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LARVICIDAL ACTIVITY OF GARLIC (*ALLIUM SATIVUM*)ON *ANOPHELES* AND *CULEX* MOSQUITO LARVAE

Muhammad Kasim[1], Ogiri Ann[2],Yahaya M.M[3]

[1]Department of Biological Sciences, Biology Unit, Faculty Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

[2]Department of Nutrition and Dietetics, College of Health Sciences and Technology, Kaltungo Gombe, Nigeria

[3]Department of Biological Sciences, Zoology Unit, Faculty Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Kasimevuti@gmail.com

Abstract

This study was designed to determine the larvicidal effect of aqueous extract of garlic against the 4th instars of *culex* and anopheles mosquito larvae. *Anopheles* and *culex* mosquito larvae were obtained using a deeper from stagnant water in the fadama at kofar Kade along illela road, Sokoto and taken to the Physiology laboratory of biological sciences, Usmanu Danfodiyo University Sokoto for further analysis. Fresh samples of garlic were obtained from central market, Sokoto state and were taken to the physiology laboratory for processing. The concentration of the extract used was 0.5mg/ml, 2.0mg/ml and 3.0mg/ml were obtained by weighing 0.5mg, 2.0mg, and 3.0mg in 10ml of water. Mortality of *Culex* the Anopheles depends on the garlic extract and increase with time of exposure and concentration of the extract. 3.0mg/ml recorded the highest mortality rate of 3 hours of exposure for both *culex* and anopheles at a mean of 10.00 each while 0.5mg/ml recorded minimum mortality after 1 hour of exposure for both anopheles and *culex* with mean of 2.33 and 3.67 respectively. The study demonstrated the potency of garlic (*Allium sativum*) in managing the larvae and thus contributes as an affordable way to control anopheles and *culex* larvae of mosquito.

Keywords: *garlic, anopheles, culex, larvae, mosquito.*

Introduction

Garlic (*Allium sativum)* appears to have originated in central Asia and then spread to China, and the Mediterranean region before moving west to Central and Southern Europe, Northern Africa (Egypt) and Mexico (Singh *et al.,* 2008).

Garlic is an important dietary and medicinal plant in human history. It is being used as food and medicine since ancient times. It is a member or alliaceae family having botanical name, *Allium sativum*. Due to being enriched with medicinal effects, it had been used in ancient Greece (Hippocrates), Egypt, Rome, India, China and Japan for multiple indications including performance enhancement, pulmonary and digestive complains, abnormal growth, cardiovascular health, emotional health, potency and as an anti- infective agent (Rivlin *et al*., 1998). It is also among the oldest of all cultivated plants. It has been used as a spice, food and folklore medicine for over 4000 years, and is the most widely researched medicinal plant (Milner, 1996).

It occupies a prominent position among human foods, not only as a condiment, but also due to its therapeutic properties, attributed to the presence of bioactive compounds (Tepe *et al.,* 2005). It is indicated as an adjuvant agent in the therapy for prevention of various chronic infirmities such as heart diseases, infections, and atherosclerosis. Most of garlic's health benefits have been attributed to the antioxidant activity of organosulfurous compounds, predominantly allicin (Queiroz *et al.,* 2009).

Mosquitoes are relatively small flying insects measuring about 3mm-6mm in length, although some species can be small as 2mm-3mm, while others may be as long as 10mm. Mosquitoes have long slender wings and are usually among flies in having small scales over most of the wing veins. Mosquitoes are group into 39 genera and 135 sub-genera (Reinet, 2001). Mosquitoes are also widely spread due to their high adaptability, higher reproductive rate. Mosquito lay their eggs in stagnant waters, pods, ditches, gutters (Busvine, 1980).

One of the most important aspects of these mosquitoes is that they are vectors of certain pathogen or micro-organisms, for instance, protozoans which may infect man and cause serious diseases and in most case may lead to death of the victims. Although some species of mosquitoes do not bite people, rather they prefer birds or amphibians host, but those that bite usually serve as vectors of diseases. Certain mosquito species prefer to feed during the day time, while others feed at night (Gillet, 1972).

Studies have shown that, in villages, town and cities the availability of suitable breeding sites for mosquitoes is related to the geographical location, size of human settlement, standard of living, level of education of the inhabitants and other socio-economic factors. Environmental management of larvae habitats can profoundly impact on mosquito-borne disease transmission, particularly when used in combination with other proven vector control measures such as indoor residual spraying and insecticide-treated nets (Keiser *et al*., 2005). Apreliminary requirement for management is to identify the habitats of the immature stages, since control is most efficiently carried out at the larvae stage when the spatial distribution is limited to water bodies (Dale *et al.,* 2003). The most productive habitat should be given a priority attention (Killeen *et al.,* 2006). Targeted environmental management is based on a sound understanding of the heterogeneity in mosquito breeding habitat productivity (Gu *et al*., 2008).

Methods

Study area

The study was conducted in the General physiology Laboratory of Biological sciences department, Usmanu Danfodiyo University, Sokoto, This is situated at latitude 13°7'44.37 N and Longitude 5°12'14.33E.

Collection and identification of plant materials

Fresh samples of *Allium sativum* were obtained from Sokoto main market, Sokoto state. The samples were to the Herbarium of Botany Unit in Biological Sciences, Department for identification.The batch number is (UDUH/ANS/0196).

Preparation of plant extracts (aqueous extract)

The fresh garlic obtained, was pilled so that the main seed will be exposed and it was slashed into smaller sizes to ease the drying process, the slashed garlic was taken to Botany Laboratory for drying which were spread on a news paper and placed in open cabinet for 14days at 40°C. The dried garlic was pounded into powder form; a sieve of pore size

0.7mm was used to sieve the powder so as to remove the residue which was discarded. A weighing balance was used to measure 50grams of the powdery form of the garlic, and was poured into a conical flask containing distilled water of 100mil of water and was covered with foil paper to avoid interaction between the environment and the mixture. It was then kept for two days with constant shaking at several intervals. After two days, the mixture was sieved in a stainless steel using a muslin cloth so as to obtain the supernatant mixture, and the residue was discarded. The supernatant mixture in the stainless steel was placed in the oven at 40°C for 2 weeks. The watery portion of the supernatant mixture evaporated leaving the main extract behind. A spatula was used to scrape the extract from the stainless steel.

The working concentrations

Three (3) varying concentrations were prepared, in accordance with to test the larvicidal activity were obtained by weighing 0.5mg, 2.0mg, and 3.0mg in 10ml of water. The control were also prepared along side, this were include: 0.1mg/ml, 0.2mg/ml, 0.3mg/ml respectively.

Collection of larvae and rearing

Anopheles and *Culex* mosquito larvae were obtained using a deeper from stagnant water in the Fadama area at Kofar Dundaye along Illela road, Sokoto. This is situated at latitude 13°4'34.25N longitude 5°13'48.77E and altitude. The mosquitoes larvae were reared with yeast and dog biscuits solution in the ratio of 1:3, they were kept in a30x30x30cm in organdy cloth cages tied onto iron frame. Cotton swabs dipped in water were kept over the cage to quench their thirst. By following the method described by (CNRFP), a medical entomology laboratory since 2004. The larvae were monitored until it attains the 4th larval stage before treating with the extract.

Mosquito larvae identification

The identification of fourth instars larvae of *Anopheles* and *Culex*mosquito was achieved by observing the size of the larval head capsule, the presence and nature of siphon and the nature of the larval contact with water surface as reported by Service (1980) as described below. *Culex* larvae are elongated with a distinct head, thorax and abdomen but without legs. The head is broad and dorsoventrically flattened and bears cresentic compound eyes, behind which are small accessory larval eye of the 10 abdominal segments in which the first 7 are similar while the 8th segment bears laterally to rows of short spines. They lay their eggs in raft and also feed below water. While *Anopheles* mosquito larvae lay flat in water, they also lay their eggs singly, there head is longer and broad, they also feed on the surface water, *Anopheles* larvae lack a respiratory siphon and for this reason they stay at the surface of the water, they breathe through spiracles located on the 8th abdominal segment (CDC, 2015).

Experimental procedures

Ten larvae of *Culex*and *Anopheles* mosquito were obtained among the larvae already kept in the laboratory and transferred into 100ml beaker containing 0.5mg/ml,2mg/ml,3mg/ml concentration of the extract each respectively for *Anopheles* and *Culex*mosquito larvae, observation on the mortality rate of the larvae were carried out subsequently from 1 hour to 5 hours respectively on each of the concentration and specie of the larvae. In each case, the number of larvae that died was recorded accordingly.

Larvicidal bioassay

Larvicidal bioassay of individual extracts was tested against 4thinstars larvae of *Anopheles andCulex.* The test were conducted in 100ml glass beakers, inaccordance with(WHO, 2005) protocol with slight modification. Three different concentrations were tested for each of the plant extracts: 0.5mg/ml, 2mg/ml, 3mg/ml, and their control. Three replicate were run simultaneously during each trial. Ten larvae were introduced in to each of the beakers. The mortality was observed at an hourly basis. The treatments were maintained at room temperature. The larvicidal activity of the extracts was determined by counting the number of dead larvae on an hourly basis interval. The dead larvae in the three replicates were recorded when they failed to move after proving with a needle, dead larvae were those enable to rise to the surface within reasonable period of time. The percentage mortality was calculated by the number of larvae dead over the total number of larvae used times hundred. The data were carried out by employing analysis of variance.

Statistical Analysis

Analysis of variance was used to determine the different of the mortality of mosquito larvae between the control solution and the experimental groups. Least significance difference (LSD) was used to separate the different means.

Results

The results obtained on the mortality of *Anopheles* and *Culex*mosquito larvae treated with different concentration of aqueous extract of garlic is presented below.

When *Anopheles* larvae were treated with 0.5mg/ml of the extract 2.33 mortality was obtained after 1 hour of exposure to the aqueous extract and at 2 hours of exposure 4.00 mortality was obtained. No significant difference was observed between the observations. Significant difference was observed after 3 hours of exposure to the extract, in which5.00 mortality of the larvae was observed similarly, at 4 and 5 hours of larval exposure, the mortality was observed to be7.00 and 10.00 respectively.

Furthermore, at 2.0mg/ml concentration after 1, 2, 3 hours of exposure, there was no significant differences but after 4 hours of exposure, significant difference was observed. However, when the extract was increased to 3.0mg/ml of concentration, there was no significant difference at 1 and 2 hours were observed but, significant difference was observed at the 3rd hour of exposure to the extract. When *Culex* mosquito larvae was treated with 0.5mg/ml of the extract 3.67 mean mortality was recorded same as to the 2nd hour and no significant different was observed after the 1st and 2nd hours but after3 , 4, and 5 hours significant different was observed which have t he mean mortality of 7.00, 8.33, 10.00 respectively.

Furthermore, when the larvae were exposed to 2mg/ml concentration, a mortality rate of 3.33 and 5.33 was recorded after 1st and 2ndhours respectively which there were no significant different but after 3 and 4 hours of exposure there was a significant difference which has the mean mortality of 7.66 and 10.00 respectively after exposure to the extract. However, when *Culex was* exposed to 3.0mg/ml of the extract there was no significant difference from the 1st 2nd, 3rd hours which al have mortality rate of 5.00, 8.67, 10.00 respectively.

Discussion

The study shows a remarkable effect of aqueous extract of garlic on the fourth instars of *Anopheles* and *Culex* mosquito larvae collected at Kofar Dundaye in Sokoto metropolis, as a case study. *Culex* larvae were affected more than the *Anopheles* which tends to resist the activity but also mortality was recorded due to the concentration, mortality rate is directly proportional to the concentration of the aqueous extract.

Mortality of larvae of *C. quinquefasciatus* and *A. gambiae* exposed to the plant extracts increased with time of exposure and concentration of extracts as was also reported for larvae of *C. quinquefasciatus* and other mosquitoes exposed to extracts of plants such as Neriumindicum and Euphorbia royleana (Srivastava *et al.,* 2003). The time at which 100% mortality was recorded for the range of concentrations of exposure for larvae of the mosquitoes indicated that the rate of mortality is directly proportional to the concentration f the extract and also *Anopheles*s pecies shows more resistivity than the *Culex.*

These results were at par with those reported by Thomas *et al.,* (2004) and Sharma *et al.,* 2005). It may be due to the fact that most plant induced 50% larval mortality after some hours of exposure to the extract.

Citrus lemon leaf extract has been reported to induce larval mortality after 24 hours of exposure (Sharma *et al.,* 2005) which is similar to this study using aqueous extract of garlic which the rate of larval mortality was based on the concentration of the extract.

Mosquito control at the larval stage is effective procedure because they are localized in space and time (Howard *et al.,* 2007) resulting in less-dangerous out comes to non-target organisms, while the fight against adult is temporary and unsatisfactory. During the last three decades, mosquito controls were directed to the use of insecticide of plant origin. Environmental safety of insecticides is of first and foremost criterion for mosquito control programmes (Rajkumar *et al.,* 2005). The leaves essential oils of T. procumbens are more effective repellents activity at 6 per cent concentration against An. Stephens (Rajkumar, 2007).

Shaalan *et al.,* 2005) reviewed different plants species, which can retard growth, inhibit reproduction, and have function as ovicides, additive, and antagonistic action of botanical mixture. In fact, many researchers have reportedthat essential oils of different plant extract with potential larvicidal activity (Sharma *et al.,* 2006).

Ethanol extract of *Alliumsativum* (garlic bulb) acts as good control agent against the filarial vector, *Cx. quinquefasciatus* (Kalu *et al.,* 2010). *Curcuma longa* L. (Zingiberaceae), *Allium sativum* L. Alliaceae, *Zingier montanum* (Koen) aqueous extracts were reported to be good larvicidal agents against *Cx. quinquefasciatus* and *Aedes, aegypti* mosquito larvae (Chansang *et al.,* 2005).

Conclusion

This research study showed remarkable larvicidal effect of aqueous extract of garlic against the 4th instar of *Culex* and *Anopheles* mosquito larvae and also demonstrates the potency of garlic (*Allium sativum*). Mortality of *Culex* and *Anopheles* depends on time of exposure and concentration of the extract.

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Table 1;Mortality of *Anopheles* mosquito larvae treated with different concentrations of aqueous extract of garlic.
Time of exposure
Mean with the same alphabets are not significantly different.

Conc.	1hr	2hr	3hr	4hr	5hr	cont	LSD	SEM
0.5 mg/ml	2.33^{a}	4.00^{a}	5.00^{bd}	7.00^{bc}	10.00^{e}	0	2.20	0.49
2.0 mg/ml	4.70^{a}	6.67^{a}	8.33^{ab}	10.00^{bc}	-	0	2.20	0.45
3.0 mg/ml	3.67^{a}	7.67^{ab}	10.00^{bc}	-	-	0	2.69	0.47

Table 2;Mortality of *Culex*mosquito larvae treated with different concentrations of aqueous extract of garlic.
Time of exposure

Conc.	1hr	2hr	3hr	4hr	5hr	cont	LSD	SEM
0.5 mg/ml	3.67^{a}	5.33^{a}	7.00^{b}	8.33^{bc}	10.00^{bcd}	0	2.80	0.63
2.0 mg/ml	3.33^{a}	5.33^{a}	7.66^{b}	10.00^{bc}	-	0	2.20	0.45
3.0 mg/ml	5.00^{b}	8.67^{b}	10.00^{b}	-	-	0	2.10	0.37

Mean with the same alphabets are not significantly different