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Bioefficacy of *Azadirachta indica* (A. Juss) and *Datura metel* (Linn.) Leaves Extracts in Controlling *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT

The aim of the present investigation is to test the larvicidal activity of *Azadirachta indica* (A. Juss) and *Datura metel* (Linn.) leaf extract against the third instar larvae of *C. quinquefasciatus* (Say) (Diptera: Culicidae). *A. indica* and *D. metel* leaf extracted by hexane and chloroform extract method at various concentrations. The hexane extract of *A. indica* and *D. metel* at 62.5, 125, 250, 500 and 1000 ppm were showed 24, 36, 55, 64 and 72.50% mortality where second one shows 9, 17.50, 30, 42 and 57% mortality, respectively. The chloroform extract of *A. indica* was showed 12, 48.50, 56.50, 73 and 87% mortality where *D. metel* shows 13.75, 27, 32, 47 and 62% mortality respectively. The hexane and chloroform extract of *A. indica* and *D. metel* had significant larvicidal effect with LC_{50} values were 246.38, 198.82, 709.96 and 562.07 ppm respectively. At 24 h post-treatment against late third instar larvae, the chloroform extracts of *A. indica* and *D. metel* were found to be more effective than hexane extracts and caused a larval mortality of 87 and 62%, respectively at 1000 ppm concentration. The larvicidal effect of *A. indica* and *D. metel* against *C. quinquefasciatus* make these plant products are potential alternative to synthetic insecticide in mosquito control plans.

Key words: *A. indica*, *D. metel*, extract, larvicidal activity, *C. quinquefasciatus* control

INTRODUCTION

Human beings have suffered from the activities of mosquito since time immemorial. It is believed that mosquitoes are ranked as the most important human health pests. They scourge him with their vicious biting and continuous singing, but most seriously they transmit malaria, filaria, japanese encephalitis, chikungunya and dengue fever to human beings. These diseases devastate Indian economy every year (Jaswanth *et al*., 2002). Worldwide, mosquitoes transmit diseases to more than 70,00,00,000 people annually and are responsible for one death for every seventeen people currently alive.

Since, prevention is better than cure, a major strategy of malarial control is to attack the vector with insecticides (Rajkumar and Jebanesan, 2009). The control of mosquito at the larval stage is necessary and efficient in integrated mosquitos management. During the immature stage, mosquitoes are relatively immobile; remaining more concentrated than they are in the adult stage (Elimam *et al*., 2009; Rutledge *et al*., 2003). Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides. But the extensive use of synthetic organic insecticides during the last five decades have resulted in environmental pollution and also in the development of physiological resistance in major vector species in addition to the increased costs of insecticides. This has necessitated the need for search and development of environmentally safer, low cost, indigenous methods for vector control. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Elimam *et al*., 2009; Mittal and Subbarao, 2003; Rajkumar and Jebanesan, 2005a, b; Promsiri *et al*., 2006). Moreover to application as general toxicant against mosquito larvae, botanical insecticides also have potential uses as growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility, larvicidal, ovicidal and oviposition activity mostly as deterrence (Elimam *et al*., 2009). Several indigenous plants in India and subtropical parts of Asia, such as *Ocimum basilicum*, *Ocimum santum*, *Azadirachta indica*, *Lantana camera*, *Vitex negundo* and *Cleome viscose* (Senthil Nathan *et al*., 2006) were studied for their larvicidal action on the field which collected fourth instar larva of *Culex quinquefasciatus* (Alouani *et al*., 2009). To date, there is no report about the *D. metel* leaf extract against the mosquito. It grows in all the warmer parts of the world and is cultivated worldwide for its chemical and ornamental properties. Thereby, we decided to investigate the larvicidal activity of *A. indica* and *D. metel* leaf extract against the third instar larvae of *C. quinquefasciatus*.

MATERIALS AND METHODS

Collection and rearing of mosquitoes: The egg rafts of *C. quinquefasciatus* were collected from Coovam River (Chetpet) in Chennai, India during 2007. The egg rafts were placed in petridishes (10.5 diameters) containing aged tap water. Larvae were fed with finely ground mixture of yeast and dog biscuits in 3:1 ratio. The pupae were transferred into mosquito cage for emergence. Blood meal from a pigeon was given to adult mosquitoes after three days of emergency. After 3-4 days of blood feeding, adult mosquitoes were provided with petridishes filled with tap water inside the cage for oviposition. The egg rafts were separated and placed in glass petridishes for hatching (Verma and Rahman, 1986).

Preparation of plant extract: The leaves of *A. indica* and *D. metel* were collected from Chetpet area in Chennai, India. The leaves were washed with tap water, shade dried at room temperature and then powdered by using electrical blender. The leaf powder (250 g) of neem and *D. metel* was sequentially extracted with hexane and chloroform after soaking for 48 h in each solvent. The excess solvent in the filtrate was evaporated under reduced pressure in rotary vacuum evaporator. One percent stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 100 mL of acetone. As of the stock, different concentrations viz., 62.5, 125, 250, 500 and 1000 ppm were prepared according to the procedures of WHO (1996).

Larvicidal bioassay: About 50 numbers of late third instar larvae of *C. quinquefasciatus* were introduced into 250 mL of dechlorinated water contained different concentrations of plant extracts in 500 mL of plastic containers. For control, 1.0 mL of acetone dissolved in 249 mL of dechlorinated water was used. Three replicates for each concentration and control were maintained. The mortality counts were made by using Abbott's formula (Abbott, 1925) after 24 h of the treatment and the LC_{50} was calculated by Probit analysis (Finney, 1971).

Statistical analysis: One way Analysis of Variance (ANOVA) was worked out to find out the significance of the treatments. The treatments were separated by Least Significant Difference (LSD) at p = 0.05 level.

RESULTS

The hexane and chloroform leaf extract of *A. indica* and *D. metel* have been studied for use as eco-friendly insecticides instead of eco-enemy synthetic insecticides. Results on the larvicidal effect of leaf extracts were reported in the present study, confirm their potential for control of larval population and management of mature *C. quinquefasciatus* mosquito population. Figure 1 shows the results on percent mortality of larvae. After 24 h of exposure, the 5 different concentrations tested 62.5, 125, 250, 500 and 1000 ppm, the hexane extract of *A. indica* at above concentration produced 24, 36, 55, 64 and 72.50% and *D. metel* produced 09.00, 17.50, 30.00, 42.00 and 57.00% larval mortality, respectively. The chloroform extract produced 12.25, 48.50, 56.50, 73.00 and 87.00% mortality in *A. indica* and 13.75, 27.00, 32.00, 47.00 and 62.00% mortality in *D. metel*, respectively. Among the two plants, the chloroform extract exhibited higher activity. The LC_{50} of hexane and chloroform extracts of *A. indica* was calculated as 246.38 and 198.82 whereas LC_{10} were 17.06 and 34.44 and LC_{90} 3557.71 and 1147.5, respectively. The LC_{50} of hexane and chloroform extracts of *D. metel* was calculated as 709.96 and 562.07 whereas LC_{10} were 65.96 and 38.59 and LC_{90} 7641.69 and 8186.26, respectively (Table 1). No mortality was recorded in the control (data not shown). These results suggest that *D. metel* was less effective than *A. indica* to the larvae of *C. quinquefasciatus*. As the concentration of the plant extracts increased, the total

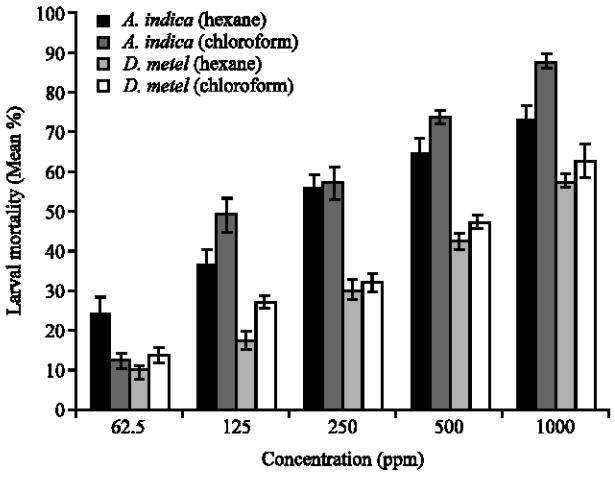

Fig. 1: Larvicidal activity of *A. indica* and *D. metel* leaf extracts against the *C. quinquefasciatus*

Table 1. Median lethal concentration (ppm) of hexane and chloroform extracts of *A. indica* and *D. metel* against *C. quinquefasciatus* larva

Plants	Solvent	LC_{10}	LC_{90}	LC_{50}	Confidence limits for LC_{50}	
					Upper	Lower
Azadirachta indica	Hexane	17.06	3557.71	246.38	314..81	192.51
	Chloroform	34.44	1147.59	198.82	336.88	103.91
Datura metel	Hexane	65.96	7641.69	709.96	1026.60	544.97
	Chloroform	38.59	8186.26	562.07	809.95	430.21

Significant at $p \leq 0.05$ level

larval mortality of the mosquitoes was also found to be increased. In *A. indica*, 125 ppm of chloroform gave a mortality of nearly 50% (48.5%), but *D. metel* extracts needed a concentration of more than 500 ppm (Fig. 1).

DISCUSSION

Today indiscriminate and continuous use of various insecticides has resulted in development of resistance in mosquitoes. Tikar *et al.* (2008) have recorded development of resistance in *A. aegypti* larvae against temephos, fenthion, malathion and DDT from different locations in India. The decrease in insecticidal susceptibility or development of insecticide resistance in populations of *C. quinquefasciatus* has been reported against temephos, fenthion, cypermethrin, α-cypermethrin and λ-cyhalothrin from Bathinda, Bikaner, Jodhpur and Jamnagar in India indicting need of search for some safe and effective alternative eco-safe control measures. Besides development of insecticides resistance, insecticides are toxic to other non-target organisms (Suman *et al.*, 2010). In contract to insecticides, the use of plant products against mosquito control is studied by Mehlhorn *et al.* (2005), Amer and Mehlhorn (2006) and Govindarajan *et al.* (2008) etc. Pharmaceutical companies tend to focus on single active anti-malarial drugs for revenue and profit including those from botanical sources (Rajkumar and Jebanesan, 2009; Burfield and Reekie, 2005), other useful actives in whole botanical extracts may be overlooked. Nowadays, the malarial control programme focused more on the elimination of mosquitoes in larval stage with plant extract. The advantage of targeting larvae is that they cannot escape from their breeding sites until the adult stage and also reduce overall pesticide use in control of adult mosquitoes by aerial application of adulticidal chemicals (Rajkumar and Jebanesan, 2009; Gleiser and Zygadlo, 2007; Senthilkumar *et al.*, 2008). The present study shows the larvicidal activity of *A. indica* and *D. metel* leaf extracts exhibits lethal effects against larvae of *C. quinquefasciatus*. The biological activity of these plant extracts may be due to various compounds, including phenolics, terpenoides, flavonoids and alkaloids existing in plant, these compounds may jointly or independently contribute to produce larvicidal activity against *C. quinquefasciatus*. The obtained results agree with some previous studies.

In the present investigation, the hexane extract of *A. indica* and *D. metel* at 62.5, 125, 250, 500 and 1000 ppm were showed 24, 36, 55, 64 and 72.50% mortality where second one shows 9, 17.50, 30, 42 and 57% mortality, respectively. The chloroform extract of *A. indica* was showed 12, 48.50, 56.50, 73 and 87% mortality where *D. metel* shows 13.75, 27, 32, 47 and 62% mortality, respectively. The hexane and chloroform extract of *A. indica* and *D. metel* had significant larvicidal effect with LC_{50} values were 246.38, 198.82, 709.96 and 562.07 ppm, respectively. At 24 h post-treatment against late third instar larvae, the chloroform extracts of *A. indica* and *D. metel* were found to be more effective than hexane extracts and caused a larval mortality of 87 and

62%, respectively at 1000 ppm concentration. The result is consistent with earlier works by Wandscheer *et al.* (2004) and Tonk *et al.* (2006), who separately reported that neem seed kernel extracts that are effective against mosquitoes were prepared with hexane, ethyl ether, acetone, ethanol and methanol. The most potent larvicide is 0.71% neem seed kernel oil obtained with hexane extract. This dosage is higher than that of Sinniah *et al.* (1994), who reported that 0.02% neem seed oil extracts caused 100% mortality in *Aedes aegypti* and *Culex quinquefasciatus* where the present investigation showed chloroform extracts of *A. indica* and *D. metel* larval mortality of 87 and 62% at 1000 ppm. Ambrose (1995) used much lower concentrations and showed that the larvicidal effects of neem oil on 3rd and 4th instar larvae of *Culex quinquefasciatus* were having the LC_{50} values of 0.99 and 1.20 ppm for neem oil for the two larvae respectively and 0.55 and 0.72 ppm for the deoiled neem cake. The present study shows that the chloroform extract of *A. indica* and *D. metel* had significant larvicidal effect with LC_{50} values were 198.82 and 562.07 ppm, respectively. These variations in susceptibility of extracts/oil to mosquito larvae may be due to variations in extraction solvents, mosquito species or exposure periods (Umar *et al.*, 2002). It reveals as the concentration of the plant extracts increased, the total larval mortality of the mosquitoes was also found to be increased. In *A. indica*, 125 ppm of chloroform gave a mortality of nearly 50% (48.5%), but *D. metel* extracts needed a concentration of more than 500 ppm (Fig. 1).

In comparison with the results of earlier studies, it was noticeable that leaf extract tested in this study exerted promising mosquito larvicidal potential. The mode of action of these leaf extract on mosquito larvae are not known, but previous studies demonstrated that phytochemicals interfered with the proper functioning of mitochondria more specifically at the proton transferring sites (Usta *et al.*, 2002) and other studies by Rey *et al.* (1999) and David *et al.* (2000) found that phytochemicals primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae. Furthermore, 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 (Rajkumar and Jebanesan, 2009; Maurya *et al.*, 2007).

CONCLUSION

In conclusion, leaves extract of *A. indica* and *D. melei* can be suggested as a natural larvicidal for controlling mosquitoes in India. Since, the both plants are economically safe and less expensive to control mosquito and the histological studies to be performed to recognize the mode of action between leaf extract and mosquito.

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