Int. J. Curr. Res. Biosci. Plant Biol. 2015, 2(7): 173-177

EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 7 (July-2015) pp. 173-177

www.ijcrbp.com

Original Research Article

Evaluation of Larvicidal Activity of Botanicals against Lymphatic Filaria Vector, *Culex quinquefasciatus* Say

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A b s t r a c t

Screening of ethanol extracts of five plants *Argemone mexicana* (leaves), *Calotropis procera* (leaves), *Ficus benghalensis* (fruit and leaves), *Lantana camara* (leaves), *Nerium oleander* (leaves and seed) were performed to evaluate larvicidal efficacy against third instar larva of *Culex quinquefasciatus* at concentration ranges of 250ppm, 500ppm, 750ppm and 1000ppm. The dose response data were analyzed with the help of SPSS (v20-64bit) probit analysis software to evaluate LC_{50} and LC_{90}, Chi-square, fiducial upper and lower limits and regression equations. Highest efficacy was observed in the extract of *A. mexicana* leaves with LC_{90} at 292.948ppm while the lowest in leaves of *N. oleander* with LC_{90} at 23741.248ppm. The study also documented the treatment behaviour and morphological changes of treated larvae. Exposure to the extract caused reduction in activity (immobility) of the larvae. Treated larvae stopped feeding after 6 h in 1000ppm of *C. procera, L. camara* and *A. mexicana* extract and after 10 h in *F. benghalensis* fruit extract treatment. Normal feeding was observed after *N. oleander* 1000ppm leaves extract treatment. Detailed examination of dead larvae revealed pore formation**,** degeneration and colour change of the integument.

K e y w o r d s

Culex quinquefasciatus

Filarial vector

Larvicidal efficacy

Plant extracts

Introduction

The vector of lymphatic filariasis, *Culex quinquefasciatus* is a markedly domestic species. It is a strong winged dipteran found in and around human dwellings all over India. Rapid urbanization and industrialization without adequate drainage facilities are responsible for its increased dispersal (Reuben et al., 1988). Lymphatic filaria is the second most common vector borne parasitic disease after malaria and is found in 81 tropical and subtropical countries (Ottesen et al., 1997). Over 120 million people are affected by filariasis; 1.3 billion live at the risk of infection and one third of them live in India (Sabesan et al., 2010). There is no specific treatment for lymphatic filaria and no vaccine has yet been discovered to protect against this disease. The only way to prevent the transmission of parasite *Wuchereria bancrofti* is to combat the vector mosquitoes.

The unrestrained use of chemical insecticides has resulted in loss of efficacy due to development of resistance in vectors along with pollution of soil and water. Further, its effects on non-target organisms has led to disruption of biological control by natural enemies and increased human health concerns (Nivsarkar et al., 2001; Ramoutar et al., 2009). Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals and are biodegradable (Warikoo et al., 2014). Many of these chemicals are selective and have little or no harmful effect on non-target organisms and environment. Although efforts have been undertaken to investigate phytochemicals as potential sources of mosquito control agents (Sukumar et al., 1991; Kumar and Maneemegalai, 2008; Elimam, et al. 2009; Ali and El-Raba, 2010; Lokesh et al., 2010; Rathi and Al-Zubaidi, 2011; Arivoli et al., 2015), there are still a large number of unexplored phytochemicals that can be used as a promising agent to control vector pests. The present study has therefore been undertaken to document larvicidal activity of various parts of five plants - *Calotropis procera, Ficus benghalensis, Nerium oleander, Lantana camara* and *Argemone mexicana* on third instar larva of *Cx. quinquefasciatus.*

Materials and methods

Plant collection

Selected parts of plants (Table 1) were collected from Nagpur city and surrounding areas and were identified by standard taxonomic references (Naik, 1998; Khare, 2007).

Table 1. List of plants selected for larvicidal efficacy study.

Plant name	Family	Part(s) used for extract preparation
Argemone mexicana L.	Papaveraceae	Leaves
Calotropis procera (Aiton) W.T.Aiton	Asclepiadaceae	Leaves
Ficus benghalensis L.	Moraceae	Fruit and Leaves
Lantana camara L.	Verbenaceae	Leaves
Nerium oleander L.	Apocynaceae	Leaves and seed

Collection and rearing of mosquitoes

Mosquitoes were collected from Nagpur city and surrounding areas. The gravid females of *Cx. quinquefasciatus* were separated and reared for egg laying. Hatched larvae were reared in de-chlorinated water (which was changed daily) and fed on finely ground dry yeast powder and reared up to third instar larva.

Preparation of extract

The freshly collected plant material was treated as described by Kumar and Maneemegalai (2008) and Lokesh et al. (2010). The material was washed with distilled water to remove residual dust and shade dried for a week. It was finely grinded in an electric grinder. Fifty grams of finely grinded material was soaked in 200 ml ethanol for a week with intermittent shaking and then filtered and the filtrate was collected. This procedure was repeated twice. The crude extract obtained was concentrated separately by rotary vacuum evaporator at 40ºC to form a paste which was stored at 4ºC in an air tight bottle until use. The obtained paste was dissolved in 5ml of ethanol and solution of 250, 500, 750 and 1000ppm was prepared in dechlorinated water. One milligram of extract in 1ml of water was considered to be the 1000ppm solution.

Larval bioassay

The method adopted by Kumar and Maneemegalai (2008) and Lokesh et al. (2010) was used for this experiment. For larval bioassay the containers were filled with respective grades of plant extract and 20 larvae of third instar were transferred to these containers with the help of a large mouthed glass dropper. The numbers of dead larvae were counted after 24 h of exposure. Three replicates of the experiment were studied. The treatment behaviour was observed after every two hours of treatment. The dead larvae were observed under binocular microscope to record morphological changes.

Statistical analysis

The dose response data from all replicates were pooled and analyzed with the help of SPSS (v20-64bit) probit analysis software to find LC_{50} and LC_{90}. The 95% confidence interval values, Chi-square, fiducial upper and lower limits and regression equations were recorded. The obtained values were analyzed and compared.

Results

The larvicidal activities of ethanol extract of five different plants and their parts namely *A. mexicana* (Leaves), *C. procera* (Leaves), *F. benghalensis* (Fruit and Leaves), *L. camara* (Leaves), *N. oleander* (Leaves and seed) concentration range of 250ppm, 500ppm, 750ppm and 1000ppm on third instar larva of *Cx. quinquefasciatus* after 24 h exposure are represented in Table 2. The analyzed data of dose versus mortality in SPSS probit analysis are represented in Table 3. No mortality was recorded in control. A positive relationship between the level of mortality and the concentration of all the extract applied was observed.

Table 2. Mortality rate of third instar larva *Cx. quinquefasciatus* induced by various plant extracts.

Name of plant		Concentration range in ppm			
		250	500	750	1000
F. benghalensis (fruit)	Average mortality	3.33	11	13	20
	Mortality %	16.66	55	65	100
F. benghalensis (leaves)	Average mortality	2	7.33	12	17
	Mortality %	10	36.66	60	85
N. oleander (seed)	Average mortality	8.33	11.33	19	19.66
	Mortality %	41.66	56.66	95	98.33
N. oleander (leaves)	Average mortality	2.33	2	1.66	2.66
	Mortality %	11.66	10	8.33	16.66
L. camara (leaves)	Average mortality	11.33	19.33	20	20
	Mortality %	56.66	96.66	100	100
A. mexicana (leaves)	Average mortality	16	20	20	20
	Mortality %	80	100	100	100
C. procera (leaves)	Average mortality	10.66	18.33	20	20
	Mortality %	53.33	91.66	100	100

Table 3. Biostatic analysis of mortality rate of third instar larva of *Cx. quinquefasciatus* induced by various plant extracts.

Test product	X^2	LC_{50} (UCL-LCL) (PPM)	LC_{90} (UCL-LCL) (PPM)	Regression Equation
F. benghalensis (fruit)	4.629	468.033*	988.786*	Y=-5.535585+3.945503X
F. benghalensis (leaves)	0.650	590.955 (541.353-644.298)	1305.866 (1127.939-1601.329)	Y=-5.315327+3.721856X
N. oleander (seed)	4.615	325.862*	748.816*	Y=-3.913170+3.546777X
N. oleander (leaves)	0.269	3457.917 (2433.809-6918.491)	23741.248 (10251.839-159900.67)	Y=-0.420586+1.531752X
L. camara (leaves)	0.054	233.750 (204.909-256.654)	386.627 (349.565-449.298)	Y=-8.891149+5.864335X
A. mexicana (leaves)	1.516	160.807 (151.284-169.724)	292.948 (266.306-334.978)	Y=-5.855777+4.920040X
C. procera (leaves)	1.987	201.186 (187.504-216.011)	459.199 (408.784-530.378)	Y=-3.237363+3.575868X

*UCL and LCL not obtained; X^2 –Chi square, UCL- upper confidence limit, LCL- Lower confidence limit.

Prior to assays, larvae were active and exhibited normal feeding. After exposure to the extract of 1000 ppm *i.e.*, highest concentration caused varied abnormal behaviour as described below. After exposure in *F. benghalensis* (fruit) extract, initially the wriggling movement was vigorous, after six hour's the movement become sluggish and after ten hours the larvae stopped feeding and become immobile. Mortality was observed from twelve hours of the treatment. But in *F. benghalensis* (leaves) extract the reaction was little late, slow movement was seen after ten hours and mortality was recorded from the sixteen hours of treatment. In *N. oleander* (seed) extract, sluggishness began from four hours and larvae were struggling to reach surface for respiration, they become immobile after ten hours. Mortality was initiated from sixteen hours of exposure. In *N. oleander* (leaves) extract normal movement and feeding was seen up to ten hours, the larvae become sluggish from twenty hours and 16.66 % mortality was recorded after twenty four hours of treatment. In exposure to *L. camara* (leaves) extract,

the larvae exhibited severe restlessness, after four hours with aggressive self-biting of their anal papillae with their mouth parts. Later they became sluggish and stopped feeding after six hours. Mortality was recorded from eight hours of treatment.

Exposure to *A. mexicana* (leaves) extract initiated irritability and after four hours curved their body to form a ring with coiling movements. After six hours larvae stopped feeding and movement. Mortality was seen from eight hours of the exposure period. Treatment of *C. procera* (leaves) extract exhibited vigorous wriggling movement initially. After four hours the larvae struggled to reach the surface for respiration and became sluggish. After six hours they stopped feeding and were paralyzed. Mortality was recorded from the eight hours of treatment. We noted pore formation, degeneration and change in colour of the integument of treated larvae.

Discussion

The secondary metabolites of plants (steroids, alkaloids, terpenoids, saponins, phenolics, essential oil etc.) are associated with a wide range of biological activities (Arnason et al., 1989) and have been the subject of thorough investigation in an effort to discover new sources of botanical insecticides. All extracts used in this investigation exhibit notable toxicity on third instar larvae of *Cx. quinquefasciatus*.

Govindrajan (2010) used methanol, benzene and acetone solvent extract of *F. benghalensis* leaves against *Cx. quinquefasciatus* and observed $LC_{50/90}$ efficacy for second instar larva in methanol extract 41.43/89.48ppm, in benzene extract-71.61/170.29ppm, in acetone extract 143.02/359.68ppm, for third instar larva in methanol extract 58.21/127.32ppm, in benzene extract 98.55/230.02ppm, in acetone extract 156.27/382.67ppm and for fourth instar larva in methanol extract 74.32/157.66ppm, in benzene extract 104.77/246ppm, in acetone extract 177.17/405.58ppm. Ali and El-Raba (2010) observed that *F. benghalensis* latex is toxic to mosquito larvae. The present study performed toxicity test of leaves and fruit extract of *F. benghalensis* and observed that extract of fruit is more toxic as compared to leaves and exhibited LC_{90} at the concentration of 988.786ppm.

Lokesh et al. (2010) reported mosquito larvicidal efficacy of 20 plants and found a maximum of 84% mortality in 3% aqueous extract of *N. oleander* leaves. Rathi and Al-Zubaidi (2011) evaluated phenolic leaves extract of *N. oleander* against whitefly *Bemisia tabaci* population and observed 82.63% adult mortality at the concentration of 2%. Hexane and aqueous extract of *N. oleander* flowers resulted in LC_{50} at the concentration of 102.54 and 61.11ppm after 24 and 48 h of exposure, while aqueous extract exhibited no significant toxicity (Raveen et al., 2014). Similarly in this study, *N. oleander* leaves revealed poor toxic nature for the third instar larva of *Cx. quinquefasciatus* as compared to seed extract which gave LC_{90} at the concentration of 748.816ppm.

Dua et al. (2010) observed that the LD50 values of the essential oil of *L. camara* treated against *Aedes aegypti*, *Cx. quinquefasciatus*, *Anopheles culicifacies*, *Anopheles fluvialitis* and *Anopheles stephensi* were 0.06, 0.05, 0.05, 0.05 and 0.06mg/cm^2, respectively. Flower extract in methanol showed 100% mortality at the concentration of 2.00mg/ml while leaves extract prepared in ethanol caused 64% mortality of third and fourth instar larvae at the concentration of 3mg (Kumar and Maneemegalai, 2008). In this study the toxicity of leaves in ethanol solvent resulted in LC_{90} value at the concentration of 449.298ppm. The observation of larvicidal efficacy of *A. mexicana* leaves against *Cx. quinquefasciatus* larvae supporting the findings of Karmegam et al. (1997) which reported 100% mortality at the concentration of 250ppm and above after the exposure period of 24 h.

Various parts of *C. procera* have been evaluated against *Cx. quinquefasciatus*. Aqueous leaves extract of *C. procera* evaluated by Elimam et al. (2009) found LC_{50} value at 187.93ppm, 218.21ppm, 264.85ppm, respectively and LC_{90} value of 433.51ppm, 538.27ppm and 769.13ppm respectively for second, third and fourth larval instars of *Cx. quinquefasciatus* while methanol leaves extract gave LC_{50} and LC_{90} at the concentration of 387.93ppm and 630.66ppm, respectively (Shahi et al., 2010).

Ramose et al. (2006) reported that latex extract of *Cx. quinquefasciatus* gave LC_{50} and LC_{90} at the concentration of 86.47 and 973.79ppm, respectively. Latex solution in distilled water caused 100% mortality of third instar larva within 5 minutes of exposure. This study reports 90% mortality in concentration of 459.199ppm for third instar larvae of *Cx. quinquefasciatus*. Although larvicidal effect of extracts from different plants presented wide range intensity, highest efficacy was observed in ethanol extract of *A. mexicana* leaves and lowest in leaves of *N. oleander*.

Acknowledgement

The authors are thankful to Dr. V. R. Zare, Assistant Director Health Services (Malaria) Nagpur and Mr. M. M. Deshpande (Zonal Entomologist), Office of the ADHS (M), Nagpur for the facilities and support. Also the authors are grateful to Dr. S. G. Deshpande, Scientist, National Chemical Laboratory-Pune (MS) for biostatic analysis of data with SPSS.

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