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BIO-EVALUATION OF LARVICIDAL ACTIVITY OF FOUR MEDICINALLY IMPORTANT PLANTS OF THE FAMILY *ASTERACEAE* AGAINST THE LARVAE OF *AEDES AEGYPTI*

S. Premalatha, K. Shri Mathi. K, Kalaiselvi, A. Muhsina

Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India-641018.

Abstract

The diseases transmitted by mosquitos' remains a major cause of the loss of human life worldwide with more than 700 million people suffering from these diseases annually. Apart from being a great source of medicine, the plants play an excellent role in various fields including insecticides. The present study, larvicidal activity of different solvent extracts[petroleum ether, hexane, chloroform, ethanol and water from asteraceae family plants -*Tridax procumbens, Synendrella nodiflora, Blumea sinuate* and *Ageratum conzoides* were against larvae-*Aedes aegypti* at various concentrations (control, 25%, 50%, 75% and 100%) and time period includes 24hrs and 48hrs to observe the mortality rate. As the exposure time increased the mortality rate also increased this shows that the crude extracts slowly affects the larvae. All the plant extracts showed larval mortality sequentially increased from lower concentration (25%) and achieved maximum activity at 100% concentration. By comparing the extraction, the chloroform shows a high mortality percentage against *Aedes aegyptii.* The least concentration of mortality was obtained in Hexane and aqueous extraction at 1.25% and 0.625% concentration. Comparing with all other crude leaf extracts the chloroform extract of *Tridax procumbens* show higher mortality rate, at 5% concentrations gives 100% mortality (LC 50 -0.70 ; LC 90 – 1.56).The current study shows that the Asteraceae family plants can be used as larvicidal agents among which *Tridax procumbens* is a good source for mosquito control.

Keywords: Asteraceae, Mosquito control, Larvicidal activity, *Aedes aegyptii*.

Corresponding Author: Dr. S. Premalatha,

Introduction:

The plants have been considered as a major source of medicine from antiquity. Various approaches were brought up to utilize the plants with ethno-botanical importance. Apart from being medicine, there are number of scientifically proven uses including pesticides. Several plants have been reported to have insecticidal activities in which the plants with aromatic odor have got significant effect. Identification of novel effective mosquitocidal compounds is essential to combat increasing resistance rates, concern for the environment and food safety (Abdel *et al.,* 2005). An increasing number of researchers is reconsidering plants containing active phytochemicals in their efforts to address some of these problems. To be highly competitive and effective, the ideal phytochemical should possess a combination of toxic effects and residual capacity (Rehman, *et al.,* 2022). Acute toxicity is required at doses comparable to some commercial synthetic insecticides while chronic or sub-chronic toxicity is required to produce growth inhibition, developmental toxicity and generational effects (Abdel *et al.,* 2005). The insecticidal activity of several plants, including *Allium sativum*, *Artemisia absinthium*, *Citrullus colocynthis*, *Laurus nobilis*, *Mentha pulegium*, *Myrtus communis*, *Nerium oleander*, *Ocimum basilicum*, and *Origanum majorana* have been reported with significant importance (Niroum *et al.*,2006).

Control of vector mosquitoes is essential for the prevention of illnesses spread by mosquitoes (Kamaraj, *et al.,* 2010). In many endemic regions of the world for vector-borne diseases during the past few decades, the issue of insecticide-resistant vectors has emerged. Therefore, researchers are looking for novel chemicals to solve the issue (Samuel *et al*., 2012). Mosquito's transmitted disease remains a major cause of the loss of human life worldwide with more than 700 million people suffering from these diseases annually. Mosquito also cause allergic responses that include local skin and systemic reactions such as angioedema in humans (Peng *et al.*, 1999). Mosquito act as a vector for most of the life threatening disease like yellow fever, malarial fever, dengue fever, chikungunya, filariasis, etc. In mosquito control, the continuous application of synthetic insecticides cause development of resistance in vector species, adverse effect on environment quality and non-target organisms including human health (Veerakumar *et al.,* 2013).These illnesses not only cause substantial morbidity and death, but they also create significant economic and social disruption in emerging nations such as India and China. With an estimated yearly economic loss of $720 billion, India alone accounts for around 40% of the global filariasis burden (Hotez *et al.*,2004).

Since plant extracts are a rich source of bioactive chemicals that may be biodegraded into harmless products that may be used to control mosquitoes, they may be an alternate source of mosquito control agent. Compounds made from plant extracts can operate as larvicides, insect development regulators, repellants, and oviposition attractants. However, plant-based pesticides have been widely utilised on agricultural pests and, to a lesser extent, on insect vectors of public health relevance. These compounds can also play a significant part in stopping the spread of mosquito-borne diseases to both individuals and the society as a whole. Many research on plant extracts against mosquito larvae have been undertaken all over the world (Govindarajan, 2010).Due to their restricted mobility in breeding habitats and simplicity of control in certain habitats, larval mosquitoes are

desirable targets for control operations. Phytochemicals have an important role in mosquito control programmes. The bioactive plant ingredient can be extracted from the entire plant or from a particular section using various polar and nonpolar solvents such as petroleum ether, hexane, chloroform, ethanol and water (Altemimi *et al*., 2017).

The order Asterales includes about 32,000 recognized species of flowering plants in over 1,900 genera in the family Asteraceae, sometimes known as Compositae. The Asteraceae family is important commercially because it supplies critical foods, garden plants, and herbal treatments. Species that are present outside of their natural habitats might be invasive or weedy. In view of an increasing interest in developing insecticides of plant origin as alternative to chemical insecticide, we tested petroleum ether, hexane, chloroform, ethanol and aqueous extracts of dried leaf powder of Asteraceae family plants *include Tridax procumbens, Ageratum conzyoides, Blumea sinuta* and *synedrella nodiflora*for larvicidal activity against III & IV instar larvae of *Aedes aegypti*.

Egunyomi *et al*. (2010) reported on the larvicidal efficacy of Tridax procumbens plant extract against Anopheles stephensi. Studies by Nazar *et al*,. (2009) have also proven Tridax procumbens' effective larvicidal action against *Culex quinquefasciatus*. Invasive weed *Synedrella nodiflora* have been reported to possess larvicidal activity against *Ae. aegypti* (Ghayal *et al*., 2010). *Ageratum conyzoides* showed larvacidal and growth-inhibitory action when tested on *Anapheles stephensi* larvae in their II and IVth instars reported by Neetu Arya *et al*., 2011.

The present study is carried out to test the larvicidal activity of selected leaf extracts of Asteraceae family plants against *Aedes aegypti* to reduce the use of chemical insecticide by botanical insecticide and to make awareness to the society about the Plant remedies in the Mosquito control.

MATERIALS AND METHODS

Collection of plants:

Plants leaves of family Asteraceae includes *Tridax procumbens, Synendrella nodiflora, Blumea sinuate* and *Ageratum conyzoides* were collected from the area Onnapalayam, Thondamuthur, Coimbatore District Tamilnadu, India, in the period of March to April 2022 and then the leaves are thoroughly washed under tap water and shade dried at room temperature.

Tridax procumbens

Synedrella nodiflora

Ageratum conyzoides

Blumea sinuate

Figure 1

Preparation of leaf extracts:

The leaves were powdered and was further macerated with petroleum ether, hexane, chloroform, ethanol and water at room temperature for three days and then filtered by suction through Whatman filter paper. The solvents are evaporated and the residues obtained were collected and stored at 4°C. The residues were then used to prepare one per cent stock solution with above solvents. From the stock solution, different dilutions were prepared with distilled water includes 5%, 2.5%, 1.25% and 0.625%

Mosquito collection and rearing:

The mosquito species *Aedes aegypti* selected for present investigation. The mosquitoes *Aedes aegpyti* are collected from garden area around the lab. The larvae were kept in plastic trays and filled with tap water. Dog biscuit and yeast were provided once a day initially for development of later stages. The pupae from culture were transferred in a cup containing tap water and placed in the oviposition cage (44x44x43). 10% sucrose solution was placed in the cage for emerged adults. Broiler chicken (*Gallus gallusdomesticus*) was used for giving blood meal to the emerged adults from third day onwards. For oviposition Small plastic bowls containing tap water lined with filter paper were placed inside the cage. The whole setup was maintained at 28 ± 2ºC and 70-80% relative humidity under the 14:10 light and dark cycles (Kamaraj *et al*., 2009).

Larvicidal activity:

The larvicidal activity of plants *Tridax procumbens, Synendrella nodiflora, Blumea sinuate* and *Ageratum conzoides* crude extracts were assessed by using the standard method as prescribed by WHO (2005). Larvae are collected from the Backyard stock water. Each extract was tested at five different concentrations at the same time. The larvae of this mosquito (5 larvae) were introduced in 200ml plastic cups containing 100 ml of aqueous medium (99 ml of de-chlorinated water) and required amount of plant extracts was added to make different concentrations. One control was maintained with each set of experiment and mortality was recorded intervals after 24 h and 48 hrs. Tests were carried out under controlled laboratory conditions against laboratory reared *Aedes aegypti* larvae. Values obtained were subjected to log probit regression analysis to obtain LC50 and LC90

values with 95% confidence limit. The percentage of mortality was calculated by using following Abbott's formula (Abbott, 1925)

Corrected Mortality = Observed mortality in treatment – Observed mortality in control * 100

100 – Control mortality

$$\text{Percentage mortality} = \frac{No\ of\ dead\ larvae}{No\ of\ larvae\ introduced} \text{ X } 100$$

Statistical analysis:

The data analysis was carried out using Microsoft word using Excel 2007. A one-way ANOVA was performed for all the experimental data; the least significant difference was calculated. Lethal Concentration (LC_{50}) represents the concentration of the test material that caused 50% mortality of all test organisms within the specified period of exposure. It was determined by exposing various developmental stages of the test organisms recorded in these bioassays, LC_{50} and LC_{90} were calculated along with their fiducal limits at a 95% confidence level by probit analysis using SPSS 16.0 (Statistical Package of Social Sciences) software.

Result:

The plant leaf extracts of *Ageratum conyzoides, Syndrella nodiflora, Blumea sinuate* and *Tridax procumbens* were tested against the fourth instar larvae of *Aedes aegypti*. The extractions are obtained by petroleum ether, hexane, Chloroform, ethanol and aqueous. The leaf extract showed larval mortality sequentially increased from Low concentration to high concentration (0.625 to 5%). The results from each plant extracts has been detailed and discussed in Table 1 and Table 2.

Table 1: LC_{50} and LC_{90} values of larvicidal activity of selected plant species against *A. aegypti*

Solvents	Hours	*Tridax procumbens*		*Ageratum conyzoides*		*Blumea sinuata*		*Synedrella nodiflora*	
		LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Petroleum ether	24hrs	3.14	5.53	4.44	6.93	5.02	8.12	15.94	32.46
	48hrs	2.80	4.65	4.11	6.34	5.02	8.12	5.96	10.16
Hexane	24hrs	15.94	32.46	15.94	32.46	0	0	15.94	32.46
	48hrs	11.87	28.60	15.94	32.46	0	0	5.02	8.12
Chloroform	24hrs	0.80	1.72	2.80	4.65	15.94	32.46	4.11	6.34
	48hrs	0.70	1.56	2.42	4.08	4.11	6.34	3.67	5.47
Ethanol	24hrs	4.44	6.93	5.02	8.12	0	0	11.29	21.04

	48hrs	3.93	5.91	5.02	8.12	15.94	32.46	11.29	21.04
Aqueous	24hrs	4.11	6.34	4.44	6.93	0	0	0	0
	48hrs	4.11	6.34	4.11	6.34	5.96	10.16	5.96	10.16

LC_{50} The concentration that kills 50% of the target is called an LC_{50} (Lethal Concentration 50) LC_{90} - The concentration that kills 90% of the target is called an LC_{90} (Lethal Concentration 90)

The chloroform extract of *Tridax procumbens* shows significant mortality in less concentration i.e., 50% mortality in the concentrations of 0.8% in 24 hours and 0.7% in 48 hours followed by petroleum ether extract *T.procumbens* which shows 50% mortality in the concentration of 3.1% and 2.8%. But the chloroform extract of *T.procumbens* at 48 hours' time duration gives higher 90% of mortality i.e., upto 1.5% at 48 hours than all other extracts.

In *Ageratum conyzoides* the highest mortality is obtained in chloroform extract that is, 50% mortality in concentration of 2.8% in 24 hours and 2.4% in 48 hours. The petroleum ether and aqueous extract of *A.conyzoides* gives equal mortality rate followed by chloroform extract (4.4% at 24 hours and 4.1 at 48 hours). In the chloroform extract of *Synedrella nodiflora*, the highest mortality % was obtained at 3.6% concentration in 48 hours and 4.1% concentration

When comparing all the four plant extracts the chloroform extract of *Tridax procumbents* shows highest 50% and 90% of mortality in less concentration, followed by petroleum ether. The aqueous extract of *Blumea sinuate* and *Synedrella nodiflora* shows 50% and 90% of mortality at same concentration (5.9% at 24 hours and 10.1% at 48 hours). The hexane extract of *S.nodiflora* gives 50% and 90% of mortality at less concentration than all other hexane leaf extract at 48 hours (5.0% and 8.1% concentration).

Table 2:Comparing the Mortality % of *Aedes aegypti* larvae by different plant species of Asteraceae family plants:

Solvent	Concentration (%)	*Tridax procumbens*		*Ageratum conyzoides*		*Blumea sinuata*		*Synedrella nodiflora*	
		24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Petroleum ether	0.625	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	1.25	40	40	Nil	Nil	Nil	Nil	Nil	Nil
	2.5	40	40	20	40	20	20	0	20
	5	60	80	60	60	40	40	20	20
Hexane	0.625	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	1.25	Nil	20	Nil	Nil	Nil	Nil	Nil	Nil
	2.5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	20
	5	20	40	20	20	Nil	Nil	20	40

Chloroform	0.625	60	60	Nil	Nil	Nil	Nil	Nil	Nil
	1.25	60	80	40	80	Nil	Nil	Nil	Nil
	2.5	80	80	40	60	0	40	40	40
	5	100	100	80	80	20	60	60	80
Ethanol	0.625	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	1.25	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	2.5	20	20	20	20	Nil	Nil	Nil	Nil
	5	60	80	40	40	Nil	20	40	40
Aqueous	0.625	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	1.25	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	2.5	40	40	20	40	Nil	20	Nil	20
	5	60	60	60	60	Nil	20	Nil	20

The chloroform extract of *Tridax procumbens* shows highest mortality % than all other extracts, at 0.625% concentration itself it gives 60% of larvae death and as the concentration increases to 5% the *Tridax procumbens* shows 100% mortality. Except hexane leaf extract all other extracts of *Tridax* gives 60% of larval death at 5 % concentration

Followed by *Tridax* the *Ageratum* chloroform extract gives 80% mortality at 5% concentration however at 2.5 % concentration itself the *Tridax* gives 80% death rate at 48 hours' time duration. The aqueous and petroleum ether extract of *Ageratum* gives 60% of mortality.

In *Synedrella nodiflora* and *Blumea sinuata* the highest mortality % was seen at 48 hours' time duration in chloroform extract (80% and 60% respectively) In aqueous extract both of these plants shows 20% of mortality rate as exposure time increases. All the hexane concentration shows least and no mortality rate in all plant extracts. The highest mortality % of hexane extract is seen in *tridax* and *synendrella* ie., upto 40%.

As from the above result the *Tridax procumbens* shows 100% of larval death. The *Blumea* and *Synedrella* show less larvicidal activity than *Ageratum.* Chloroform extract gives positive result towards all plant extracts followed by pethroleum ether. The aqueous, ethanol and hexane extracts gives larval death at least amount.

Discussion and conclusion:

The leaf extract showed larval mortality sequentially increased from Low concentration to high concentration (0.625 to 5%). Comparing with all other leaf extracts the chloroform extract of *Tridax procumbens* show higher mortality rate, at 5% concentration gives 100% mortality (LC 50- 0.80 and LC 90 -1.76) at 24hrs

itself. Hexane shows lower mortality percentage in all plant samples and no mortality in *Blumea sinuata*. As petroleum ether extracts concentration increases the rate of mortality also increased. The 5% concentration gives 80% (LC 50 -2.80 and LC 90 -4.65) of mortality at 48 hrs time duration in *Tridax* followed by *Ageratum conyzoides, Blumea sinuate,* and *Synedrella nodiflora*. This shows that as exposure time increases the mortality also increases. The aqueous extract shows mortality rate at 2.5% conc only on all leaf extracts. *Tridax procumbens* and *Ageratum conyzoides* shows equal amount of mortality rate on aqueous extract. By comparing these plants extraction the hexane and ethanol extraction shows a least % mortality.

The results of this present study suggest that the chloroform extract of all plant species gives a positive result on larval mortality. The chloroform extract of *Tridax procumbens* gives 100% mortality at higher concentration which shows a promising activity in mosquito control. *T. procumbens* extracts killed *C.quinquefasciatus, Aedes aegypti*, and *Anopheles stephensi* larvae, which can be linked to the presence of terpinoids, an insecticide (Devan *et al.,* 2013). Followed by *Tridax procumbents*,the petroleum ether extracts shows good mortality rate. *Blumea sinuate* gives a least mortality rate among other leaf extracts. Results revealed that duration of exposure time increases the rate of mortality also increases respectively. The maximum larval mortality was found in 48 hrs.

Anopheles larvae treated with plants showed some juvenomimetic effects, according to Kalyansundrum and Babu (1982) . Vasudevan *et al*., (2009) reported similar outcomes. From the findings of the present works, it has been concluded that, the selected plants possess mosquitocidal properties against the vector mosquitoes. Thus, hypothesis proposed in the present study is accepted since the chloroform extracts of *Tridax procumbens*showed significant activities against the mosquito vector. Furthermore, generally the phytochemicals are eco-friendly in nature and safer to non-target organisms and the utilization of the selected plant phytochemicals after a thorough screening to the cause by various vector mosquitoes in the near future.

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