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LARVICIDAL EFFECT OF TRIDAX PROCUMBENS AND AZADIRACHTA INDICA PLANT EXTRACT AGAINST AEDES AEGYPTI AND CULEX QUINQUEFASCIATUS

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ABSTRACT

Aedes aegypti is a vector for transmitting several tropical fevers which include dengue virus and the virus spread to other disease including mosquito salivary gland. Whereas *Culex quinquefasciatus* found in eutropic water of artificial containers and in open pond, diectuss train of sewage. It is important to develop the non-toxic and inexpensive method to conclude the mosquito larvae using medicinal important plant extract such as *Azadirachta indica* and *Tridax procumbens*. The larvae were categorized based on their prey size such as 4th instar larvae of both species. *Tridaax procumbens* revealed larval growth incubation were as *Azadirachta india* extracts as bio-pesticides and as natural insecticides proved to be an efficient and inexpensive and non-toxic method for control of mosquito. The percentage of prey killed of *culex quinquefasciatus* at 5% concentration revealed 82% at 10mg/ml and At different concentration of the *Azadirachta indica* leaf observed for the period of 24 hours exposed to the prey density of 25 showed an increased percentage of prey killed of 90%. The larvae mortality rate at 10 mg/ml of leaf extract found exhibit at maximum activity against *Aedes agypti*. The results demonstrate and excellent activity which contributes to rare opportunity of natural control. Similary, *Tridaax procumbens* showed increased the higher larval (*culex quinquefasciatus*) mortality was found to 62% at the concentration of 10mg/ml and *Aedes agypti* percentage of prey killed was found to be 74% at the concentration of 10mg/ml.

KEYWORDS: *Culex quinquefasciatus*, *Aedes aegypti*, *Tridax procumbens* and *Azadirachta indica.*

INTRODUCTION

Mosquitoes play a major role in the transmission of dengue fever, yellow fever, malaria, filariasis. Mosquito are one of the most medically significant vectors, and they transmit parasites and pathogens, which continue to have a devastating impact on human beings and other animals (Elumalai *et al*., 2013 a, b). Some mosquito species like *Culex and Aedes* are the vectors for the pathogens of various disease and contribute significantly to poverty and social debility in tropical countries (Jiang et al., 2009).

Culex quinquefasciatus is a peridomestic mosquito seldom found far from human residence or activity, and readily feeds on avian, mammalian or human hosts. The larvae are typically found in the eutrophic water of artificial containers or man-made impoundments including open ponds, ditches and drains containing human or animal sewage. As such, *Culex quinquefasciatus* was uniquely adapted to the environs of historical sailing ships outfitted for long voyages where polluted water and livestock were common. Since adult mosquitoes can fly short distances to shore **(Subra, 1981; LaPointe, 2008)** and immature forms could be carried ashore in water casks taken to be refilled **(Hardy, 1960),** it is likely that this mosquito was spread worldwide by commercial sailing vessels involved in the Atlantic slave trade, Old China trade and American whale oil industry between the 17 and 19th centuries **(Lounibos, 2002).**

Dengue virus is primarily transmitted by *Aedes* mosquitos, particularly *A. aegypti*. *Aedes aegypti* is a vector for transmitting several tropical fever and only the female bites for blood which she need to mature her eggs **(Hahn et al., 2001).**They typically bite during the early morning and in the evening, but they may bite and thus spread infection at any time of day **WHO (2009**). An infection can be acquired via a single bite. A female mosquito that takes a blood meal from a person infected with dengue fever, during the initial 2- to 10-day febrile period, becomes itself infected with the

virus in the cells lining its gut **Georgiev, Vassil (2009).** About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life. *Aedes aegypti* is particularly involved, as it prefers to lay its eggs in artificial water containers, to live in close proximity to humans, and to feed on people rather than other vertebrates. **Gubler (2010).**

***Azadirachtaindica* (Neem)**

Every part of *Azadirachta indica* (Neem) has been advocated to possess medicinal properties. **Pruthi (1937)** first proved scientifically the insecticidal effect of neem. Naturally occurring bio pesticides could be an alternative to chemical pesticides. **(Abdelouaheb *et al.,* 2009**). *Azadirachta indica* is a natural insecticide and is nonhazardous to man and other mammals **(Oudegans, 1991**). Therefore simple nonhazardous and inexpensive methods of extraction should be developed to enable practical use of neem **(Feuerhake, 1984).** The aims and objectives of the present work is to develop a simple inexpensive and nontoxic method for the control of mosquito larvae by *Azadirachta indica* (neem), which can be applied easily by the ordinary man without use of costly spraying equipment.

Tridax procumbens

Tridaxprocumbens is commonly used in Indian traditional medicine as anticoagulant, antifungal and insect repellent, in bronchial catarrh, diarrhoea and dysentery **(Ali, *et al.,* 2001)**. The entire plant is used for the treatment of malaria, leishmaniasis, vaginitis, dysentery and gastrointestinal disorders **(Caceres *et al.,* 1994).**

Tridaxprocumbens showed growth inhibitory and juvenile hormone mimicing activity to the treated larvae of *Culex quinquefasciatus*. It has been extensively used in Indian traditional medicine for wound healing, as anticoagulant, antifungal and insect repellent, in diarrhea and dysentery. **(Ali *et al.,* 2001).**

MATERIALS AND METHODS

Collection of prey (*AedesAegypti* and *Culex quinquefasciatus*)

Larvae of *Aedes aegypti* were taken as the prey item throughout the investigation. *Aedes aegypti* and *Culex quinquefasciatus* egg craft were collected from ICMR, Chinnachokkikulam, Madurai, India. There were brought to the laboratory and culture to a plastic bucket containing clean water. The bucket was covered with the mosquito mesh. Larvae were categorized based on their prey size as large and small class the covered with nylon mesh. They were fed with the dog biscuits and yeast powder in the ration of 3:1 as food.

Collection of Plant

Fresh leaves of *Tridax procumbens* and *Azadirachta indica* were collected from Government Arts college, Melur, Madurai District, Tamil Nadu.

Preparation of the Leaves extract

The leaves of the *Tridax procumbens* and *Azadirachta indica* were shade dried 18°C ground and sieved to get fine powded from which the extracts were prepared. Acetone extracts of the plant was obtained by taking 25g dried leaf powder with 250 ml of acetone and kept for 24hrs with periodic shaking.

The leaves were dried to a constant weight at in an enclosed air conditioned research laboratory. The dried leaves were blended to powder to increase the surface area for extraction.

Soxhelt method of extraction was carried out. In a conventional soxhlet system, the powdered leaf material is placed in a thimble-holder and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying extract solutes into the bulk liquid. In the solvent passes back into the plant solid bed. The operation is repeated until complete extraction is achieved (**Wang and Weller, 2006).**

Phytochemical Analysis

Phytochemical test were carried out on the aqueous extract of the powdered leaves from soxhlet using standard procedures.

Larvicidal Bioassay

The larvicidal activity was assessed using Acetone-leaf extract of *Tridaxprocumbens* and *Azadirachtaindica* by the procedure of **WHO (1996)** with some modification and as per the method of **Rahuman *et al.,* (2000)**, For the stock (50 mg/ml) preparation 5ml of desired plant extract was taken and 95 ml of dechlorinated tap water. For the bioassay test 25 larvae/concentration were used for all the experiment. For mortality studies, 25 larvae each of 4th instar larvae introduced in different concentration (2, 4, 6, 8 and 10 mg/ml) of leaf extract were added. Percentage of mortality was observed in acetone leaf extract of *Tridaxprocumbens* and *Azadirachtaindica* against *Aedesaegypti*and *Culex quinquefasciatus*. The number of dead mosquito larvae was counted after 24 hrs of exposure, and the percentage mortality was reported from five replicates. The percentage of mortality was calculated by (No of larvae dead / Total no. larvae)*100. Corrected mortality was calculated by **Abbott (1925).**

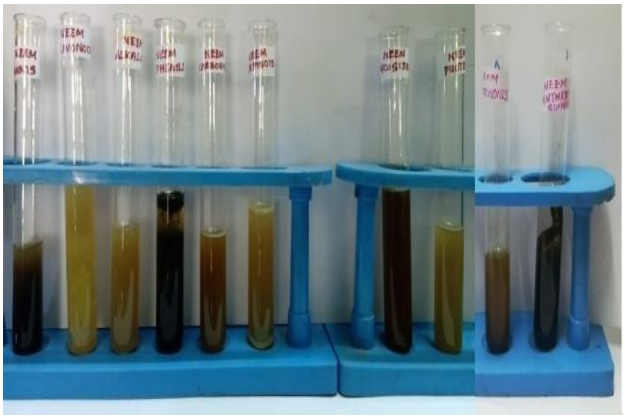

Fig. 1: Phytochemical Analysis of*Azadirachtaindica* Extract.

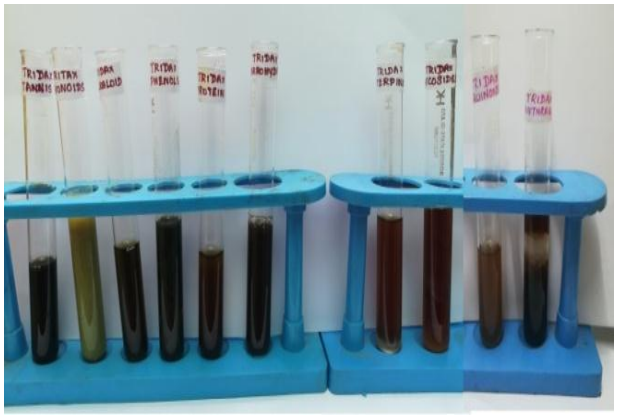

Fig. 2: Phytochemical Analysis of *Tridax procumbens* Extract.

Statistical analysis

From the results Statistical analysis such as Mean, Median, Mode, Standard Deviation and Percentage was performed using from MS Excel 2007. The larvicidal mortality were subjected to probit analysis **(Chandra *et al.*, 2014)** for compute lethal concentration of 50% (LC_{50}) values were calculated by using the Regression Equationfor hasty determination of lethal concentration.

RESULTS

Larvicidal activity of *Tridaxprocumbens* and *Azadirachtaindica leaf extract against Aedes aegypti* and *Culex quinquefasciatus*

The percentage mortality and LC_{50} rates of the fourth instar larvae of *Culex quinquefasciatus* and *Aedes Aegypti* exposed to the acetone extracts of *Azadirachta indica* presented in **Table 2**.

The percentage of prey killed of the adult mortality of *culex quinquefasciatus* at 5% concentration of leaf extract in a prey density of 25 showed an increased percentage of prey killed with 82% at 10mg/ml. The result of the study showed that plant based components of *Azadirachta indica* leaf extract demonstrated an excellent activity which contributes to an increased opportunity for the natural control.

At different concentration of the *Azadirachta indica* leaf observed for the period of 24 hours exposed to the prey density of 25 showed an increased percentage of prey killed of 90%. The larvae mortality rate at 10 mg/ml of leaf extract found exhibit at maximum activity against *Aedesagypti*.

Results on the larvicidal activity of the medicinally important plant *Tridax procumbens* as plant extract have the potential to be used as eco-friendly approach to control the mosquito. The effect of larvicidal activity of different prey density of *Aedes aegypti* treated with the concentration of the *Tridax procumbens* leaf extract were depicted in the **Table 3**.

The higher larval mortality was found to be 62% at the prey density of 25 was recorded at the concentration of 10mg/ml. Among the different prey density, the larvicidal efficiency of the leaf extract *Tridax procumbens* at a lower prey density of 25 exhibited better high activity of 62%.

The larvicidal activity of *Aedes aegypti* have been tested with plant extract *Tridax procumbens*. It is presumed the extract of *Tridax Procumbens* containing essential oil which source as effective repellent activity against larvae of *Aedes aegypti* at the prey density of 25. The percentage of prey killed was found to be 74% at the concentration of 10mg/ml.

Table 1: Phytochemical Analysis of Plant Extracts.

Phytoconstituents	Aqueous Extract	
	Azadirachtaindica	***Tridaxprocumbens***
Tannins	+	+
Flavonoids	+	+
Alkaloid	-	-
Phenols	+	+
Protein	-	-
Carbohydrate	+	-
Terpinoids	-	-
Glycosides	-	-
Quinones	+	+
Anthro-quinones	-	-

Table 2: Larvicidal activity of *Azadirachtaindica* leaves against *Aedesaegypti*and *Culex quinquefasciatus*.

plant	Mosquito	Prey Density	Concentration (mg/ml)	% of Mortality	LC_{50}
Azadirachtaindica	*Culex quinquefasciatus*	**25**	2	34	**5.79**
			4	42	
			6	66	
			8	73	
			10	82	
	Aedesaegypti	**25**	2	48	**2.59**
			4	58	
			6	68	
			8	79	
			10	90	

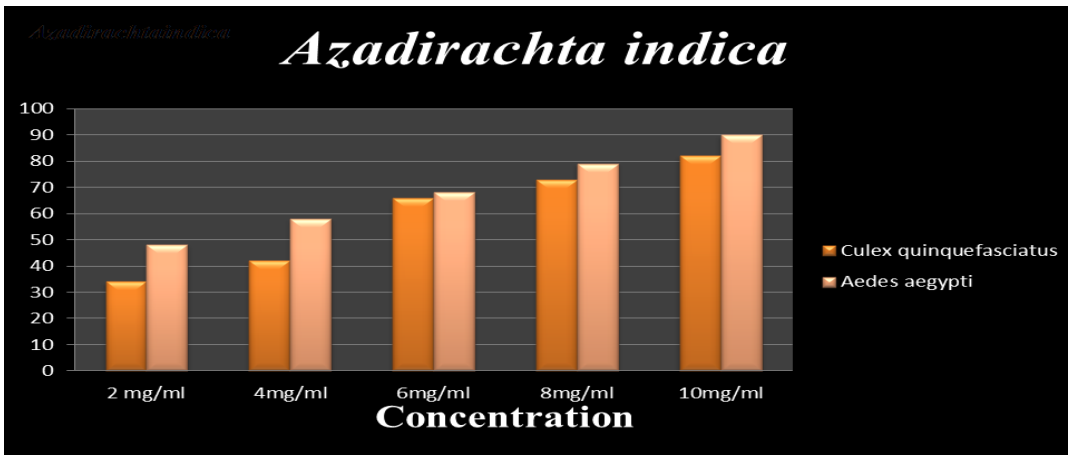

Figure 4: Larvicidal Activity of *Aedesaegypti*and *Culex quinquefasciatus* in *Azadirachta indica* Extract.

Table 3: Larvicidal activity of *Tridax Procumbens* leaves against *Aedesaegypti*and *Culex quinquefasciatus*.

plant	Mosquito	Prey Density	Concentration (mg/ml)	% of Mortality	LC_{50}
TridaxProcumbens	*Culex quinquefasciatus*	**25**	2	26	**6.31**
			4	38	
			6	48	
			8	55	
			10	62	
	Aedesaegypti	**25**	2	38	**3.63**
			4	50	
			6	60	
			8	69	
			10	74	

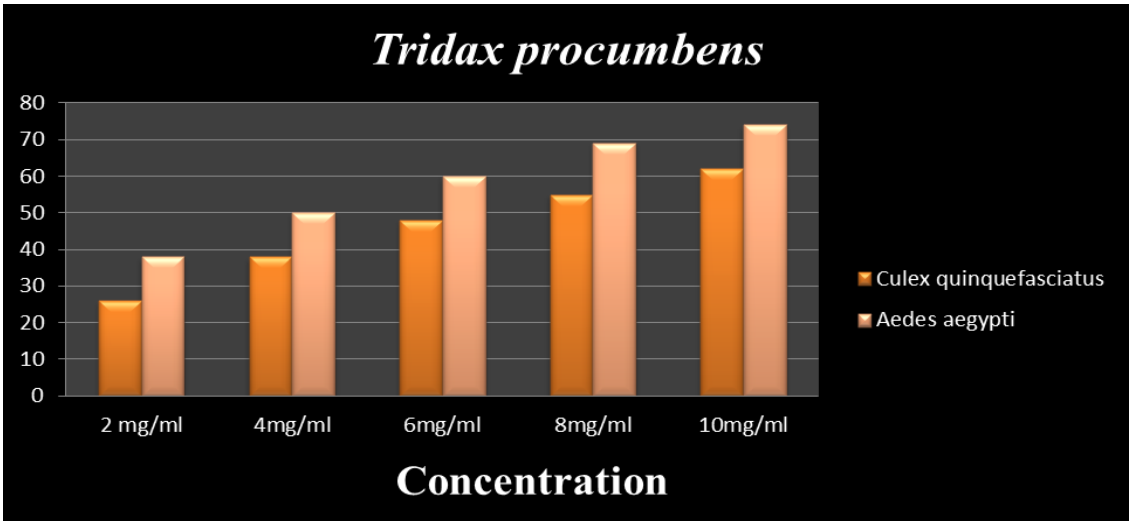

Figure 5: Larvicidal Activity of *Aedes aegypti*and *Culex quinquefasciatus* in *Tridax procumbens* Extract.

DISCUSSION

Larvicidal activity of *Azadirachta indica* against various species of mosquitoes has been observed by various researchers **(Wandscheer, 2004; Chavan, 1984; Virendra *et al.*, 2009; Aliero *et al.*, 2003; Vatandoost and Vaziri, 2004; Abdelouaheb *et al.*, 2009; Senthil *et al.*, 2006).** The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids and phenolic compounds. Presently many scientist and organizations are search of traditional remedies as alternative medicines. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants **(Puri, 1999)**.

The extracts produced some abnormalities in larvae. Larval pupal intermediates were observed. Partially emerged adults showed crumpled legs and entangled in pupation. All these abnormalities were also reported by **Naqvi (1987).** everal studies have focused on natural products for controlling Aedes mosquitoes as insecticides and larvicides, but with varied results.[14,15,16,17,18,1]

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Several studies have focused on natural products for controlling *Aedes* mosquitoes as insecticides and larvicides, but with varied results **(Rajan and Savarimuthu, 2012: Raveen, et al., 2012; Govindarajan and Karuppannan, 2011; Govindarajan, 2010; Radhika, 2011).**

The phytoconstituents of *Tridax procumbens* responsible for effect on cellular and hormonal functions in mice were attempted to explore. The *in vitro* (phagocytosis) and *in vivo* (haemagglutination and delayed hypersensitivity) were used to study the effect of extract and fraction on the cellular and hormonal immunity. The results obtained indicate the ability of flavonoidal and saponin fraction of *Tridax procumbens* to modulate both cell mediated and the hormonal components of the immune system and explored the phytoconstituents responsible for immunomodulatory potential of *Tridax procumbens*. **(Agarwal et al., 2010).**

The leaves essential oils of *T. procumbens* are more effective repellents activity at 6 per cent concentration against *An. Stephens*i **(Rajkumar and Jebanesan, 2007).**

Leaf acetone extract of *Tridax procumbens* against the larvae of An. subpictus (LC50=93.80, 39.98, and 51.57 mg/l; LC90=524.90, 145.70, and 226.56 mg/l) respectively. Ethyl acetate extract of *Tridax procumbens* have the potential to be used as an ecofriendly approach for the control of the *An. subpictus*, and *Cx. tritaeniorhynchus*. **(Kamaraj *et al.*, 2011).**

CONCLUSION

In this study, the acetone extracts of *Tridax procumbens* and *Azadirachta indica* leaves showed potential larvicidal activities agaist *Aedes aegypti* and *Culex quinquefasciatus*. The biological activity of the experimental aqueous *Tridax procumbens* plant extracts may be recognized to the presence of various compounds, including Tennis, Flavonoids, Phenol and Quinones. *Azadirachta indica* plant extracts attributed to the present of various compounds, including Tannins, Flavonoids, phenols, Carbohydrate and Quinones, which may be jointly or independently contribute to produce larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus*. The aceton extract of *Azadirachta indica leaves showed good larvicidal activity against larvae of Aedes aegypti* ($LC_{50=}$2.59 mg/ml) *Culex quinquefasciatus* ($LC_{50=}$5.79mg/ml at 24 hrs). The aceton

extract of *Tridax procumbens* also showed potential larvicidal activity agaist 4th instar larvae of *Aedes aegypti* ($LC_{50=}$6.31 mg/ml) *Culex quinquefasciatus* ($LC_{50=}$3.63 mg/ml at 24 hrs). The results demonstrate and excellent activity which contributes to rare opportunity of natural control.

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