016), *Citrus aurantifolia* (Christm. & Panz.) Swingle (Rutaceae) (Soonwera, 2015) and fruit and leaf extract of *Tamarindus indica* L. (Leguminosae (Fabaceae)) (Afrin Jumana *et al.*, 2018; Burman *et al.*, 2019) were tested against *Culex* spp..

Different plant parts consist of various phytochemicals, which are proven to have beneficial effects for human health. Phytochemicals can be released from the plant materials into water by immersing them for a certain period of time, in which this method is widely known as water infusion (Thiagarajah *et al.*, 2029). However, so far no one tested the impact of plant stem infused water against any mosquitoes and particularly, *C. quinquefasciatus* life stages. In this study, four different plants like *A. indica*, *C. aurantium*, *C. aurantifolia* and *T. indica* stem infused water tested against *C. quinquefasciatus* third and fourth instar larvae under laboratory conditions to record larvicidal and pupicidal activities, and also to record their impacts on development. In addition, preliminary chemical composition and total tannin quantity of the plant stems infusion water was also recorded.

MATERIALS AND METHODS

Collection and maintenance of insects

Mosquito larvae were collected from sewage water at St. Xavier's College and also from household waste water in Rhamathnagar (8.7242° N, 77.7617° E) of Tirunelveli district, Tamil Nadu, India. The larvae were fed with dog biscuits (Doggy Day, Zee Track Food Factory Pvt Ltd., Alappakkam, Tamil Nadu, India) and yeast powder in the 3:1 ratio (w/w). Adults were identified by Dr. S. Balasubramaian, Central Vector Control Board, Alaby of Kerala, India. A laboratory colony of *C. quinquefasciatus* was used for the larvicidal, pupicidal and adulticidal activities. Adults were provided with 10% sucrose solution. Mosquitoes life stages were maintained at 29±2°C, 70±5% relative humidity and a photo regime of 16:8 h (L: D). Laboratory emerged 0-day old, third and fourth instar larvae were utilized for the experiment.

Plant materials

Neem tree and lemon tree stems were collected from St. Xavier's College campus (8.7236° N, 77.7359° E,). Tamarind tree stem, bitter orange tree stem was collected from Nazareth (32° 41' 58.686" N 35° 18' 12.766" E) of Tuticorin district. Collected tree stems were dried for 3-days under shade in Crop Protection Research Center, St. Xavier's College, Palayamkottai. Then the dry weight of the stem was cut into small size, their total length (cm), breath (cm) and weight (g) were recorded and presented in Table 1. Vernier calliper was used to measure the length and breathe, whereas electronic balance (US–series precision electronic balance, CyberlabTM Corporation, USA) was used to record the weight.

Table 1. Length and breadth (cm) and weight (g) of the plant stem parts utilized in the study.

Plants	Weight (g)	Breath (cm)	Length (cm)
Citrus aurantifolia	10.23±.16a	0.34±0.006abc	6.22±0.008abc
Citrus aurantium	9.45±0.63ab	0.39±0.011a	6.22±0.0094abc
Tamrindus indica	5.99±0.08c	0.35±0.010ab	6.28±0.0072ab
Azadiracta indica	5.84±0.13cd	0.24±0.003d	6.30±0.0081a

Means with different small letters in the same column differ significantly ($p<0.05$), while means with different capital letters in same row differ significantly ($p<0.05$)

Mosquitocidal activities

White plastic cups of 300 mL capacity (Essee, Chennai) were used for the study. Initially they were washed with warm soap water and rinsed thoroughly with tap water thrice. Then, the plastic cups were placed in the sunlight from 10 a.m. to 4 p.m. for sterilization purposes. The test material (plant stem) was then place on to the plastic cup, 4-5 cm just above the bottom. It was filled with 250 mL water and kept undisturbed for an hour. Stems which floated on the water surface were replaced with new stems. Ten uniform sized *C. quinquesfaciatus* third instar larvae were introduced into the water. Ten replications were maintained for each test material. The larvae were provided with dog biscuit (Pedigree, India) and yeast powder (Merck, India) mixture (3:1 w/w) daily as mentioned above. After 24, 48 and 72 hours, a number of insects died and moulting of insects was recorded. During our observations dead insects were removed and preserved in 70% alcohol for morphological deformities if any.

Alive larvae were maintained till they attained adulthood. Adults were maintained with 10% sucrose solution as mentioned above.

Qualitative and quantitative profiling of phytochemcial

In another experiment, *A. indica*, *C. aurantium*, *C. aurantifolia* and *T. indica* stems were maintained on to the water for 8-days, and then water passed onto the filter paper was the filtered water was used for preliminary phytochemical screening (Brindha *et al.*, 1981; Horbone, 1984). The total tannin content was determined using the methodology of Aparna (2000) using tannic acid as standard. In brief a 1 mL of sample water was mixed with 75 mL of distilled water in a 100 mL volumetric flask and recorded total tannin content following Folin–Danis methods. After colour development, the optical density read at 700 nm against the reagent blank (1 mL of distilled water instead of sample). A standard graph of known samples was prepared by following the same procedure. From the standard graph the concentration of tannic acid in the test sample was calculated.

Data analyses

The mortality in the treatments and control was corrected using Abbott's formula (ABBOTT 1925). Larval mortality and pupal mortality data (%), larval developmental data, adult period data (days) were subjected to SPSS (IPM SPSS 20) to find out mean value, standard error, degrees of freedom (df), F and significance (P) values. All significances were represented at 5% level.

RESULT AND DISCUSSION

Qualitative and quantitative profiling of phytochemcial

The result of preliminary phytochemical screening of the test plant stems which infusion in water for 8-days is presented in Table 2. *Tamarindus indica* showed the presence of tannin, flavonoid and cardiacglycyde. Previously Prajapathi *et al.* (2003) also recorded all these observed chemicals in the tested plants. Tannin level was recorded in *C. aurantifolia* (Okwu and Emenike 2006) and recorded anticancer (Narang and Jiraungkoorskul 2016), insecticidal (Effiom *et al.*, 2012) and mosqitocidal (Soonwera 2015) activities.

To know the water nature of mosquito larval rearing, the optical density (OD_ was recorded and presented in Figure 1 The O.D value observed at 400 nm reveals the increasing trend, from first day to day eight day (Fig 1).

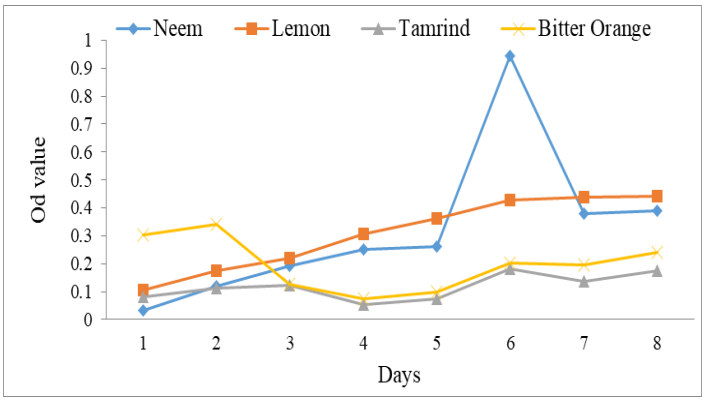
It indicates phytochemical constituents particularly tannins of plants gradually dissolved into the water as a result the O.D. value increased. Total tannin content was high in *C. aurantifolia* (139.5 mg/100 mL) followed by *C. aurantium* (120.9 mg/100 mL), *A. indica* (80.92 mg / 100 mL) and *T. indica* (74.4 mg / 100 mL) (Fig. 2).

Table 2. Preliminary phytochemical screening of the test plant stems which infusion in water for 8-days

Phytochemicals	*Az. indica*	*Ci. aurantifolia*	*Ci. aurantium*	*Ta. indica*
Alkaloids	-	-	-	-
Steroids	-	-	-	-
Phenolic compound	++	++	++	++
Total tannins	+++	+++	+++	+++
Phylloptamim	--	--	--	--
Saponin	-	-	-	-
Flavounoids	+	+	+	+
Terpenoids	-	+	-	-
Cardiac glycosides	-	--	--	+
Aromatic acid	--	--	--	--
Xanthoprotein	--	--	--	--

+ indicates low level, ++ indicates medium level,, +++ indicates maximum level, and – indicate absence of the compound

Figure 1. Optical Density (OD) value for test plants infusion in water from 1 to 8 days



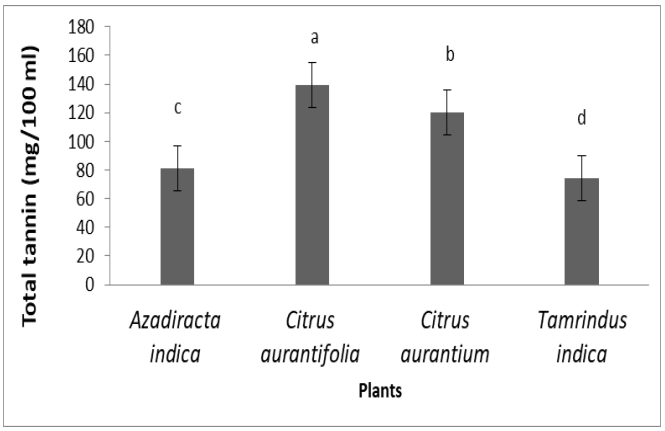


Figure 2. Total tannin content (mg/100 mL/g plant) of various plants stems infusion water for 8 days

Mosquitocidal activities

Initially mortality of *C. quinquefasciatus* third and fourth instar larvae was recorded one, two and three days after exposure continuously and 72hrs data is presented in Table 3.

Table 3. Impact of various plants stems infused water on mortality of *C. quinquesfaciatius* third and fourth instar larval stages at 72 hrs

Plants	Mosquito larval stages	
	Third instar	Fourth instar
A. indica	11.7±1.6eB	30.0 ±1.1dA
C. aurantifolia	46.6±2.8cB	55.0 ±4.3bA
C. aurantium	61.8±2.2bB	73.5 ±2.0aA
T. indica	40.5±1.7dA	45.0 ±2.3cB
Vijayneem	100.00±1.7aA	74.0±4.26aB
One way ANOVA	Df1,8; F=0.04; p =0.846471	

Means with different lower case letters in the same column differ significantly ($p<0.05$) for each third and fourth instar separately, while means with different upper case letter in rows differ significantly ($p<0.05$) for the same plants

Result showed that *Cu. quinquefasciatus* larval mortality was gradually increased from first day to third day after the exposure. The mortality was highly significant for *C. aurantifolia* (d. f. 2, 17; F=25.761; P<0.0005) than other three tested plants (Table 4). Among the other three plants the least significance was observed for *A. indica* (df 2, 17;F=0.233; P=0.795). Though *C. aurantifolia* showed significant mortality for individual days (data not included) its total mortality was 46.6±2.8% and 55.0 ±4.3% for third and fourth instar larvae respectively only considering the total mortality *T. indica* significantly more mortality (70.55%) followed by *C. aurantium* (61.66%) and *A. indica*. Also the results agree with the finding of Pushpanathan *et al.* (2006) and Elimam (2009) who had reported that early instar larvae of *Cx. quinquefasciatus* was more susceptible than later instars when treated with *Cymbopogan citratus* and *Calotropis procera* plants respectively.

Significantly higher pupal mortality was caused by *T. indica* (50.0%) (Table 4). Least mortality was caused by *A. indica* (23.33%) however; it was in significant when compound to impact of *C. aurantifolia* since *T. indica* caused significantly high mortality this plant can be utilized to control *C. quinquefasciatus* at field level *T. indica* is a common, economically important tree can be possible to be integrated in pupal control. Less pupal mortality (=50%) suggesting that the effects of the stem infused water on the pupal stage appear after more than 72 hrs exposure.

The total number in days of larval duration was premeditated from hatching to the stage of pupation. Result of life traits of *C. quinquefasciatus* reveals that larval total developmental period and pupal period was significantly prolonged by the plant stem infused water suggesting the growth regulatory activity as suggested by Sivagnaname and Kalyanasundaram (2004) and Gad *et al.* (2018). Similarly, ethyl acetate extract of *Solarium suratense* leaf (Muthukrishnan *et al.,* 1997), Petroleum ether extracts of *Ageratum conyzoides*, *Argemone mexicana*, and *Azadirachta indica* (Sharma *et al.,* 2009), ethanol extracts of *Cinnamomum osmophloeum* (cinnamon) bark, *Matricaria chamomilla* (chamomile) whole plant, *Seasamum indicum* (sesame) seeds, and *Nigella sativa* (black seed) (Gad *et al.,* 2018) and *Avicennia marina* (Forssk.) vierh (Karthi *et al.,* 2020).

Table 4. Total pupal mortality (%); larval period, pupal period and adult longevity (days) of *C. quinquesfaciatius* in relation to various plant stem infused water treatments

Test plant	Larval period (days)	Pupal period (days)	Total pupal mortality (%)	Adult longevity (days)
Third instar larva				
Control	10.9± 1.1e	3.2 ± 0.8c	0	6.1±0.4a
Azadiracta indica	11.3±0.1d	4.0±0.2b	28.1±2.2d	4.6±0.3b
Citrus aurantifolia	12.3±0.6c	4.6±0.3b	34.4±1.8c	4.5±0.2b
Citrus aurantium	14.0±0.2a	6.5±0.3a	50.0±2.9a	5.7±0.2a
Tamrindus indica	13.0±0.5b	3.7±0.2c	38.3±0.4b	3.5±0.5dc
Vijay neem	14.4±0.2a	6.9±0.3a	51.7±2.9a	5.2±0.2a
Fourth instar larva				
Control	11.0± 0.8e	3.6 ± 0.7d	0	6.7±0.1a
Azadiracta indica	13.7±0.5c	5.6±0.4c	23.3±1.2a	5.6±0.6b
Citrus aurantifolia	14.3±0.2b	6.4±0.5b	17.4±1.0c	5.5±0.5b
Citrus aurantium	16.0±0.1a	7.1±0.6a	48.3±1.4a	4.1±0.1c
Tamrindus indica	12.8±0.1d	4.7±0.3c	30.0±1.6d	6.1±0.3b
Vijay neem	16.2±0.1a	7.7±0.2a	50.79±2.9a	4.9±0.4a

Means with different lower case letters in the same column differ significantly ($p<0.05$) for each life time parameters separately

Also, a remarkable reduction in adult emergence was obtained (Table 3). Crude latex and ethanolic leaves extract of *Calotropis procera* (Asclepiadaceae) also reduced adult emergence of *C. Quinquefasciatus* (Mashlawi *et al.,* 2017). *Culex quinquefasciatus* were maintained under laboratory condition using 10% sugar solution. Adults were lived up to 10 days. However, minimum adult longevity recorded in *T. indica* treated insects (3.0 days) than *C. aurantium* (3.7 days), *A. indica* (4.64 days) and *C. aurantifolia* (5.0 days).

The mosquitocidal activity of the plant stem infused waters may be due to the presence of more levels of phenolic compound total tannins, these secondary metabolites responsible for the biological activities against *C. quinquefasciatus. Previously,* Wongo (1998); Isayama *et al.* (2011); Na Young (2015) and Yuan *et al.* (2020) also reported that tannins were either directly or indirectly responsible for the insecticidal activity. Literature reveals the mode of action of tannins in insect pests shows that tannins formed complexes with digestive enzymes in the insect gut, reduced digestive physiology and retarded insect growth (CHEN *et al.,* 2018).

In conclusion *Culex quinquefasciatus* populations can be managed using the locally available plants such as *Tamarindus indica, Citrus aurantium, Azadiracta indica* and *Citrus aurantifolia,* because they possess: phenolic compounds, Tannins, alkaloids, and flavonoides. Tannin content was very high in *Citrus aurantifolia.* When the plant stem is infused in water, the plant releases mucilage which in turn increases the viscosity of the water. Third instar *C. quinquefasciatus* larvae was more susceptible than fourth instar larvae. Plant phytochemicals are responsible for *C. quinquefasciatus* larval and pupal mortality and delay the adult longevity. These native plants stem infused water can be utilized for *C. quinquefasciatus* management and considered as the best alternative to synthetic pesticides.

Authors' contributions

Conceived and designed the experiments: CR and DN Performed the experiments and analyzed the data: CR. CR and DN analyzed and interpreted the data and wrote the manuscript. Revision of the

manuscript: KS and NKD Finally, all authors have read and approved the final manuscript.

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