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Effect of methanolic leaf extract of *Calotropis procera* on the longevity of Adult *Culex quinquefasciatus* after ten generations

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Abstract
Mosquitoes constitute a serious Public Health menace, resulting in millions of death worldwide each year. Emergence of insecticide resistant strains of the mosquitoes poses a serious threat and hence calls for alternative control measures. This study assessed the larvicidal efficacy of the methanolic leaf extract of *Calotropis procera* against the post emerged Adult of *Culex quinquefasciatus* after ten generations. Adulticidal activities of the leaf, of the plant was therefore studied on laboratory reared Adult of *Culex quinquefasciatus.* The result of longevity of post emerged adult *Culex quinquefasciatus* after ten (10) generations shows a higher longevity value recorded in females compared to the males and in both the males and females, decrease in longevity value was recorded from the first to the tenth generation. The results show a significant difference in the mean longevity values between the male and the female at P<0.05 level of significance and also between the test group and the control at P<0.05 with the control having the highest longevity values.
The results of this research therefore under-scores the efficacy of the plant and further suggest the use of methanolic leaf extracts of *Calotropis procera* as an eco-friendly alternative in the control of filarial worm vector as Adulticide.

Keywords: mosquitoes constitute, *Calotropis procera, Culex quinquefasciatus*, methanolic leaf

1. Introduction

Culex quinquefasciatus Say is an important vector of a wide variety of pathogens and parasites of medical and veterinary diseases worldwide and is a vector of the filarial worm *Wuchereria bancrofti*, the agent of bancroftian filariasis (Triteeraprapab *et al*.,2000; Pumidonming *et al*., 2005) [15, 9]. It also helps in transmitting avian malaria, dengue, chikungunya, filariasis, Japanese encephalitis etc. which lead to thousands of deaths yearly. World Health Organization (WHO) 2005 [17, 18].

Culex quinquefasciatus is one of the most widespread mosquitoes in the world. It is found throughout most of pan and subtropical Americas (Andreadis *et al*., 2010) [1], the Neotropics, Afrotropics (Diaz-Badillo *et al*., 2011) [2], Indomalayan, Australian (David *et al*., 2012) [3] and Eastern Asian regions of the world (Rios-Ibarra *et al*., 2010) [10]. It is also present in the United Kingdom and parts of the Middle East such as Pakistan, Iran among others. It is an important vector of periodic filariasis in parts of the world (Rios-Ibarra *et al*., 2010) [10] and is known to transmit *Wuchereria bancrofti* to some degree in many regions of the globe. Of the estimated global 128 million lymphatic filarial cases, 91% are caused by *Wuchereria bancrofti* (Andreadis *et al*., 2010) [1]. Currently, the main tool for mosquito control is the use of diverse synthetic chemicals as larvicides and adulticides (Govindarajan and Rajeswary, 2014) [5]. The drastic effects of synthetic insecticides in the environment have received wide public apprehension (Morin and Comrie, 2010) [6]. Indeed, misuse of synthetic insecticides in agriculture and public health programmes has caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans and other non-target organisms (Sarwar *et al*., 2009) [11]. Chemical insecticides have the advantage of speedy action and easy application. However, their continuous use causes the development of resistance in insects in many parts of the world such as South America, Sudan, Sri Lanka, Nigeria, Burkina Faso, Egypt, Guatemala, USA, Turkey, and Syria (Malcolm,1988; WHO,1992). Consequently, several insecticides have been withdrawn. To overcome the problem of development of resistance in insects, attention is being given to natural products because of their biodegradable nature, non-toxic to other organisms, safer to use, easily available and cost effective (Rattan, 2010) [14]. The botanical insecticides have been used as alternative methods to control vectors of biological importance (WHO, 2002). A botanical phytochemical, with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, ovicidal, adulticidal and repellent properties (Govindarajan *et al*., 2008). The resistance shown by *Culex* mosquito larva and adults to insecticides has prompted researches on botanicals to discover new human and eco-friendly insecticides (WHO, 2002) [18].

Calotropis procera belongs to the family *Asclepiadaceae* and is a soft wooded, evergreen perennial shrub. It is a xerophytic erect shrub, bearing purple spotted pink scented flowers (Shrivastava *et al*., 2013) [13]. The latex of *C. procera* is used as purgative, while the flower and dried leaves are considered as digestive aids, useful in cough, asthma and anorexia. The root bark is useful in treating skin diseases and intestinal worms; it also possesses analgesic, anticonvulsant, and sedative effect. It is highly recommended in the treatment of leprosy and hepatitis. Oil extracted from leaves of *C. procera* is very efficacious in treating cases of paralysis. Fresh leaves are utilized to relieve rheumatic pains and inflammation in joints (Verma *et al*., 2011). *Calotropis procera* possesses alkaloids, cardiac glycosides, anthraquinone, tannins, saponins, flavonoids, steroids, terpenoids, reducing sugars, and resins which are supposed to have significant antibacterial activity (Shaalan *et al*.,2005). The extracts of *C. procera* possess good larvicidal activity against mosquitoes and more studies shows that the extract contained more active compounds for future use against other stages in mosquito control (Shahia *et al*., 2010) [12]. The phytochemicals present in *C. procera* extracts has been found to act as antioxidants by scavenging free radicals and thus possessing therapeutic potentials (Patel *et al*., 2014) [8].

2. Materials and Methods

2.1 Collection and processing of plant materials

Fresh healthy leaves of *Calotropis procera* was collected from Aminu Saleh College of Education, Azare. Authentication of plant samples was done at the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria and was assigned a Voucher no of 009219.

The leaves were thoroughly washed with tap water to avoid dusts and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were then allowed to dry under shade at room temperature (28 ± 2 ºC) for about 20 days. The dried *Calotropis procera* was powdered by using mortar and pestle, sieved with a mesh size of 0.002mm to collect fine powder.

2.2 Extraction and preparation of plant material

The dried *Calotropis procera* leaves were pulverized and the material, thus obtained was cold macerated in organic solvent (methanol). Stock solution of the extract was prepared from 100mg of the solid residue dissolved in one litre (1L) of distilled water to obtain $100mgL^{-1}$concentrations. Serial dilution of this was made with distilled water to obtain test concentrations of $10mgL^{-1}$, $20mgL^{-1}$, $30mgL^{-1}$ and $40mgL^{-1}$. Control concentration, devoid of the plant extract, consisted of 99ml of distilled water and 1ml methanol.

2.3 Collection and Rearing of Mosquitoes

Blood fed Female *Culex quinquefasciatus* were collected from within Aminu Saleh College of Education, Azare using mechanical aspirators and brought to the laboratory. They were placed in entomological cage containing dechlorinated water for egg laying. The larval stages were fed with mouse pellet and the third instar larvae (L3) were exposed to various concentrations of methanolic leaf extract of *Calotropis procera*. The concentration that killed 50% of the larvae was determined. The survived larvae were reared to adult stage. A quail was introduced into the cage for the emerged female adult to have source of blood meal for about 6hours, and allowed to lay eggs. This process was repeated until 10 generations were tested and resistance ratio was determined in each case.

2.4 Larval bioassay

Bioassay was performed according to WHO guidelines (2005) [18]. After making test concentrations, 25 third instars larvae were introduced into each plastic bowel (125 ml capacity). The healthy mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the materials under test by determination of the larva mortality after 24hours of exposure. Batches of 25 third instars larvae were transferred by means of droppers to small disposable test cups, each containing the test concentrations of 10, 20, 30 and 40mg/L while the control devoid of the extract contained 99ml of water and 1% methanol.

2.5 Longevity of *Culex quinquefasciatus*

To determine the impact of sub lethal dose of the methanolic leaf extract of *Calotropis procera* on the longevity of adult *Culex quinquefasciatus*, both males and females of the emerged adults of *Culex quinquefasciatus* treated at the 3rd instars larval stage (plant extract and control) were maintained in foldable cages separately (15 males and 15 females per cage). Adult of *Culex quinquefasciatus* were fed with 10% sucrose using cotton pad and kept at standard rearing conditions. Mortality was recorded daily until the death of the last individual. This was conducted on the F1 generation and the same procedure was repeated until the tenth generation was observed.

The longevity of *Culex quinquefasciatus* was calculated according to a modified formula of (Fletcher *et al.,* 1990) [4]

$$\text{Longevity} = \frac{(\text{Number of dead mosquitos' } \times \text{ Number of days' mosquitoes survived})}{\text{Total number of mosquitoes}}$$

2.6 Statistical Analysis

Student t-test was used to determine the differences in the mean longevity value between male and female based on gender and based on group.

3. Results

Adult *Culex quinquefasciatus* were selected for 10 generations and each generation was tested for susceptibility to methanolic leaf extract of *Calotropis procera* using the dosage- mortality relationship (Table 1). The LC_{50} values increased steadily in subsequent generations. Little increase in LC_{50} values in larvae was recorded over 10 generations. There was RR value of approximately1.311-fold in the F10 generation compared to the parent generation (F0) = 1.050-fold.The results of longevity of post emerged adult *Culex quinquefasciatus* after ten (10) generations revealed higher mean longevity values in females (8.50 and 2.00 days) for F1 and F10 respectively compared to the males (3.50 and 0.50 days) for F1 and F10 respectively. And in both the males and females, decrease in longevity value was recorded from the first to the tenth generation. The result from (Table 2) shows a statistical significant difference in the longevity between the male and the female at $P<0.05$ level of significance and also between the test group and the control at $P=0.000<0.05$ shown in (Table 3) with the control having the highest longevity values.

Table 1: Effect of methanolic leaf extract of *Calotropis procera* on the longevity of Adult *Cx. quinquefasciatus* after ten (10) generations.

Generation	%Adult emergence	Sex ratio Male	Female	No, of days Male	Female	Longevity(days) Male	Female
1st	45	22	23	7	17	3.5	8.5
2nd	48	21	27	7	15	3.5	7.5
3rd	53	24	29	6	14	3.0	7.0
4th	57	30	27	5	12	2.5	6.0
5th	61	25	36	4	12	2.0	6.0
6th	65	30	35	4	10	2.0	5.0
7th	70	32	38	3	9	1.5	4.5
8th	74	34	40	2	7	1.0	3.5
9th	81	39	42	1	5	0.5	2.5
10th	87	46	41	1	4	0.5	2.0
Control	100	45	55	9	30	4.5	15.0

Table 2: Summary of independent sampled t-test of the Longevity Based on Gender

	Sex	Mean	Std. Dev	df	t-cal	t-crit	p-value	Remark
Longevity	Male	2.000	1.1304	18	4.230	2.101	0.001	Significant
	Female	5.250	2.1506					

Significant at $p \leq 0.05$

Table 3: Summary of paired sampled t-test Based on group

Group	Mean	Std. Dev	Df	t-cal	t-crit	p-value	Remark
Longevity	3.625	2.3614	38	6.526	2.021	0.000	Significant
Control	9.595	5.382					

Significant at $p \leq 0.05$

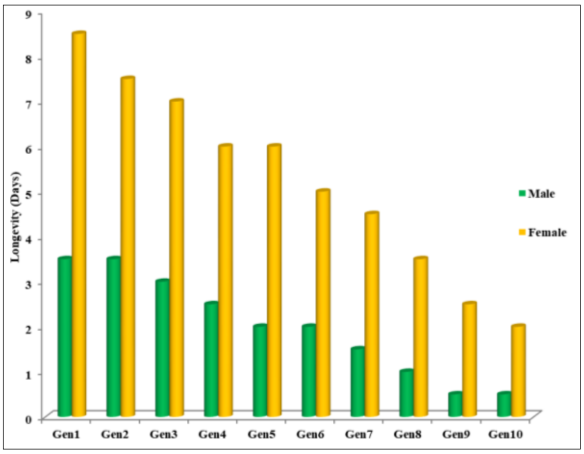

Fig 1: Graphical Representation of Longevity (Days) between Male and Female mosquitos from Generation1 to Generation10

4. Discussion

From the graph of the longevity values obtained, Fig:1 the life span of the emerged adults of the treated larvae decreases as the generation increases with females having higher longevity values than the males across the generations with the control having the highest longevity value compared to both the males and the females. The decrease in the longevity values from F1-F10 was probably as a result of the accumulation of the extract at different larval stages of the mosquitoes which may lead to changes in the behavior and physiology in the mosquitoes and other more.

5. Conclusion

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. *Calotropis procera* leaf was found to be very effective in the control of mosquito and it is relatively safe, inexpensive and readily available in many parts of the world. The longevity of the emerged adult *Culex quinquefasciatus* was determined and found to be decreasing from generation one (F1) with the mean value of 2.000 days in males and 5.250days in females. And the p-value of ($P < 0.05$).

6. Recommendations

Considering mosquito resistance and other problems being associated with synthetic insecticides, *Calotropis procera* leaf could be good botanical substitutes to synthetic insecticide in view of its excellent efficacy and slow larval resistance development demonstrated. It is therefore recommended that:

1. Adoption of *Calotropis procera* leaf for mosquito control operations after safety tests.
2. There is need to investigate the effects of this leaf on mosquito-cohabitant non-target aquatic organisms to ensure specificity.
3. Further studies are needed to unravel the mode(s) of action of the leaf on mosquito larvae.

There is need to investigate the effects of the leaf on other life stages (eggs and pupae) of mosquito species.

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