FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 3 No. 4, December, 2019, pp 20 -23

LARVICIDAL ACTIVITY OF *Lawsonia inermis* L. AND *Senna obtusifolia* L. AGAINST FOURTH INSTAR LARVAE OF *Culex quinquefasciatus* (SAY) (CULICIDAE: DIPTERA)

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ABSTRACT

Ethanolic leaves extract of two plants, *Lawsonia inermies* L. and *Senna obtusifolia* L. were tested on against 4th instar larvae of *Culex quinquefasciatus.* Three different concentration; 25000, 5000 and 75000 ppm of the plant extracts were made and tested to assess their toxicity and to determine the lethal concentrations required to kill 50% of the insect. Results show that mortality increased as the period of exposure and concentration level increased. The mortality recorded in *L. inermis* and *S. obtusifolia* at 25000 ppm was 70% and 80%, respectively, while 100% mortality was caused by 75000 ppm of each of the two plants leaf extracts. The lethal concentration causing 50% (LC_{50}) mortality of 4th instars larvae of *C. quinquefasciatus* varied. These results indicated that *L. inermis* and *S. obtusifolia* extracts were toxic to immature stages of *C. quinquefasciatu* and could developed as a biological control agent for mosquitoes' management.

Keywords: Biocontrol, *Culex quinquefasciatus*, *Lawsonia inermis*, *Senna obtusifolia*, Toxicity.

INTRODUCTION

Mosquitoes are one of the most leading causes of death of animals in the world. They cause millions of deaths of humans due to their ability to carry and spread diseases (WHO, 2019). One of these mosquito's species is the tropical and subtropical *C. quinquiefasciatus*, the vector of lymphatic filariasis, commonly known as elephantiasis. Infection occurs when filarial parasites are transmitted to humans through *C. quinquiefasciatus* (WHO, 2015). Most of the infected persons are asymptomatic, but infections can lead to lymphedema, hydrocele and swellings of the breast in women (Eneanya *et al.*, 2018).

According to report of mapping survey conducted in Nigeria, lymphatic filariasis is prevalent in all states and geopolitical zones of the country and a total of 241 lymphodema and 205 hydrocele cases have been recorded (Okorie *et al.*, 2015). Mosquito control using insecticides, sanitation, habitat disruption or personal protection from mosquito bites are the most widely measures employed to control and protect people from infection of these diseases (Sani *et al*., 2017). However, over reliance on these measures to control mosquitoes have often led to some problems such as resistance to insecticides and even biopesticides (Mbatchou *et al.*, 2017). In recent years, there has been an increasing interest in the possibility of controlling mosquitoes at immature stages, because mosquitoes breed in water where they are easy to control than at adult stages. Over the past few decades, many researchers have demonstrated the effectiveness of some plant extracts against larvae of mosquitoes, where the result showed greater than 50% mortality over a 96-hours of test period (Remia and Logaswamy, 2010; Arivoli *et al.*, 2012). Plant extracts are harmless natural products that control insects with no effect on non-target organisms and environment (Dass and Mariappan, 2014). The objective of this study is therefore to evaluate the efficacy of extracts of *L. inermis* and *Senna obtusifolia* against larvae of *C. quinquefasciatus* in the laboratory**.**

MATERIALS AND METHODS

Study Area and Sampling Site

The study was conducted at Umar Musa Yar'adua University Katsina (UMYUK), Nigeria. Fresh leaves of plants were collected from their natural habitat (bushes) around the University.

Collection and Preparation of Plant Materials

The fresh leaves of *L. inermis* and *S. obtusifolia* were collected at their natural habitat (bushes) around Umaru Musa Yar'adua University, Katsina (UMYUK) located at the latitude of 12º 53N and longitude of about 7º 35E. The plant species were brought to the Department of Biology, UMYUK, for identification. They were then rinsed with distilled water and shade-dried. The dried leaves were ground using laboratory blender (Model 8010ES) and sieved using 1 mm (Sani and Suleiman, 2017). The powders were then separated and kept in polythene bags at room temperature in the laboratory.

One hundred grams of plant powders were then dissolved in 400 ml of ethanol and kept in the laboratory shelf for 48 hours at room temperature. The extracts of the two plants were filtered separately using muslin cloth and Whatman No.1 filter papers (Suleiman *et al.*, 2018). The filtrate was then concentrated by evaporating excess solvents using rotary evaporator followed by

air-drying the extracts and stored in the refrigerator at 4°C prior to use for laboratory experiments.

Collection and Rearing of Mosquito Larvae

C. quinquefasciatus larvae were collected from stagnant water close to Block C of the male hostel, UMYUK. The larvae were transported to the Biology laboratory 1 and identified using reference text (Littig & Stojanovich, n.d.) identification. They were then maintained in the laboratory at 27ºC and 70% r.h. in plastic buckets. The larvae were separated based on the instars level and fed with yeast powder as a food media in every 24hr. The upper of the plastic bucket was tied with muslin cloth to prevent escape of the adult ones.

Preparation of Test Solutions

Three solutions were made by dissolving 0.5g, 1.0g, and 1.5g of each of the crude leaves extracts in 20 ml of distilled water in separate beakers of 150ml capacity to give three concentrations of 25000ppm, 50000ppm, and 75000ppm, respectively. There were three replicates laid down.

Larvicidal Bioassay

Ten 4th instar larvae of *C. quinquefasciatus* were placed in glass beakers (150 ml capacity) containing 20 ml of distilled water, separately. Each beaker was inoculated with 1 ml of plant extract of *L. inermis* or *S. obtusifolia* at different concentrations of 25000 ppm, 50000 ppm, and 75000 ppm, separately. Twenty ml of distilled water only was added to another set of beakers containing the 4th instar of *C. quinquefasciatus* to serve as control. Each assay was replicated three times in a completely randomized design. Larvae were fed with yeast powder and their mortality was observed in a 24 hr interval for 7 days.

Statistical Analysis

Lethal concentration causing 50 % mortality (LC_{50}) were estimated by fitting mortality data to probit analysis using statistical computer programmed, Statistical Package of Social Sciences (SPSS)-20.

RESULTS AND DISCUSSION

Results of this study indicate that mortality increased with increase in both the exposure period and concentration levels. The mortality recorded in the lowest dose of 25000ppm after 72 hours post treatment was 70 and 80% in *L. inermis* and *S. obtusifolia*, respectively. At 50000 ppm, the mortality ranged from 80 to 90%, whereas it was 100% at the highest concentration of both the extracts (Table 1).

Table 1: Percentage mortality (%) of *C. quinquefasciatus* larvae exposed at different concentrations of *L. inermis* and *S. obtusifolia.*

Plant extract	Concentration (ppm)	Exposure Period (hrs)		
		24	48	72
L. inermis	25000	60	70	70
	50000	80	90	80
	75000	80	100	100
S. obtusifolia	25000	40	70	80
	50000	60	80	90
	75000	70	90	100
Control	0	0	0	0

The results indicate that LC_{50} values of *L. inermis* were 7045.880 ± 0.493, 5944.392±0.580 and 10185.735±1.936 ppm after 24, 48 and 72 hrs of exposure, respectively. Further, the LC_{50} values of *S. obtusifolia* extract were 3511.502±0.378, 1510±0.439 and 2561.925±1.057 ppm after 24, 48, and 72 hours of exposure, respectively (Table 2).

Table 2: LC_{50} value of *L. inermis* and *S. obtusifolia* against 4th instar larvae of *C. quinquefasciatus* after 24, 48, and 72 hrs of exposure.

Plant extracts	Time of exposure (hrs)	Probit equation	LC_{50} ± S. E (ppm)
L. inermis	24	3.3x – 13.84	7045.880 ± 0.493
	48	3.41x – 11.09	5944.392 ± 0.580
	72	6.29x – 24.90	10185.735 ± 1.936
S. obtusifolia	24	1.64x – 7.07	3511.502 ± 0.378
	48	0.75x – 2.10	1510.427 ± 0.439
	72	3.04x – 10.93	2561.925 ±1.057

The result of the present study indicates the potential of two indigenous plant extracts in controlling *C. quinquefasciatus.* This study is comparable to that of Dass and Mariappan (2014) who studied the methanolic leaf extracts of *L. inermis* and

Murraya exotica on *C. quinquefasciatus.* This result further supports the findings of Ubulom *et al* (2013) who tested the efficacy of *Senna alata on Anopheles gambiae, C. quinquefasciatus and Aedes aegypti.*

The results showed that both the two botanicals were effective and toxic against fourth instar larvae of *C. quinquefasciatus*. Percentage mortality of mosquito larvae was found to be directly proportional to the concentrations of the two plant extracts. Similar trend was reported by Raheli *et al.* (2015) with the use *Indigofera arrecta* leaf extract against culex mosquito larvae.

The mortality effect of the plant extracts against mosquito larvae might be due to the presence of some secondary metabolites such as the saponin, alkaloids and tannins which were reported to have a wide range of biological activity with great impact on interaction with the cuticle membrane of the insects (Ebuka, *et al.*, 2017)

The lethal concentration required to kill 50% (LC_{50}) however, had an inverse relation with time; thus, they decreased with the increase of time of exposure from 24 to 48 hrs after treatment. Such relation was reported by Amer and Mehlhorn (2006) who tested the effectiveness of 41 oil derived plant against *Aedes*, *Anopheles*, and *Culex* larvae and recorded lethal concentration (LC_{50}) values of13 selected plants. Among which LC_{50} value of *C. quinquefasciatus* range between 1 to 50.2ppm. Findings of the present study indicated that both two ethanolic leaf extracts of *L. inermis* and *S. obtusifolia* caused significant mortality on immature stage of *C. quinquefasciatus* which could effectively be used to control the filarial vector. which is prevalent in all states and geopolitical zones of our country as reported by Federal ministry of Health.

Further studies on effects of *L. inermis* and *S. obtusifolia* leaf extracts on biochemical composition of mosquito larvae are hereby recommended.

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