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International Journal of Current Research
Vol. 10, Issue, 02, pp.65303-65306, February, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

LARVICIDAL EFFICACY OF *AZADIRACHTA INDICA* AGAINST *ANOPHELES* MOSQUITO LARVAE

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ARTICLE INFO

Article History:
Received 27th November, 2017
Received in revised form
18th December, 2017
Accepted 06th January, 2018
Published online 28th February, 2018

Key words:
Anopheles,
Azadirachta indica,
Extracts, Larvicidal,
Mosquito, Jalingo.

ABSTRACT

The aim of this study was to test the larvicidal efficacy of stem and leaf extracts of *Azadirachta indica* on *Anopheles* mosquito larvae. Leaf and stem of *A.indica* were pulverized and sieved. The sieved stem and leaf were extracted with methanol and water respectively and evaporated in a Rotary evaporator. The extracts were tested against *Anopheles* mosquito larvae in 24 hours each in replicate of 24 to 100mg/l,150mg/l,200mg/l and 250mg/l. The percentage mortality of methanol extract of stem bark at 86.6%at 250mg/lwhile methanol extract of leaf was96.5% at 250mg/l. The LC_{50} of the methanol leave was the lowest at 1.031mgl^{-1} and LC_{90} of 3.56mgl^{-1} with a relatively more toxic characteristic. This shows that *A. indica* has high mortality potentials on the larvae of *Anopheles* mosquito and hence can be used to control malaria vectors.

Citation: Garba, L. C., Chintem, D. G. W., Anuye, R. J. and Joro, A. A. 2018. "Larvicidal efficacy of *Azadirachta indica* against *Anopheles* mosquito larvae", *International Journal of Current Research*, 10, (02), 65303-65306.

INTRODUCTION

Malaria is a protozoa disease and is transmitted from one person to another through the bite of infected female *Anopheles* mosquito vectors during blood meal (Abbey, 2010). Among the approximately 4000 known mosquito species, less than 10% are regarded as efficient vectors of pathogenic agents of infectious diseases with high impact on human welfare and health. Mosquito-transmitted diseases remain a major cause of the loss of human life worldwide with more than 700 million people suffering from these diseases annually (Igweh, 2012).The high burden of malaria is a significant drawback of economic and social development in endemic countries.The 2015 goals of the (WHO) Roll back malaria partnership are to reduce global malaria cases by 75% and to reduce the malaria deaths to nearly zero percent through universal coverage by effective prevention and treatment interventions. The attempt to prevent malaria through other anti-malaria drugs and insecticides is threatened due to the emergence and spread of drug resistant malaria parasite and insecticide resistant mosquito vectors (Afolabi, 2004) thus, a search for ecologically friendly and biodegradable. The most essential medical value of plant extracts is due to the presence of certain bioactive substances such as alkanoids, glycerides, resin, volatile oil, saponin and tannin (Grainage, 1988).

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Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities. The use of different parts of locally available plants and their various products in the control of mosquitoes have been well established globally by numerous researchers. The larvicidal properties of indigenous plants have been documented in many parts of India along with the repellent and anti- juvenile hormones activities (Singh, 2003). Plant extracts are safer for non-target organisms including man, therefore, plant based formulations would be more feasible from environmental perspective than synthetic insecticides (Bhat, 2009). Many plant species have been found to contain relatively high amount of secondary metabolites with probable insecticidal repellent, antifeedantor insect growth regulatory activity. However, there is no record of such studies in Jalingo this therefore, necessitates this study. The present investigation was aimed to study the larvicidal efficacy of *A.indica* extracts on *Anopheles* mosquito species and to compare the performance of extract from different parts of the plant.

MATERIALS AND METHODS

Study area

The study was conducted in the laboratory of Department of Biological Sciences, Taraba State University, Jalingo main campus.

It lies roughly between latitude 6° 30° and 9° 36° north and longitude 9° 10° 50° east. The Location covers a land area of about 60, 291km^2 with a population of about 2,300,736.

Plant Collection and Preparation of Plant Sample

Leaves and stem bark of *A. indica* were collected at the Taraba State University campus areaand air dried at room temperature. The dried leaf and stem materials were pounded to powder using a mortar and pestle. About l00g of the plant powdered materials were suspended in 800ml of methanol and water for 48 hours. A rotary evaporator was used to remove excess solvents from each extract and further expose to air at room temperature for the extract to solidify. Residues of each extract were labeled appropriately and stored at room temperature prior to use.

Preparation of Test Solution

Different test concentration of extracts from leaves and stem bark of *A. indica* were prepared into 100mg/l, 150mg/l, 200mg/l and 250mg/l and 100ml/l of distilled water was also prepared as control.

Collection of the mosquito larvae

A dipper was used to collect *Anopheles* mosquito larvae from breeding sites along with the breeding site water into a plastic container with the help of a funnel. The mouth of the plastic container was covered with net material to allow for ventilation and transported to the Department of Biological Sciences Laboratory. At the laboratory the larvae were transferred into white plastic bowls and fed with baker's yeast.

Bioassay

Fifteen mosquito larvae were isolated from white plastic containers into the bowls using a dropping pipette. These were introduced into the bowls containing 100ml of the test concentrates of the solvent extracts and water extract of the plant (Adebote *et al*., 2011).

The test concentrations were maintained in triplicates as well as corresponding control. Experimental bowls were labeled according to the extract types and concentrationused. The set up was observed for 24hours after which mortality was recorded.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and the means separated by Duncan's multiple range test (DMRT). Statistical software SPSS 16 was used for data analysis.

RESULTS

Percentage mortality and lethal concentration (LC) of crude methanol and aqueousextracts of *Azadirachta indica s*tem bark against *Anopheles* mosquitoes larvae. The result showed that methanol stem extract presented at 100mgl^{-1} produced 31.33% mosquito larvae mortality, at 150mgl^{-1} produced 60% mosquito larvae mortality, at 200mgl^{-1} produced 73.3% mosquito larvae mortality, at 250mgl^{-1} produced 86% larval mortality, on the other hand water stem extract of the plant at100mgl^{-1} produced 20% mosquito larvae mortality, at 150mgl^{-1} produced 26.6% mosquito larvae mortality, 200mgl^{-1} produced 40% mosquito larvae mortality, and 250mgl^{-1} produced 62% mosquito larvae mortality.

The LC_{50} of the methanol was the lowest at 1.54mgl^{-1} and LC_{90} of about 5.18mgl^{-1} followed by aqueous extract with LC_{50} of 3.48 mgl^{-1} and LC_{90} of 19.47mgl^{-1}. On the other hand aqueous stem extract had the highest LC_{50} with poor mortality while methanol stem extract had the lowest LC_{50} with a relatively more toxic characteristic. Percentage mortality and lethal concentration of crude methanol and aqueous of *Azadirachta indica* was represented in table 2, leave against *Anopheles* mosquitoes larvae is shown in The result showed that methanol stem extract presented at 100mgl^{-1} produced 65% mosquito larvae mortality, at 150mgl^{-1} produced 67% mosquito larvae mortality, at 200mgl^{-1} produced 87% mosquito larvae mortality, at 250mgl^{-1} produced 96% larval mortality, on the other hand aqueous leave extract of the plant at100mgl^{-1} produced 33% mosquito larvae mortality, at 150mgl^{-1} produced

Table 1. Mortality of *A. indica* (Neem) stem bark extracts on *Anopheles* mosquito larvae

SOLVENT TYPE	CONC.(mgl^{-1})	MEAN (%) MORTALITY	LC_{50}	LC_{90}	R^2(R-equation)
Methanol	100	4.7(31)	1.54	5.18	0.7257
	150	9.0(60)			(y = 7.98x - 0.88)
	200	11.3(75)			
	250	13.0(87)			
Aqueous	100	3.0(20)	3.482	19.47	0.5793
	150	4.7(31)			(y = 37.54x - 6.25)
	200	6.0(40)			
	250	9.3(62)			

No. of replicates 3 @ 15 larvae/replicate

Table 2. Mortality of *A. indica* (Neem) Leave extracts on *Anopheles* mosquito larvae

SOLVENT TYPE	CONC.(mgl^{-1})	MEAN (%) MORTALITY	LC_{50}	LC_{90}	R^2(R-equation)
Methanol	100	8.33(56)	1.031	3.56	0.7177
	150	10.0(67)			y = 5.55x - 0.6364
	200	13.0(87)			
	250	14.33(96)			
Aqueous	100	5.0(33)	1.808	11.74	0.5405
	150	8.0(53)			y = 24.13 - 4.23
	200	10.0(67)			
	250	12.0(80)			

No. of replicates 3 @ 15 larvae/replicate

53% mosquito larvae mortality, 200mgl^{-1} produced 67% mosquito larvae mortality, and 250mgl^{-1} produced 80% mosquito larvae mortality. The LC_{50} of the methanol was the lowest at 1.031mgl^{-1} and LC_{90} of about 3.56mgl^{-1} followed by aqueous extract with LC_{50} of 1.808=mgl^{-1} and LC_{90} of 11.74mgl^{-1}. On the other hand methanol leave extract had the lowest LC_{50} with a relatively more toxic characteristic and was considered to be the best solvent system for the extraction of Larvicidal active principle in the leave of *A.indica*. The above Figure 1 shows the comparative LC50 and LC90 values of Stem bark and leave using methanol and aqueous extraction, Methanol Leave extract had the lowest LC value indicating to be the most toxic extract followed by methanol stem bark, Aqueous leave extract and aqueous stem bark with the highest LC value with low toxicity on the mosquito larvae respectively.

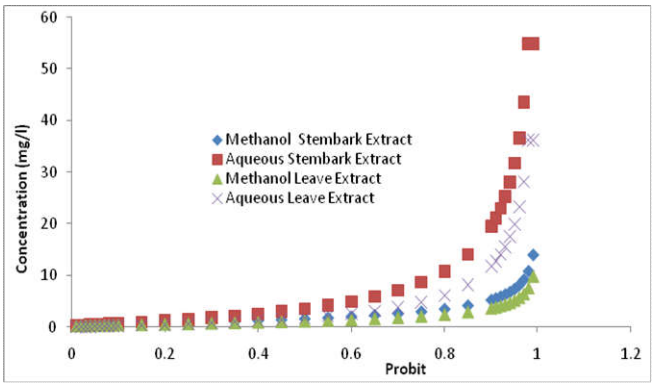

Figure 1. Comparative Lethal Concentration of different *A. indica* extracts

DISSCUSION

The result obtained from this study demonstrates a high susceptibility rate over the larvae of anopheles mosquito in various concentrations of methanol and water. It is also noticed that the mean mortality rate increase with increased concentration all the solvent base used in the extracts. The plant extract from water in leaf and stem recorded the least mortality rate in all the various concentrate. The highest mortality rate was recorded in the methanol extract of both the leaf and stem of *A. indica*. This observation is consistent with Raji and Akinkurolere (Raji, 2010) who found ethanol extracts from various plant part more toxic than water extracts of the same plant parts as against mosquito larvae. Aina *et al.* (2009) who found out that there was significant difference in the results obtained in the effectiveness of both the ethanolic and water extracts of *X. aethiopica* on the larvae of *Aedesaegypti*.
The recorded mortality rate in each concentration reflect slight difference as it was recorded in chapter four of this work., potency rate is mainly as a result of the solvent base used in the extract, all the ones with numerous rate of mortality with increasing concentration in various replicate. Comparing the values obtained in the stem and leaf to the above values it show that the bioactive ingredients present in *A. indica* are weaker than those in convention insecticides already in used against the control of Anopheles mosquito larvae. from the result obtained , it shows that methanol in all the extract acted as the best solvent for extracting active ingredient in *A. indica* since it shows the highest mean larvicidal effect against anopheles mosquito larvae. The result is comparable to results of Al Dakhil and Mory (1999) using *Cardiospermum halicocabum* and *A. indica* extracts against *Culexpipiens* larvae. Egunyomi *et al*., (2010) demonstrated that hexane plant extracts were more effective than methanol plant extracts this indicates that the active compounds are more soluble in methanol than water. Nzelibe and Chintem (2013) also reported that ethanol leaf extracts of *Datura stramonium* leave were very effective as mosquito larvicides. The insecticidal activity of *A. indica* has been attributed to a compound called nimdim ($C_7H_{10}O_2$) with active antiparasite substance Butterworth and Morgan (1971). This study shows high efficacy of *A. indicia* against *Anopheles* mosquito larvae with highest mortality rate in leaf than stem extract which shows that active properties of plants are more concentrated in the storage parts of the plant like leaf, rootetc (Evenson, 1995). Ebe *et al*. (2015) also reported that there was higher mortality in leaf than stem.

Conclusion

In Conclusion, extracts from *A. indica* showed potent larvicidal activity against *Anopheles* mosquito, with the methanol extract of leaf showing the greatest mortality effect. The stem and leaf of *A.indica* could thus be added to the growing list of botanicals with anti-mosquito properties that could be harnessed for the control of noxious mosquito species and as replacement for synthetic pesticides.

Conflict of interest: The authors wish to declare there is no conflict of interest in this research article.

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