Larvicidal activity of *Tridox procumbens* against *Aedes aegypti Larvae*

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Abstract

Mosquitoes are vectors that transmit many dreadful diseases. Repeated use of synthetic insecticides for the control of mosquitoes causes adverse effects on target organisms. So alternate control measures are being explored from plant based insecticides. Present investigation was carried out the larvicidal activity of solvent extracts (chloroform, petroleum ether and acetone) *Tridox procumbens* against fourth instar larvae of *Aedes aegypti*. Mortality counts were made after 24, 48 and 72hrs of incubation for different concentrations (50, 75, 100, 125 and 150ppm). Chloroform and petroleum ether extracts show 100% mortality after 48 and 72hrs of incubation for 150ppm concentration. Petroleum ether and acetone extracts of *Tridox procumbens* has least LC50 of 101.62 and 103.92ppm respectively at 24hrs of exposure. At 72hrs of incubation, high LC50 (169.10ppm) values were observed in petroleum ether extracts of *Tridox procumbens*. Extracts of *Tridox procumbens* were more effective vector control agent against *Aedes aegypti* mosquito larvae.

Key words: mosquitoes, *Aedes aegypti*, *Tridox procumbens*, solvents and larvicides

1. Introduction

Mosquitoes are tiny assassinate vectors that responsible for the transmission of many dreadful diseases such as malaria, chikungunya, yellow fever, filariasis and dengue haemarrhagic fever. These diseases are the major public health concerns in TamilNadu because of their tropical climate and poor drainage facilities particularly rainy season. Irrigation ditches, water stagnation in polytene bags, coconut tenders, earthen pots, fallen parched leaves and tanks are the major places act as a breeding ground for mosquitoes. *Aedes aegypti* is a freshwater breeding vector to transmit dengue fever, dengue haemarrhagic fever (DHF), dengue shock syndrome (DSS). It is difficult to control during rainy season. WHO (2011) estimates, there may be 50 millions of people are at high risk from dengue in worldwide every year. In the past 4 months, majority of the peoples (including infants, child and adults) in TamilNadu faces at high risk even mortality also occurs due to dengue haemarrhagic fever. Vaccines are not yet been developed for dengue but the incidence of disease is reduced only by control of mosquitoes. Controlling measures are either by killing or prevention of biting mosquitoes or causing large scale larvicidal mortality at breeding places (Rajmohan *et al.*, 20017). Householders use variety of synthetic insecticides to control the mosquitoes. But the vector diseases endure because of genetically resistance of mosquitoes against insecticides and changes in biting habits of vectors. Synthetic pesticides are xenobiotic (persisted) in nature that cause respiratory problems, allergic reaction, some types of cancer and also affect immune system. Therefore a necessary step to develop alternative source of pesticide to the synthetic pesticides and could be safe, ecofriendly and low cost.

Large number of plants with strong herbal tradition has been reported as insecticidal activity (Nazar *et al.*, 2009; Nataya *et al.*, 2010; Pankaj *et al.*, 2010 and Kamaraj *et al.*, 2011). Traditionally medicinal plants and their products have been used in human communities to reduce mosquitoes for many years. Bioactive compounds present in the plant act as insecticidal, antifungal, antibacterial, ovipositon reductors and antimoulting agents. Bioactive compounds are ecofriendly, less toxic and easily biodegradable. Present investigation was carried out to validate the larvicidal potentiality of different solvent extracts *Tridox procumbens* against fourth instar larvae of *Aedes aegypti*. *Tridox procumbens* is an ayurvedic traditional herb with multiple properties. This plant is used as anticoagulant, antifungal

agent and for the treatment of bronchial disorder, diarrhea, malaria, leishmaniasis, vaginitis and gastrointestinal disorders.

2. Materials and Methods

2.1 Collection of plant materials

Leaves of *Tridox procumbens* was harvested, washed thoroughly, blotted and dried in shade for one week.

2.2 Preparation of extracts

Dried plant materials were powdered and extracted by different solvents (chloroform, petroleum ether and acetone) in soxhlet apparatus for 18hrs and the extract were filtered through Whatmann no: 1 filter paper. The extracts were concentrated at 40^0C in vacuum and stored at 4^0C for experiment.

2.3 Rearing of *Aedes aegypti*

Eggs of *Aedes aegypti* were procured from the Entomological department. Egg crafts of *Aedes aegypti* were kept in the culture medium (water) at room temperature. After 24hrs of incubation, egg hatches out into first instar larvae. The larvae were collected and reared in the culture medium containing yeast and dog biscuit in 3:1 ratio at room temperature, fourth instar larvae were used for bioassay test.

2.4 Larvicidal Bioassay

Dried extracts were used for larvicidal bioassay as per the standard method WHO (2005). One gram of each extract was dissolved in the selected solvents to get stock solution. Various concentration (50, 75, 100, 125 and 150ppm) of solvent extracts *Tridox procumbens* were taken in 250ml beaker. 30 fourth instar larvae were released into different concentrations of solvent extracts. Four replicates were obtained for each concentration. Percentage of mortality was calculated after 24, 48 and 72hrs of incubation. Percentage of mortality was calculated by following formula

Percentage of mortality= Number of dead larvae/ Total number of larvae *100

Toxicity and activity were reported as LC_{50} and LC_{90}, representing the concentrations in ppm that killed 50 and 90% of larvae at 24, 48 and 72h respectively. Percentage of larval mortality were subjected to probit analysis (Finney, 1971), for calculating LC_{50} and LC_{90}, 95% confidence limits and regression equations.

3. Results and Discussion

Larvicidal activity of chloroform, petroleum ether and acetone extract of *Tridox procumbens* on the *Aedes aegypti* larvae were studied by counting the larval mortality at 24, 48 and 72hrs of incubation for different concentration i.e., 50, 75,100,125 and 150ppm. Percentage of mortality and the lethal concentration were depicted in the tables 1 and 2. The data has also been presented in the form of chart (Figure: 1.1 to 1.3). It was found that chloroform and pertroleum ether extract *Tridox procumbens* has 100% mortality after 48 and 72hrs of incubation at 150ppm concentration. But in 24hrs of incubation, percentage of mortality were 13.33, 26.66, 40, 66.67, 83.83% (chloroform) and 10, 30, 43.33 63.33 and 80% (petroleum ether) at 50, 75,100,125 and 150ppm concentration respectively. Acetone extract of *Tridox procumbens* exhibited 80% of mortality after 48hrs of incubation and it achieved 100% mortality after 72hrs at 150ppm concentration respectively. Above result indicate that the selected solvent extracts of *Tridox procumbens* does not causes rapid larval mortality, but it can cause certain changes in their activity during experimental time. Most obvious sign of behavioural changes observed in the *Aedes aegypti* was restless, loss of equilibrium, whirling movement and finally lead to death. This may be due to the presence of secondary metabolites in the plant act as a neurotoxin. It knocks down the central nervous system and cause paralytic reaction in mosquito larvae. According to Bowers *et al.*, (1995), biological activity of the plant extract is due to various compounds like alkaloids, terpenoids etc. synthesized within plants in varying proportions to protect themselves from miroorganisms and fungi. These compounds either independently or jointly contribute to larvicidal activity of mosquitoes.

Table No: 1

Larvicidal activity at different concentration of solvent extracts T*ridox procumbens* against *Aedes aegypti* larvae applied for different time exposure

Solvents	Concentration (ppm)	Percentage of mortality		
		Period of exposure		
		24hrs M±SD	48hrs M±SD	72hrsM±SD
Chloroform	50	13.33±1.87	26.67±2.44	33.33±0.07
	75	26.66±2.45	36.67±1.41	43.33±1.41
	100	40±1.58	53.333±0.07	60±1.41
	125	66.67±3.16	73.33±1.58	80±0.07
	150	83.33±1.41	100±1.58	100±2.16
Petroleum ether	50	10±1.22	30±1.41	36.67±1.41
	75	30±1.41	43.33±1.41	46.67±0.07
	100	43.33±1.41	60±1.41	66.67±4.33
	125	63.33±1.41	80±2.91	83.33±1.58
	150	80±2.91	100±2.54	100±1.58
Acetone	50	6.67±0.07	20±1.41	30±1.41
	75	26.67±2.44	33.33±0.07	50±1.41
	100	46.67±0.07	50±1.41	66.67±3.16
	125	60±1.41	63.3±1.41	86.67±1.41
	150	76.67±1.41	80±2.91	100±1.58

M±SD: Mean ± Standard deviation of four replicates

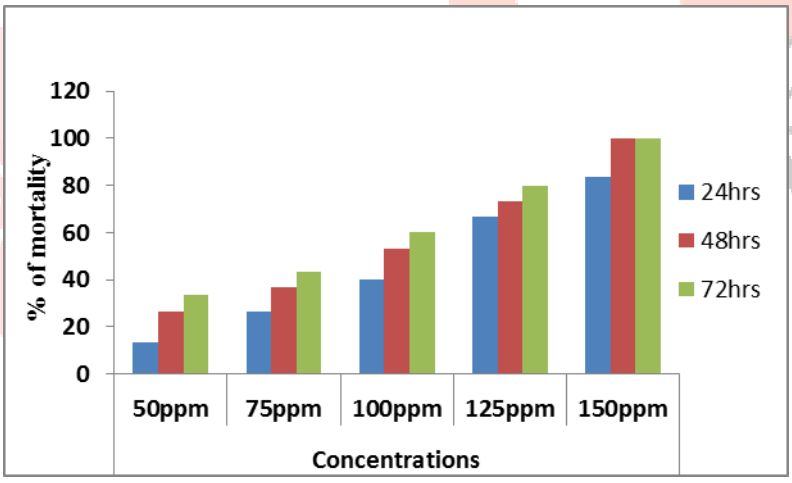

Figure: 1.1. Larvicidal activity of chloroform extract T*ridox procumbens* against *Aedes aegypti* larvae.

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities which have been observed by many researches (Joish madhasudhana Murthy *et al.*, 2009 and Venkatachalam, 2011).

LC_{50} values for chloroform, petroleum ether and acetone extracts *Tridox procumbens* at 24hrs of incubation was 89.12, 101.62 and 103.92ppm respectively. Similarly LC_{50} values for chloroform, petroleum ether and acetone extracts at 48hrs of incubation was 80.54, 73.11 and 94.54ppm respectively. At 72hrs of exposure, LC_{50} values of chloroform, petroleum ether and acetone extracts *Tridox procumbens* were 72.14, 73.11 and 70.47 respectively. LC_{90} values for chloroform, petroleum ether and acetone extracts *Tridox procumbens* at 24hrs of incubation was 100, 125 and 125ppm respectively.

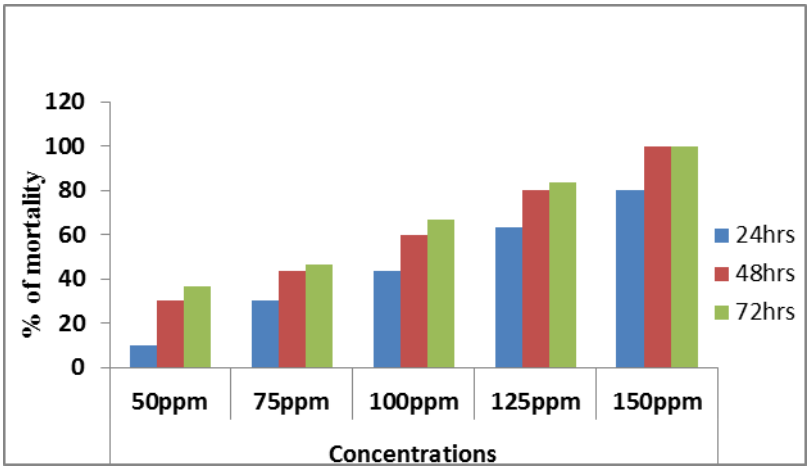

Figure:1.2. Larvicidal activity of petroleum ether extract *Tridox procumbens* against *Aedes aegypti* larvae.

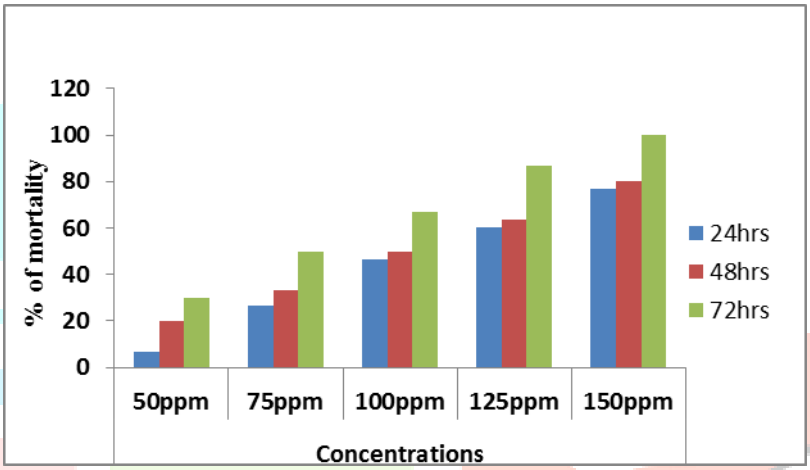

Figure: 1.3. Larvicidal activity of acetone extract *Tridox procumbens* against A*edes aegypti* larvae.

Table No: 2

Lethal concentrations of *Tridox procumbens* against *Aedes aegypti* larvae at different period of exposure

Solvents	Period of Exposure (Hrs.)	LC50±SE	95% confident limit		LC90±SE	Regression equation	r^2	F
			Lower	Upper				
Choloroform	24	89.125±0.214	2.469	6.082	100±0.08	Y=4.28X-3.55	0.95	0.004
	48	80.54±0.69	0.37	6.20	117.5±0.15	Y=5.49X-5.39	0.75	0.058
	72	72.44±0.62	14.93	55.36	102.32±0.35	Y=5.23X-4.73	0.77	0.049
Petroleum ether	24	101.62±0.11	-5.54	5.26	125.89±0.28	Y=4.27X-3.56	0.98	0.008
	48	73.114±0.58	0.486	10.31	107.15±0.13	Y=5.40X-5.39	0.80	0.039
	72	69.18±0.56	0.420	9.898	107.15±0.52	Y=5.16X-4.50	0.80	0.040
Acetone	24	103.92±0.185	2.859	5.998	125.89±0.48	Y=4.43X-3.93	0.96	0.002
	48	94.54±0.139	2.233	4.591	125.99±0.57	Y=3.41X-1.74	0.97	0.002
	72	70.47±0.485	1.411	9.618	107.15±0.68	Y=5.51X-5.19	0.86	0.02

LC50: Lethal Concentration that kills 50% of exposed larvae, **LC90**: Lethal Concentration that kills 90% of exposed larvae, SE: Standard Error

LC_{50} values for chloroform, petroleum ether and acetone extracts *Tridox procumbens* at 24hrs of incubation was 89.12, 101.62 and 103.92ppm respectively. Similarly LC_{50} values for chloroform, petroleum ether and acetone extracts at 48hrs of incubation was 80.54, 73.11 and 94.54ppm respectively.

At 72hrs of exposure, LC_{50} values of chloroform, petroleum ether and acetone extracts *Tridox procumbens* were 72.14, 73.11 and 70.47 respectively. LC_{90} values for chloroform, petroleum ether and acetone extracts *Tridox procumbens* at 24hrs of incubation was 100, 125 and 125ppm respectively. Similarly LC_{90} values for chloroform, petroleum ether and acetone extracts at 48hrs of incubation was 117.5, 107.15 and 125.99ppm respectively. At 72hrs of exposure, LC_{90} values of chloroform, petroleum ether and acetone extracts *Tridox procumbens* were 102.32, 107.15 and 107.15 respectively. Similar result was observed by Anitha *et al.*, (2012) on larvicidal activity of plant extracts against *Aedes aegypti*. Present observation clearly revealed that the extract of chloroform and petroleum ether was more effective against *Aedes aegypti* larvae than acetone extract of *Tridox procumbens*. It can be concluded that extract of *Tridox procumbens* showed toxic effect on *Aedes aegypti* larvae and can be used for control of mosquito larvae in the mosquito's breeding grounds.

4. Conclusion

It could be an alternative source to synthetic pesticides on *Aedes aegypti* mosquito larvae because they are generally free from harmful effects. Use of *Tridox procumbens* for mosquito control would generate local employment, reduce dependence on synthetic pesticidal products and to enhance health of the humans. Further studies such as identification, isolation of bioactive components from the plant extracts and its systematic effects on target mosquitoes with the biocides under field condition are needed.

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