International Journal of Bioassays
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Original Research Article

The larvicidal activity of *Indigofera arrecta* leaf extract against culex mosquito larvae

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Received for publication: September 04, 2014; **Revised:** September 18, 2014; **Accepted:** November 13, 2014

Abstract: The present study was done to assess the larvicidal activity of the Java indigo plant against the larvae of culex mosquito. The plant leaves were extracted using methanol and water (9:1). After extraction a bioassay was conducted to test its activity in the larva of culex mosquito. The mortality data was subjected to probit analysis to determine the lethal concentration. The Java indigo leaf extract can be used as a natural insecticide control against the Culex mosquito larvae.

Key Words: Larvicidal activity, *Indigofera arrecta*, culex

INTRODUCTION

Mosquitoes transmit several public health problems, such as malaria, filariasis, and dengue causing millions of deaths every year (Vatandoost H, 2001). Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat. The use of herbal products is one of the best alternatives for mosquito control (Nandita C, 2008).

Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against a number of species including specific target-insects and are eco-friendly. Traditionally plant based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. These bioactive chemicals may act as insecticides, anti-feedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, anti-moulting hormones as well as attractants. Plant based pesticides are less toxic, delay the development of resistance and are easily biodegradable (Srinivasan *et al.,* 2013)

Plant based products do not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant derived compounds, such as saponins, steroids, flavonoids, essential oils, alkaloids and tannins, are potential mosquito larvicides. Plant's secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitoes biodegradable (Srinivasan *et al.,* 2013).

Secondary metabolites from plants have been explored for mosquito larvicidal properties. Indigofera species are known to contain secondary metabolites such as flavonoids, glycosides, saponins and steroids which are known to be active against mosquito larva.

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However, there is no known mode of metabolite activity on the mosquito larvae. In this study, the plant *Indigofera arrecta*, a member of *indigofera* species, was selected for the larvicidal activity against the culex mosquito. The *Indigofera arrecta* is commonly known as the java indigo, a member of leguminosae family. The plant is an erect, woody, large shrub up to 3m tall. The leaves are used in traditional medicines for epilepsy and nervous disorders and to heal sores and ulcers. It is also a soil improver whereby it is also used as a cover crop and a green manure. (Orwa C, 2009)

MATERIALS AND METHODS

Area of study

The research was conducted within the University of Eastern Africa, Baraton.

Plant and specimen collection

The leaves of Java indigo were collected from the nature preserve of the University. The leaves were dried at room temperature, grounded into fine powder and kept in transparent polythene bags.

The larvae of Culex mosquitoes were collected with the help of a dropper from stagnant water found at the University. They were transferred into the lab and cleaned several times with distilled water.

Leaf extract and phytochemical screening

The powdered leaves of Java indigo were placed in a conical flask. Then methanol: water solvent (9:1) was added until the powdered leaves were completely submerged in the solvent. The mixture was agitated for thorough mixing for twenty-four hours on a shaker for effective extraction of the plant components. The extract was filtered, evaporated and brought to dryness using a vacuum and pressure pump at room temperature. The dried crude extract was stored in a refrigerator at 4 C.

Larvicidal bioassay

A stock solution of the extract was prepared in distilled water. From the stock solution, 100 ml each of 2000, 1000, 500 and 250ppm were prepared in different beakers and labeled accordingly. For bioassay of each extract, 10 larvae were transferred to each beaker using a dropper. Larvae were fed with a diet of yeast and biscuits. A control was maintained by adding only larval food and 1ml of acetone. The numbers of dead larvae were counted after 24, 48 and 72 hours of exposure (WHO, 2005).

Data analysis

Data was taken for analysis using Log dosage-probit mortality regression line computer software program to calculate the lethal concentration.

$$\text{Percentage mortality} = \frac{n}{m} \times 100$$

Where

n= is the average number of all dead larvae in the replicates

m= number of all larvae introduced into the experiment

RESULTS

The overall objective of the present study was to determine the ability of leaf extract *Indigofera arrecta* inhibit the survival of the mosquito larvae. Larvicidal bioassay was performed with the extract of the leaves of *Indigofera arrecta* against the larvae of culex mosquito.

The data was analyzed by computer using the probit software program. The results of this study had two parts. Part 1 was the phytochemical screening which was done to test the phytochemical compounds in the plant. Part 2 contained the bioassay which was tested against the mosquito larvae. Data were analyzed descriptively.

Table 1: Phytochemical screening of the plant *Indigofera arrecta*

Phytochemicals	Observation	Inferences
Tannins	Green color	Present
Saponins	stable foam	Present
Flavonoids	Yellow color	Present
Terpenoids	Grey color	Present
Glycosides	Brown color	Present
Alkaloids	Ppt formed	Present
Steroids	Blue green ring formed	Present
Phenols	No color change	Absent

The table above shows phytochemicals present in the methanol water extract of the leaves of *Indigofera arrecta.* The leaves contain tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids and steroids.

Table 2: Mortality rates of mosquito larvae

Concentration (mg/l)	Percentage mortality of the larvae at an interval of 24 hours (%)		
	24	48	72
5000	70	100	100
2500	40	60	90
1250	30	50	70
625	20	40	60
Control	0	0	0

The table above shows that the *Indigofera arrecta* leaves were found to have toxicity against culex larvae with the percentage mortality of 70% in a period of 24 hours at a concentration of 5000mg/l, 40% at a concentration of 2500mg/l, 30% at a concentration 1250mg/l, and 20% at a concentration of 625mg/l. The plant showed increased mortality after 48 hours with highest after this period being 100% at a concentration of 5000mg/l, 60% at a concentration of 2500mg/l, 50% at a concentration of 1250mg/l and 40% at a concentration of 625mg/l. After 72 hours the plant showed the highest larvicidal activity as compared to preceding periods, However the data recorded at a concentration of 5000mg/l was similar to that recorded at the same concentration after 48 hours since all the larvae had died after the later period. The highest activity after 72 hours was recorded at a concentration of 5000mg/l and the lowest recorded at a concentration of 625mg/l.

Table 3: Statistical analysis using Probit software Table shows the lethal concentration

Observation Period	LC_{50}	LC_{90}
24 Hours	2752.6206	20630.3242
48 Hours	1097.5875	7823.8955
72 Hours	551.2507	2290.4746

The table above shows the statistical analysis of the raw data where by the LC_{50} after 24 hours were 2752.6 and LC_{90} 206330.3. The LC_{50} after 48hours was found to be 1097.6 and LC_{90} was 7823.9. The LC_{50} after 72 hours was found to be 551.3 and LC_{90} was 2290.5.

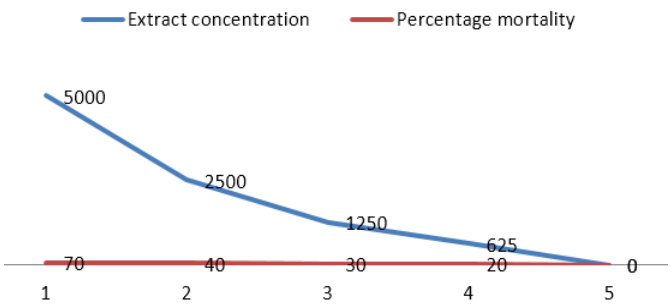

Figure 1: Graphs for extract concentration against percentage mortality after 24, 48 and 72 hours

The figure below shows that the effect of the plant against the mosquito larvae is directly proportional to

the concentration of the extract. From the graphs above shows that the *indigofera arrecta* leaves extract showed dose dependent increase in larvicidal activity against the larvae of culex mosquito since the percentage mortality was observed to increase with increasing concentration of plant extract.

The increase of percentage mortality of the treated mosquito larvae is supported by the presence of phytochemicals in the plant extract which have insecticidal properties.

DISCUSSION

Mosquito are the vector for large number of human pathogens. They cause borne diseases that causes high levels of morbidity and mortality. Mosquitoes are the most deadly vectors for these disease causing organisms. Plant derived natural products have traditionally been used against mosquitoes Aziz *et al.,* (2013)

According to Pedro *et al.,* (2014) phytochemicals of plants serve as storage of compounds that have biological action. Alkaloids, flavonoid, tannins and saponins are known to possess medicinal and pesticidal properties and are also responsible for insecticidal and toxicity to other animals.

Phytochemicals may serve as a suitable alternative to synthetic insecticides since they are safe and are readily available. The screening of locally available medicinal plants for mosquito control would reduce dependence on expensive imported products Aziz *et al.,* (2013)

Researchers have come up with more selective modes of action and reduced risks for non-target organisms and the environment. Progress has been made in the development of natural products capable of interfering with the process of growth, development and metamorphosis of the target species Aziz *et al.,* (2013)

Phytochemicals present in the *I. arrecta* leaf extract could show larvicidal action of mosquito larvae as supported by the previous study on the larvicidal activity of selected plants extracts against *Aedes aegypti* mosquito. The derivates of three families tested, (meliaceae, annonacease and solanaceae) showed wide range of mode of action standing out the insecticidal and anti-feeding activity in the meliaceae family and insecticide in the annonacease and solanaceae families. (Luis ES, 2010)

Indigofera suffruticosa a plant of the same family with *I. arrecta* was tested for the oviposition and embryo toxicity on early development of *Aedes aegypti*. The results showed repellent activity, specific embryo toxicity, and general growth retardation in *A. aegypti* (Vieira, 2012)

According to Govindarajan (2013), different parts of plants contain a complex of chemicals with unique biological activities which are thought to be due to toxins and secondary metabolites that act as self-defense agents.

It was noted that the percentage mortality increased with increasing concentrations of the leaf extract. The mortality of mosquito larvae was also inc34reased in relation to time of exposure. The mortality of the mosquito larvae was not due the solvent used during extraction instead it was due to the toxic compound found in the plant. According to Pedro. (2014), higher concentration of the plant extracts would lead to greater number of mortality in the mosquito larvae and not the use of methanol as the extracting solvent.

Results of the present study were found to be effective against the culex mosquito larvae. The presence of secondary metabolites can be attributed to the plant extract killing agent against mosquito larvae which is compatible with a study conducted by (Rawani *et al.,* 2009) that revealed the presence of phytochemicals that are accountable for their larvicidal efficacy potential.

CONCLUSION

This study revealed that *Indigofera arrecta* plant leaves extract contained phytochemicals compounds such as tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids and steroids that are responsible for larvicidal activities of insects. The plant leaves showed larvicidal activity to culex mosquito larvae as seen in higher concentration with high percentage mortality.

RECOMMENDATION

Further study needs to be done to get the specific compounds which are toxic to the larvae and also their mode of action against the larvae.

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Cite this article as:
Raheli Neema M, Ojunga M and Ramesh F. The larvicidal activity of I*ndigofera arrecta* leaf extract against culex mosquito larvae. International Journal of Bioassays, 2015, 4 (01): 3666-3669.

Source of support: Nil
Conflict of interest: None Declared