Asian Journal of Agriculture & Life Sciences Vol. 2(4),October 2017: 41-47

Website: www.crsdindia.com/ajals.html e-ISSN: 2455-6149

ORIGINAL ARTICLE

Botanicals as Potential Insecticides against *Culex quinquefasciatus* Say and *Aedes aegypti* Linnaeus

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Received: 2nd July 2017, Revised: 15th September 2017, Accepted: 18th September 2017

ABSTRACT

Aedes aegypti is one of the mosquito species responsible for the transmission of Dengue fever, chikungunya, yellow fever and the worst, dengue hemorrhagic fever while Culex quinquefasciatus vectors Japanese encephalitis (JE) Wuchereria bancrofti and is responsible for several human and animal diseases. World Health Organization stated that about 2/5 of the global human population are currently threaten of dengue and the best way to control the transmission of dengue virus is fight the mosquitoes that cause the disease. Indiscriminate use of several conventional mosquitocidal agents though effective cause several problems to non-target organism including human and affect the ecological balance as well. Thus there is a need to develop an alternative strategy to manage mosquito populations. Biological products like plant extracts are one of the ways to deal with mosquito control. The secondary metabolites of several plants due to their co-evolution with insects are known to have novel Mosquitocidal molecues. The objective of the present study is to evaluate the bioactive potential of Lantena camera, Menthapiperita L. and Eucalyptisgrandis against Culex quinquefasciatus and Aedes aegypti. The leaf extracts were assessed for its larvicidal and repellency activity by standard methods.The highest larvicidal activity was obtained at LC_{50}=96 ppm and 103 ppm against C. quinquifasciates and Aedes aegypti respectively with M.piperita extractwhile 100 % repellency was exhibited by Eucalyptisgrandis extract upto 240 minutes for both the mosquito species. These results reveal that the selected plants have potential to be considered in mosquito control program.

Key words: *Lantena camera, Menthapiperita L., Eucalyptisgrandis, Aedes aegypti, Culex quinquefasciatus, larvicide, repellency*

INTRODUCTION

Mosquitoes are well known vectors which spread several pathogens causing human diseases such as malaria, dengue, filariasis, and several types of encephalitis including West Nile fever (Service, 1993). They are markers of poor sanitation and unhygienic conditions. Mosquito transmitted diseases are the major cause of human death worldwide. No part of the world is free from vector borne diseases (Fradin and Day 2002). About 700 million people suffer from these diseases every year (Taubes 1997). Mosquitoes are recognized vectors of various human diseases in tropical and subtropical countries (Kovendan 2011). In addition to their role as vectors they cause lot of nuisance and conditions like allergic reaction that includes local skin and systemic sensitivity. Global warming, periodic flooding and deforestation have opened new habitats to mosquitoes which show high plasticity in their breeding behavior and readily spread their distribution. In fact, sporadic malaria outbreaks have been reported in non-endemic countries and transmission of the disease was caused by the bite of a locally infected Anopheles species (Zucker, 1996).

More than two billion people, mostly in tropical countries, are at risk from mosquito-borne diseases, such as malaria, dengue, haemorrhagic fever and filariasis (Snow, 2005). The widely and commonly used chemical method though effective, has some major demerits making insect control practically difficult. The major drawbacks with these synthetic insecticides are that they are generally non-biodegradable, toxic to non-targets, and vectors develop resistance against them (Evans and Raj, 1988).In view of the above, it is unavoidable to search for new molecules, which are eco-friendly, cheaper, and safer (Khandagle, *et al*, 2011) Recently, the environmental friendly and biodegradable natural insecticides of plants origin have been receiving attention as an alternative green measure of control of arthropods of public health importance (Nathan, *et al.* 2005).

Compared to other synthetic compounds, natural products are presumed to be safer for human use, justifying therefore a broad search for eco-friendly biological materials to be used for the control of vectors of medical importance. The chemical contents extracted from plant materials can be useful as repellents, larvicides, oviposition attractants, insect growth hormone regulators and deterrent agents (Kilonzo, *et al.* 2001). Plant products have been used in many parts of the world for killing or repelling mosquitoes either as extracts or oils or as whole plant. Many plant extracts have been studied for their efficacy in controlling larvae of different mosquito species (Kumar and Dutta, 1987; Evans and Raj, 1988; Markouk, *et al,* 2000). Plant products can be used, either as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess. A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors, but very few plant products have shown practical utility for mosquito control. Some indigenous plant based products are very promising against mosquitoes and can be used as insecticides and/or repellents. In the present study *Menthapiperita L., Lantana camara* Linn. and *Eucalyptisgrandis*were selected to evaluate their potential as mosquitocideagainst *Culex quinquefasciatus*and *Aedes aegypti.*

Lantana camara Linn. (Fig 1.a) is a flowering ornamental plant belonging to family Verbenaceae. *L. camara* is also known as Lantana, Wild Sage, Surinam Tea Plant, Spanish flag and West Indian lantana. It is a well-known medicinal plant in traditional medicinal system.

L. camara is a low erect or subscandent vigorous shrub with tetrangular stem, stout recurved pickles and a strong odour of black currents. Plant grows up to 1 to 3 meters and it can spread to 2.5 meter in width. Leaves are ovate or ovate oblong, acute or sub-acute, crenate serrate, rugose above, scabrid on both sides. The leaves are 3-8 cm long by 3-6 cm wide and green in colour. Leaves and stem are covered with rough hairs. Small flower held in clusters. Colour usually orange, sometime varying from white to red in various shades and the flower usually change colours as they age (Khare,2007; Kirtikar and Basu 2006; Chopra, *et al.* 1956) Different parts of *L. camara* are reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids, triterpens, sesquiterpenoides and tannin as major phytochemical groups (Ganjewala, *et al* 2009; Kensa, 2011)

M. piperita (Fig 1.b) is commonly called as peppermint. It is widely used in food, cosmetics, and medicines. It has been proven helpful in symptomatic relief of the common cold. It may also decrease symptoms of irritable bowel syndrome and decrease digestive symptoms, such as dyspepsia and nausea. It is used topically as an analgesic and to treat headaches. Peppermint is on FDA's generally recognized as safe list. Its essential oil contains 44 % menthol, 15–30 % methone, and 5 % esters, in addition to various terpenoids. Other compounds found in the peppermint are flavonoids (12 %), polymerized polyphenols (19 %), carotenes, tocopherols, betaine, and choline (Murray 1995).

Eucalyptus grandis (Fig 1.c) attains a height of 45-55 m, usually with an excellent trunk and a wide-spreading, rather thin crown; most of the bark and branches are smooth, white or silvery, sometimes greenish, rough on lower stem, smooth above, debark easily. Juvenile leaves are petiolate, opposite for several pairs then alternate, ovate up to 16 x 8.5 cm, green to dark green and slightly wavy; adult leaves are petiolate, alternate, stalked, lanceolate to broad lanceolate, up to 15x3 cm, green on topside and pale green on underside, slightly wavy, with a long point. Inflorescence axillary and simple, 7 flowered; peduncules flattened, to 1.8 cm long; buds have a bluish bloom. Fruit or seed capsules several, short stalked, pear shaped or conical, slightly narrowed at the rim, thin, 8x6 mm, with whitish waxy coating, narrow sunken disc, and 4-6 pointed, thin teeth, slightly projecting and curved inward, persisting on twigs. (Orwa, *et al,* 2009) The main constituents of the oil of the *E. grandis* are α-Pinene (29.69%), p-Cymene (19.89%), 1,8-cineole (12.80%), α-Terpineol (6.48%), Borneol (3.48%) and 3.14% D-Limonene (Oluwagbemiga, *et al.* 2013). The methanolic extracts of the selected plant leaves were assessed for their larvicidal and repellency potential against the mosquito species.

Fig. 1: (a). *Lantena camera* (b). *Mentha piperita* (c). *Eucalyptis grandis*

(a). *Lantena camera* **(b).** *Mentha piperita* **(c).** *Eucalyptis grandis*

MATERIAL AND METHODS

PREPARATION OF THE PLANT SAMPLES AND EXTRACTION OF PLANTS:

Leaves of the selected plants were dried for four weeks and 50-gram powder were used for extraction through Soxhlet apparatus in 400 ml methanol for about 36 hours and a concentrated solution was obtained. After evaporation of solvent, the extracted compound in dried form was obtained. The extracted compound was stored in air-tight desicator and further used for experiments.

MOSQUITO REARING:

The mosquitoes, *A. aegypti and Culexquinquefasciatus*were reared (as per WHO guidelines) in the Department of Zoology, Prof. Ramkrishna More College, Akurdi Pune -44. Mosquitoes were kept at (28± 2) °C, 75%-85% relative humidity (RH), with a photo period of 12 h light, 12 h dark. The adults were reared in separate metal cages 24"x24"x12" with cotton sleeve at one end to have an easy access to the culture. The larvae were fed on dog biscuits and yeast powder. Adults were provided with 10% sucrose solution and blood meal.

LARVICIDAL BIOASSAY:

The larvicidal activity was performed according to the guidelines for laboratory and field testing of mosquito larvicides published by WHO (who/cds/whopes/gcdpp/2005.13), with minor modifications.

Initially, the mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the extracts under test. Batches of 25 third-instars larvae of the mosquitoeswere placed in a small plastic container with200 ml dechlorinated water small, unhealthy or damaged larvae were removed and replaced. The depth of the water in the containers was between 8 cm and 10 cm. Larval bioassays were carried out using the desired concentration of the extract. Five replicates per concentration and 6 concentrations in the activity range of the extract were used. Larval mortality was recorded after 24 h exposure and was calculated using the following formula-

Percentage mortality = Number of dead larvae / Number of larvae introduced x 100

Results were subjected to statistical analysis.

REPELLENT ACTIVITY:

The repellent activity was evaluated using the human bait technique (WHO 1996; Schreck and Mcgovern 1989). Evaluation was carried out in net cage 30×30×30 cm containing 30 blood-starved female mosquitoes. The volunteer had no contact with lotions, perfumes, oils, or perfume soap on the day of assay. Dose used was 1.0 mg/cm2. The surgical glove with a window of 2 cm×2 cm was used. A 2 cm×2 cm muslin cloth uniformly treated with the test extracts were fixed on window of the glove while for control only the solvent was used to treat the muslin cloth. After every 30 minutes, the hand with the glove was offered to the mosquitoesseperately to check number of bites for 5 min. This was continued till the bites were received. The experiment was repeated five times. Method used for this assay was same for both mosquito species

RESULT AND DISCUSSION

The disadvantages of the conventional chemicals used to control mosquitoes have made it imperative to search for new safer and cheaper methods. Plant products due to their metabolites have always been preferred to be considered as an eco-friendly alternative to conventional chemical method of insect control.

Table 1: Larivicidal activity of plant extracts against *A. aegypti* and *C. quinquefasciatus*

Extract	Mosquito	LC_{50}±SE (ppm)	95% Confidential limit		Regression equation	LC_{90} (ppm)
			LCL	UCL		
Lantena camera,	*A. aegypti*	167±0.77	124	291	Y=3.82X-4.06	297
	C. quinquefasciatus	159±1.05	118	284	Y=4.32X-5.24	269
Menthapiperita	*A. aegypti*	103±0.45	88	143	Y=3.98X-6.76	161
	C. quinquefasciatus	96± 0.67	74	132	Y=6.24X-4.87	130
Eucalyptisgrandis.	*A. aegypti*	121± 0.91	91	162	Y=3.29X-5.22	171
	C. quinquefasciatus	116±0.88	82	152	Y=2.84X-5.77	161

Table 2: Repellent activity of plant extracts against *A. aegypti* and *C. quinquefasciatus*

Extract	Mosquito	Percent Repellency after								
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min
Lantena camera,	*A. aegypti*	100	100	95	87	82	75	70	64	56
	C.quinquefasciatus	100	100	94	89	84	79	73	68	62
Menthapiperita L.	*A. aegypti*	100	100	100	97	91	85	79	73	69
	C.quinquefasciatus	100	100	100	96	90	84	80	76	70
Eucalyptisgrandis	*A. aegypti*	100	100	100	100	100	100	100	100	94
	C.quinquefasciatus	100	100	100	100	100	100	100	100	93

The results of the present study reveal that all the three selected plant extracts have considerable larvicidal activity and repellency. The most larvicidal potential (Table 1) was observed in the extract of *Menthapiperita*against both the mosquito species viz. *Culexquinquefasciatus*and *Aedesa egypti* with LC $_{50}$ value of 96 ppm and 103 ppm respectively, while LC_{50} of 116 ppm and 121 ppm was exhibited by the extract of *Eucalyptisgrandis* against *Culex quinquefasciatus*and *Aedes aegypti* respectively. As compared to the other two extracts the extract of *Lantena camera* showed weaklarvicidal potential viz, LC_{50}= 159 ppm against *Culex quinquefasciatus* and LC_{50}= 167 ppm against *Aedes aegypti*. These results are more significant or at par with the several reported findings, Rahuman and Venkatesan (2008), reported thelarvicidal activity of methanolic leaf extracts of *Cocciniaindica, Momordicacharantia, Trichosanthesanguina,* and*Cucumissativus* against *A. aegypti*, i.e., LC_{50}=309.46, 199.14, 554.20, and 492.73 ppm, respectively. They have also reported larvicidal activity of the same plant extracts against *C. quinquefasciatus*, i.e., LC_{50}=377.69, 207.61, 842.34, and 623.80 ppm, respectively.Ethanol fractionate of *E. crassipes* showed the highest larvicidal and pupicidal activity against *C. quinquefasciatus* with LC_{50} =71.43, 94.68,120.42, 152.15 and 173.35 ppm for I, II, III, IV instars and pupae, respectively (Jayanthi et al 2012).The first- to fourth-instar larvae and pupae of *A. stephensi* had values of LC_{50} = 272.50, 311.40, 361.51, 442.51, and 477.23 ppm, and the LC_{90} = 590.07, 688.81, 789.34, 901.59, and 959.30 ppm; the *A. aegypti*had values of LC_{50} = 300.84, 338.79, 394.69, 470.74, and 542.11 ppm, and the LC_{90} = 646.67, 726.07, 805.49, 892.01, and 991.29 ppm, respectively. (Panneerselvam, *et al.* 2012) Leaf extract of *Vitexnegundo* showed an LC_{50} of 212.57 ppm against the fourth instar larvae of *C. quinquefasciatus* (Kannathasan, *et al.* 2007). Mullai and Jebanesan 2007 have reported larvicidal activity against the third instar larvae of *C. quinquefasciatus* (LC_{50} = 118.74 ppm). Methanol extract of *Jatropha curcas* and *Bacillus thuringiensis israelensis* against I, II, III and IV instar larvae of filarial vector showed promising larvicidal activity (Kovendan, *et al.* 2011). LC_{50}= 456.29 ppm was reported by Gunabalan Madhumitha *et. al.* 2012 against *C. quinquefasciatus*. Sharma, *et al.* (2005) reported that the acetone extract of *Neriumindicum* and *Thujaoriertelis* has been studied with LC_{50} values of 200.87, 127.53, 209.00 and 155.97 ppm against III instar larvae of *A. stephensi* and *C. quinquefasciatus,* respectively. The LC_{50} values of aqueous extract from leaves of *Ricinuscommunis* were 1,091.44; 1,364.58; and 1,445.44 ppm against 2nd, 3rd, and 4th larval instars of *C. quinquefasciatus* (Elimam, *et al.* 2009). Murugan, *et al.* (2012) described the LC_{50} for *C. sinensis*, was determined for the larvicidal and pupicidal activities against mosquito vector species from first to fourth larval instars and pupae the values for *A. stephensi* were 182.24, 227.93, 291.69, 398.00 and 490.84 ppm; *A. aegypti* values were 92.27, 106.60, 204.87, 264.26, 342.45, 436.93 and 497.41 ppm; and *C. quinquefasciatus* values were 244.70, 324.04, 385.32, 452.78 and 530.97 ppm, respectively. Shahi, *et al*. 2010, reported the alcoholic extract of *C. procera* showed to be less toxic than latex in both mosquito species. The LC_{50} values were 109.71 and 387.93 mg/l for *An. stephensi* and *C. quinquefasciatus*, respectively. These figures were 13.06 and 86.47 mg/l respectively for latex of the plant. The 512 ppm concentration of plant extract didn't show a mortality rate >78% in *C. quinquefasciatus* after 24 h. But in the case of *An. stephensi* we observed >95% mortality after 24 h from 256 ppm. Tests with latex showed 99% mortality at 64 ppm for *An. stephensi*, only 44% mortality against *Cx. quinquefasciatus* and a maximum of 67% in 256 ppm.

Gandhi et al 2016, have reported the bioactive potential of both the extract and the isolated compound and upon screening one of the fraction from the methanol extract of *R. cordifolia* showed good mosquitocidal activity against *C. quinquefasciatus* and *A. aegypti*. LC_{50} and LC_{90} values of fraction 2 were 3.53 and 7.26 ppm for *C. quinquefasciatus* and 3.86 and 8.28 ppm for *A. aegypti* larvae, and 3.76 and 7.50 ppm for *C. quinquefasciatus* and 3.92 and 8.05 ppm for *A. aegypti* pupae, respectively. Further, the isolated compound alizarin presented good larvicidal and pupicidal activities. LC_{50} and LC_{90} values of alizarin for larvae were 0.81 and 3.86 ppm against *C. quinquefasciatus* and 1.31 and 6.04 ppm for *A. aegypti*larvae, respectively. Similarly, the LC_{50} and LC_{90} values of alizarin for pupae were 1.97 and 4.79 ppm for *C. quinquefasciatus* and 2.05 and 5.59 ppm for *A. aegypti* pupae, respectively. The highest larval mortality was found in the hexane extract of *Z. zerumbet*, ethyl acetate extract of *D. biflorus*, and methanol extracts of *A. indica* against *C. gelidus* (LC_{50} = 26.48, 33.02, and 12.47 ppm; LC_{90} = 127.73, 128.79, and 62.33 ppm) and against *C. quinquefasciatus* (LC_{50} = 69.18, 34.76, and 25.60 ppm; LC_{90} = 324.40, 172.78, and 105.52 ppm), respectively, after 24 h (Kamaraj, *et al.* 2010).

Table 2 depicts the repellency potential of the extracts of the selected plants against both the mosquito species and the obtained results show that *Eucalyptisgrandis*extract has 100 % repellency upto 240 minutes against both mosquito species. The maximum repellent activity was observed at 450 ppm in ethanol extracts of *C. sinensis* and the mean complete protection time ranged from 150 to 180 min. The ethanol extract of *C. sinensis* showed 100 % repellencyupto 150 min and showed complete protection upto 90 min at 350 ppm against *A. stephensi, A. aegypti*and *C. quinquefasciatus,* respectively (Kadarkarai Murugan, *et al.* 2012).Amer and Mehlhorn, 2006, have reported that the five most effective oils were those of Litsea (*Litseacubeba*), Cajeput (*Melaleuca leucadendron*), Niaouli (*Melaleuca quinquenervia*), Violet (*Viola odorata*), and Catnip (*Nepetacataria*), which induced a protection time of 8 h at the maximum and a 100% repellency against *A. aegypti*, *An. stephensi,* and *C. quinquefasciatus*. The essential oil of *Zingiber officinalis* showed repellent activity at 4.0 mg/cm2 which provided 100% protection up to 120 min against *C. Quinquefasciatus* (Pushpanathan, *et al.* 2008)

Effiom, *et al.* 2012, reported the mosquito repellent activity of diethyl ether extracts from Peels of five citrus fruit species, *Citrus sinensis, Citrus limonum, Citrus aurantifolia, Citrus reticulata*and *Citrus vitis,* at five different concentrations, 5%, 10%, 15%, 20% and 25%. Topical application revealed that 20% and 25% repelled mosquitoes 2 hours and 5 hours, respectively. At a dose of 0.1 mg/cm2, potent repellency against mosquito adults was obtained with the extracts of *Cinnamomum cassia* Blume bark (9l%), *Nardostachyschinensis* Batalin rhizome (81%), *Paeoniasuffruticosa* Andrews root bark (80 %), and *C. camphora* steam distillate (94 %). *Eugenia caryophyllata* Thunb extract provided 75 % repellency (Young, *et al*. 2004)

Kamaraj et al 2011 reported for maximum repellent activity at 500 ppm in methanol extracts of *N. nucifera*, ethyl acetate and methanol extract of *P. nigrum* and methanol extract of *T. ammi* the mean complete protection time ranged from 30 to 150 min with the different extracts tested. These plants certainly have bioactive potential that can be further explored with different field trials. Toxicological tests should also be done before including them in the mosquito control strategy. These extracts may also be used in combinatorial way to step up the larvicidal potential.

Though the knockdown effect of synthetic chemicals is remarkable but they bring irreversible environmental hazard, severe side effects and pernicious toxicity to human being and beneficial organisms. In the light of the recognized demerits of the chemical control method, emphasis on controlling mosquito vectors has shifted steadily from the use of conventional chemicals toward alternative insecticides that are target-specific, biodegradable, and environmentally safe. Recently, use of environment friendly and biodegradable natural insecticides of plant origin have established renewed consideration as agents for vector control as they are rich in bioactive chemicals, active against a limited number of species including specific target insects. Among the biopesticides in practice plant extracts and essential oils are of choice and great help to control mosquitoes. These results indicate the potential of the plant extracts and their possibility to be included in the mosquito management program. It can also be a part of IPM practice to control mosquitoes. Further exploration of these plants for their active principle and insecticidal potential even against other vectors can be thought of. With further field trials and toxicity tests these extracts may be a helpful addition to the mosquito control strategy.

ACKNOWLEDGEMEMT

The authors wish to thank the Principal of the college, Dr. M.G. Chaskar for making the infrastructure available for this work. I also wish to thank my colleagues to support me throughout the period of the experimentation.

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