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Mosquito larvicidal properties of *Impatiens balsamina* (Balsaminaceae) against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)

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PEER REVIEW

Peer reviewer

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Comments

The research work is very much important to the society to control morbidity and other defects caused by mosquitoes and other insect vectors as well. This work reports a novel approach for the control of vector mosquitoes.
Details on Page 224

ABSTRACT

Objective: To assess the larvicidal potential of the crude benzene, chloroform, ethyl acetate and methanol solvent extracts of the medicinal plant *Impatiens balsamina* against *Anopheles stephensi (An. stephensi)*, *Aedes aegypti (Ae. aegypti)* and *Culex quinquefasciatus (Cx. quinquefasciatus)*.
Methods: Twenty five third instar larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to various concentrations and were assayed in the laboratory by using the protocol recommended by WHO. The larval mortality was observed after 24 h of treatment.
Results: Among extracts tested, the highest larvicidal activity was observed in leaf methanol extract of *Impatiens balsamina* against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with the LC_{50} and LC_{90} values 98.04, 119.68, 125.06 and 172.93, 210.14, 220.60 mg/L, respectively.
Conclusions: From the results it can be concluded that the larvicidal effect of *Impatiens balsamina* against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* make this plant product promising as an alternative to synthetic insecticide in mosquito control programs.

KEYWORDS

Impatiens balsamina, Larvicidal activity, *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus*

1. Introduction

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. World Health Organization has declared the mosquitoes as 'public enemy number one'[1]. They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya ferver, filariasis, encephalitis, West Nile virus infection, *etc*. in almost all tropical and subtropical countries and many other parts of the world. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides such as organophosphates (*e.g*. temephos and fenthion), insect growth regulators (*e.g*. diflubenzuron and methoprene), *etc*. Although effective, the repeated use of these insecticides has disrupted natural biological control systems and sometimes resulted in the widespread development of resistance. These problems have warranted the need for developing alternative strategies using eco-friendly products[2]. These steadily growing problems demand an intensive search for new products that are environmentally safe, target specific and degradable. The above facts prompted us to undertake investigations of some plant species traditionally used as insecticidal agents, as well as other endangered plant species, with the aim of identifying lead compounds for the development of new plant based insecticidal agents[3].

Impatiens balsamina L. (*I. balsamina*) is a plant belonging to the family Balsaminaceae. *I. balsamina* has been traditionally used for the treatment of thorn or glass-puncture wounds, abscesses, ingrown nails and chronic ulcers caused by allergic reaction of detergents. The groups of compounds commonly found in this plant are naphthoquinones, coumarins, phenolic acids, flavonoids, anthocyanidins and steroids. Flavonoids and naphthoquinones have strong antimicrobial, anti-anaphylaxis, anti-allergic and anti-inflammatory

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activities[4]. As far as the literature survey we ascertain, no information was available on the larvicidal activity of the experimental plant species given here against *Anopheles stephensi (An. stephensi), Aedes aegypti (Ae. aegypti) and Culex quinquefasciatus (Cx. quinquefasciatus)*. Therefore, the present study was carried out to determine the larvicidal efficacy of *I. balsamina* leaves extract against malaria, dengue and filariasis vector mosquitoes.

2. Materials and methods

2.1. Collection of plants

The leaves of *I. balsamina* were collected in and around Gingee (12°25′N, 79°41′E), Tamil Nadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of Plant Phytochemistry Division, Department of Zoology, and Annamalai University, India.

2.2. Extraction

The dried leaves (1 kg) were extracted with four different solvents, namely, benzene, chloroform, ethyl acetate and methanol (500 mL), individually and the extract was evaporated in a rotary vacuum evaporator. Standard stock solutions were prepared at 1% by dissolving the residues in ethanol, which was used for the larvicidal bioassay.

2.3. Mosquitoes

Cx. quinquefasciatus, *Ae. aegypti* and *An. Stephensi* were reared in the Vector Control Laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the ratio 3:1. Adults were provided with 10% sucrose solution and membrane feeding on goat blood. Mosquitoes were held at (28±2) °C, 70%-85% relative humidity with a photoperiod of 12 h light : 12 h dark.

2.4. Larvicidal activity

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by World Health Organization[5]. Batches of 25 third instar larvae were transferred to a small disposable paper cups, each containing 200 mL of water. The appropriate volume of dilution was added to 200 mL water in the cups to obtain the desired target dosage, starting with the lowest concentration (40-350 mg/L). Four replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. To this, 1 mL of ethanol was added. The lethal concentration that kills 50 percent of the exposed larvae (LC_{50}) and lethal concentration that kills 90 per cent of the exposed larvae (LC_{90}) were calculated after 24 h by probit analysis[6].

3. Results

The results of the larvicidal activity of crude benzene, chloroform, ethyl acetate, and methanol solvent extracts of leaf of *I. balsamina* against the larvae of three important vector mosquitoes, *viz.*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* are presented in Table 1. Among extracts tested, the highest larvicidal activity was observed in leaf methanol extract of *I. balsamina* against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, the LC_{50} and LC_{90} values were 98.04, 119.68, 125.06 and 172.93, 210.14, 220.60 mg/L, respectively.

4. Discussion

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friendly in nature. Phytochemicals may serve as eco-friendly insecticide because they are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activity in many parts of the world. Our result showed that the crude benzene, chloroform, ethyl acetate and methanol solvent extracts of leaf of *I. balsamina* have significant larvicidal properties against three vector mosquitos *viz.*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. This result is also comparable to earlier reports of the LC_{50} values of benzene, hexane, ethyl acetate, methanol, and chloroform extract of *Eclipta alba* against early third-instar larvae of *Ae. aegypti* were 151.38, 165.10, 154.88, 127.64, and 146.28 mg/L, respectively[7], and for the larvicidal efficacy of benzene, hexane, ethyl acetate, methanol, and chloroform leaf extract of *Cardiospermum halicacabum* against *Cx. quinquefasciatus* and *Ae. aegypti*, the LC_{50} values were 174.24, 193.31, 183.36, 150.44, 154.95 and 182.51, 200.02, 192.31, 156.80, and 164.54 mg/L, respectively[8]. The LC_{50} values of *Ficus benghalensis*

Table 1
Larvicidal activity of different solvent leaf extract of *I. balsamina* against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

Mosquito species	Solvent used	LC_{50} (mg/L) (LCL-UCL)	LC_{90} (mg/L) (LCL-UCL)	Slope	Regression equation	χ^2 (df)
An. stephensi	Benzene	134.21 (112.99-155.96)	232.23 (202.83-280.88)	2.015	Y=-0.538+0.381x	9.612*(4)
	Chloroform	122.91 (98.94-146.48)	221.09 (190.19-274.54)	2.230	Y=3.071+0.382x	11.547*(4)
	Ethyl acetate	110.47 (77.41-141.23)	205.73 (169.11-281.71)	2.495	Y=6.886+0.383x	19.647*(4)
	Methanol	98.04 (81.09-114.74)	172.93 (150.83-209.03)	2.110	Y=2.038+0.489x	9.814*(4)
Ae. aegypti	Benzene	157.18 (130.92-183.70)	275.98 (240.24-335.60)	2.090	Y=0.752+0.317x	9.927*(4)
	Chloroform	141.24 (110.27-171.05)	255.99 (217.98-324.11)	2.280	Y=4.300+0.322x	13.305*(4)
	Ethyl acetate	129.24 (91.24-164.52)	237.73 (196.06-322.69)	2.395	Y=6.900+0.326x	19.320*(4)
	Methanol	119.68 (97.86-141.03)	210.14 (182.45-256.17)	2.090	Y=2.262+0.397x	10.708*(4)
Cx. quinquefasciatus	Benzene	169.68 (139.52-199.22)	303.21(264.00-367.35)	2.190	Y=2.905+0.277x	9.826*(4)
	Chloroform	155.84 (129.49-182.45)	272.98 (237.39-322.53)	2.070	Y=0.671+0.319x	10.173*(4)
	Ethyl acetate	136.54 (104.17-167.39)	249.10 (210.57-319.90)	2.330	Y=5.248+0.324x	14.535*(4)
	Methanol	125.06 (103.69-146.26)	220.60 (192.35-266.93)	2.110	Y=1.657+0.388x	9.791*(4)

UCL: upper confidence limit, LCL: lower confidence Limit; χ^2: *Chi* square, * : Significant at P<0.05, df: degrees of freedom.

against early second, third, and fourth instar larvae of *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* were 41.43, 58.21, and 74.32 mg/L; 56.54, 70.29, and 80.85 mg/L; and 60.44,7 6.41, and 89.55 mg/L, respectively[9]. The adulticidal activity of the essential oil of *Lantana camara* was evaluated against different mosquitoes species on 0.208 mg/cm^2 impregnated papers, the knockdown time 50% and 90% values of the essential oil were 20, 18, 15, 12, 14 min and 35, 28, 25, 18, 23 min against *Ae. aegypti*, *Cx. quinquefasciatus*, *Anopheles culicifacies*, *Anopheles fluvialitis*, and *An. stephensi* with their percent mortality of 93.3%, 95.2%, 100%, 100%, and 100%, respectively[10]. The LC_{50} values of extracts of medicinal plants *Plumbago zeylanica* and *Cestrum nocturnum* against *Ae. aegypti* were less than 50 mg/L. The larvicidal stability of the extracts at five constant temperatures (19 °C, 22 °C, 25 °C, 28 °C and 31 °C) evaluated against fourth instars larvae revealed that toxicity of both plant extracts increases with increase in temperature[11]. Compared with earlier reports, our results revealed that the experimental plant extracts were effective to control *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. From these results, it was concluded that the plant *I. balsamina* exhibits larvicidal activity against three important vector mosquitoes. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, and Japanese encephalitis. Mosquitoes also cause allergic responses in humans, including local skin and systemic reactions such as angioedema. This study was carried out to investigate the efficacy of *I. balsamina* against larvae of three important mosquitoes in the laboratory.

Research frontiers

This study aimed to determine the control of vector mosquito against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* by the plant *I. balsamina*.

Related reports

Numbers of scientists have worked (Senthilkumar, 2009; Elango *et al*., 2011) on the mosquito control using plant products. Some researcher also reported that toxicity of some plant extracts increases with increase in temperature.

Innovations and breakthroughs

Control of rural vector mosquitoes is an important issue. The author use plan products as a natural insecticide without causing any percentage of destruction to environment, which is very important to the society.

Applications

The findings of the present study provide eco-friendly approaches to control vector mosquito of Dengue, malaria, and fillariasis.

Peer review

The research work is very important to the society to control morbidity and other defects caused by mosquitoes and other insect vectors as well. This work reports a novel approach for the control of vector mosquitoes.

References

[1] World Health Organization. Report of the WHO informal consultation on the evaluation on the testing of insecticides. Geneva: World Health Organization; 1996. [Online] Available from: http://whqlibdoc.who.int/hq/1996/CTD_WHOPES_IC_96.1.pdf. [Accessed on 16 November, 2013].

[2] Govindarajan M. Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes. *Asian Pac J Trop Med* 2010; **3**(9): 691-695.

[3] Govindarajan M, Sivakumar R, Rajeswary M, Yogalakshmi K. Adulticidal activity of *Pithecellobium dulce* (Roxb) Benth against *Culex quinquefasciatus* (Say). *Asian Pac J Trop Dis* 2012; **2**(2): 124-128.

[4] Oku H, Ishiguro K. Cyclooxygenase-2 inhibitory 1,4-naphthoquinones from *Impatiens balsamina* L. *Biol Pharm Bull* 2002; **25**: 658-660.

[5] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005. [Online] Available from: http://whqlibdoc.who.int/hq/2005/who_cds_whopes_gcdpp_2005.13.pdf. [Accessed on 16 November, 2013].

[6] Finney DJ. *Probit analysis*. London: Cambridge University Press 1971, p. 68-72.

[7] Govindarajan M, Karuppannan P. Mosquito larvicidal and ovicidal properties of *Eclipta alba* (L.) Hassk (Asteraceae) against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**: 24-28.

[8] Govindarajan M. Mosquito larvicidal and ovicidal activity of *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) leaf extract against *Culex quinquefasciatus* (say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci* 2011; **15**(7): 787-794.

[9] Govindarajan M. Larvicidal efficacy of *Ficus benghalensis* L. plant leaf extracts against *Culex quinquefasciatus* Say, *Aedes aegypti* L. and *Anopheles stephensi* L. (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci* 2010; **14**(2): 107-111.

[10] Dua VK, Pandey AC, Dash AP. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian J Med Res* 2010; **131**: 434-439.

[11] Patil CD, Patil SV, Salunke BK, Salunkhe RB. Bioefficacy of *Plumbago zeylanica* (Plumbaginaceae) and *Cestrum nocturnum* (Solanaceae) plant extracts against *Aedes aegypti* (Diptera: Culicide) and nontarget fish *Poecilia reticulata*. *Parasitol Res* 2011; **108**(5): 1253-1263.