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EVALUATING THE EFFECTIVENESS OF *OCIMUM GRATISSIMUM* (L) AS AN INSECTICIDE AGAINST ADULT ANOPHELES MOSQUITOES

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Abstract: The insecticidal effect of *Ocimum gratissimum* (Lamiaceae) on wild-caught adult *Anopheles* (WAA) mosquitoes was investigated. The aim of this investigation was to find an eco friendly, affordable and available means of physically combating mosquitoes near our homes. Sixty (60) wild - caught adult mosquitoes were exposed to three concentrations, 1%, 2% and 3% , of the aqueous extract of the leaves of *Ocimum gratissimum* in an insect box. Investigations were based on the World Health Organization (WHO) susceptibility test for adult mosquitoe bioassay procedure which involves exposing the mosquitoes at different time intervals to different concentrations of the suspected insecticidal extract. 0.75% of 2, 2-dichlorovinyl di methyl phosphate (DDVP), was used as a reference insecticide. Results obtained showed a time dependent insecticidal effect of the extract on WAA mosquitoes, such that a t 60 minutes there was 100% mortality of mosquitoes at all concentrations of exposure, indicating the insecticidal potentials of the aqueous extract of *O. gratissimum* against malaria – causing mosquitoes. Further investigations to ascertain and isolate the active ingredient with this potentiality in the herb extract, is being recommended as an initial step in the formulation of a cost effective and eco- friendly bio-insecticide.

Keywords: *Ocimum gratissimum*; *Anopheles* mosquitoes; WHO susceptibility test; insecticidal potentials.

Introduction

Malaria, a mosquitoe-borne disease, has always posed a major public health problem and accounts for about 1-3million deaths annually worldwide, with most of this occurring in children under 5yrs (WHO, 1996). In developing countries close to the tropics like sub-Saharan Africa, it is one of the most prevalent causes of direct or indirect infant, child and even adult morbidity and mortally which results in about two million confirmed cases and above 1000 deaths recorded annually (Coker *et al*, 2000). Blood feeding mosquitoes are responsible for the intolerable biting nuisance and the transmission of a large number of diseases such as malaria, yellow fever, dengue fever, filariasis amongst others, causing serious health problems to humans and obstacles to

Original Article

socioeconomic development of developing nations (Murugan *et al*, 2007). Despite much ongoing research, mosquitoes still remain a huge problem in society both as nuisance and disease vector and this is highly mitigated by economic and logistical problems associated with conventional method of control such as insecticide spray or fumigation (Pates and Curtis, 2005). For blood sucking insects, olfaction is the principal sensory modality used in host recognition and location. This is especially true for disease - vector mosquitoes, many of which locate their vertebrate host during scotephase (Takken, 1999). Thus, it's this characteristic that has prompted the production of several insecticides, larvicides, etc. for control of mosquitoes.

Ocimium gtratissimum, commonly known as African basil (Kei'ta *et al*, 2001) or scent leaf (Nwaeze and Eze, 2009), belongs to the family laminaceae and is found widely in warm regions of the world especially in Africa (okigbo and ogbonna, 2006). It is called "Ntong" by the Efiks, "Abuji", by the Igbos, "Effirin-nla" by the Yorubas and "Daidoya" by the Hausas in Nigeria (Akinmoladun, *et al*, 2007). The rich composition of the plant has aided its application for both culinary and medicinal uses (Aziba *et al*., 1999). It can be used as an additive to extend the shelf life of foods; leaves extract are also used for treatment of diarrhea, dysentery and pile (Idie *et al*, 2005). Also traditionally, it is believed that planting it around the house can repel mosquitoes as the basis for the prevention of malaria (Aguiyi *et al*, 2000).

Mosquitoes are members of the family of nematocerid flies. Females of most species lays their eggs in stagnant waters, some close to water edges while others attach their eggs to aquatic plants. The actual breeding ground though is determined by situation and ecological adaptations of each species (kasova, 2003). Many methods have been employed to control disease - causing mosquitoes, depending on the situation. These are grouped into; source reduction, bio control, trapping or using insecticides to kill adult or larvae and exclusion (Delvin, 2010).

Insecticides are chemicals or biological substances that are used to kill or disable insects (IUPAC, 2006). Most can significantly alter the ecosystem, some concentrate along the food chain and are toxic to humans and wild life. They can be classified as systemic (residual or long –term effect) or contact (fast mode of action) insecticides.

Methods

Test Substance - *O. gratissimum*

Whole plants of *O. gratisissum* was obtained from from a private farm in OkonInok in Calabar, Cross River State. It was taken to the botanic garden, Biological Science department, Cross River University of Technology, Calabar where it was identified and authenticated by the curators.

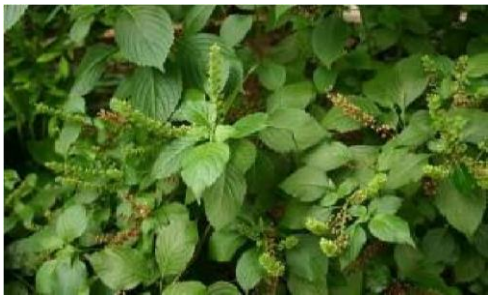

Fig. 1: *Ocimum gratissimum*

Fig. 2: Pulverized *O. gratissimum*

Original Article

Dead leaves, stems and insects larva were carefully separated from fresh leaves, then the fresh leaves were washed and rinsed with tap water before twigs and stems were removed. The fresh leaves were then room- dried under laboratory conditions at room temperature ($28+1^0$c) for 5 days (120hrs). The air-dried leaves were pulverized in an electric blender (Lapriva, 3000), after which 20g of the pulverized leaves were soaked in 100ml di water and stirred continuously for even spread of the solution. The aqueous extract with a 0.2g/ml concentration, was then filtered using a fine mesh muslin cloth into a 50ml beaker. From this stock solution, three concentrations of 1%, 2% and 3% of the *O. gratissimum* aqueous extract were prepared for the WHO susceptibility tests.

Wild-caught Adult Anopheles Mosquitoes (WAA)

Sixty (60) WAA mosquitoes were collected from a bushy garden behind the students' hostel in Cross River University of Technology, Calabar, with an aerial net and housed in an insect holding jar containing sugar solution- soaked cotton wool, for transfer to the laboratory. The sex of the mosquitoes was confirmed by an entomologist in the Department of Biological Sciences of the same institution. The study was carried out under optimum laboratory conditions of 25 °C temperature and 80% relative humidity.

WHO susceptibility test

The WHO susceptibility test for adult Mosquitoe bioassay procedure was employed for this research (WHO, 2013) with slight modifications. Three concentrations of the plant extract and 0.75% of a reference insecticide, DDVP or 2,2-dichlorovinyl dimethyl phosphate, were used to test the susceptibility of 60 adult mosquitoes, with15mosquitoes exposed at each concentration. These were grouped into A (control – 0.75%), B (1%), C(2%) and D(3%). Four sheets of clean white paper were impregnated with the extract and the control, and rolled into cylinder shapes and inserted into four exposure tubes. These tubes were fastened in an upright position with copper wire inside an insect exposure box (Fig. 3). The mosquitoes were then carefully aspirated into the netted, compartmentalized exposure box and covered with cardboard for 60mins. At the end of this period, data was recorded for further analyses.

Results

The number of mosquitoes knocked down, either moribund or dead, were recorded at timed intervals. The observed mortality was calculated using the WHO 2013 mortality of test sample formula:

$$\text{Observed Mortality} = \frac{\text{Total number of Dead Mosquitoes}}{\text{Total Sample Size/group}} \times 100\%$$

(WHO, 2013)

Results of the susceptibility test are recorded on Table 1. From the table, at 10 mins. 2, 3, 9 and 3 moribund and dead mosquitoes were observed for control group A, group B, group C and group D respectively. At 20 mins after aspirating the mosquitoes into the exposure box, the same number was recorded. At 30 mins, 4, 3, 9 and 8 mosquitoes were found dead at the bottom of the exposure box respectively. At 40 mins. 4, 6, 12 and 13 insects were observed for the control, group A, group B, group C and group D respectively. At 50 mins. 8, 9, 12 and 13 deaths were recorded, respectively. Finally, at 60 mins. , which is the termination period, all 15 insects in each cage were observed to be either moribund or dead.

Original Article

TABLE 1: Susceptibility Test for Adult Female Mosquitoes to Aqueous extract of *O. gratissimum*

	A (0.75 %)		B (1%)		C (2%)		D (3%)	
Exposure (Min)	**Time (pm)**	**No. dead**	**Time (pm)**	**No. dead**	**Time (pm)**	**No. dead.**	**Time (pm)**	**No. dead**
10	**1.45**	**2**	**1.49**	**3**	**1.53**	**9**	**2.00**	**3**
20	**2.05**	**2**	**2.09**	**3**	**2.13**	**9**	**2.20**	**3**
30	**2.15**	**4**	**2.19**	**3**	**2.23**	**9**	**2.30**	**8**
40	**2.25**	**4**	**2.29**	**6**	**2.33**	**12**	**2.40**	**13**
50	**2.35**	**8**	**2.39**	**9**	**2.43**	**12**	**2.50**	**13**
60	**2.45**	**15**	**2.49**	**15**	**2.53**	**15**	**3.00**	**15**

The results were interpreted based on the WHO 2013 guidelines as stated below:

- A mortality in the range 98–100% indicates susceptibility.
- A mortality of less than 98% is suggestive of the existence of resistance and further investigation is needed.
- If the observed mortality (corrected if necessary) is between 90% and 97%, the presence of resistant genes in the vector population must be confirmed.

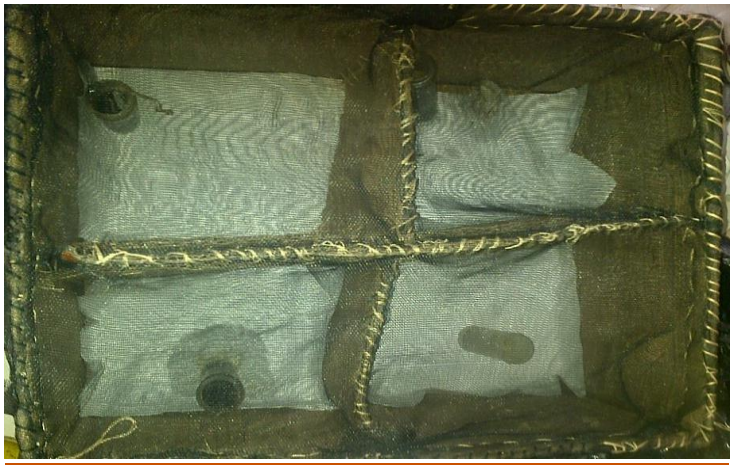

Fig. 3 Compartmentalized Insect Exposure Box

Using the standard WHO 2013 mortality of test sample formula given above, the observed mortality at the timed intervals were calculated and results presented on Table 2. On Table 2, at 10 mins. 13.3%, 20%, 60% and 20% mortality were observed respectively. At 20 mins. only group D had a different mortality rate of 53%. At 30 mins. exposure time, 26.7%, 20%, 60% and 86% mortality rate were observed respectively. At 40mins., 26.7%, 40%, 80% and 86% mortality were observed respectively. At 50 mins. exposure time, 53%, 60%,80% and 86% mortality were recorded. Finally at 60mins., 100% mortality was observed for all the treatments and the positive

control. From the analyzed results, susceptibility was dependent on time of exposure. 100% mortality was observed in the treated groups and the positive control 1 hr (60 mins) after exposure. This indicates susceptibility of the WAA to the test substance. This results agree with reports by Koom *et al*., (2003) on insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis.*

Table 2: Observed Mortality of Mosquitoes at Timed Intervals

	Observed Mortality (%)			
Time Of Exposure (mins.)	**A(Control)**	**B**	**C**	**D**
10	**13.3**	**20**	**60**	**20**
20	**13.3**	**20**	**60**	**53**
30	**26.7**	**20**	**60**	**86**
40	**26.7**	**40**	**80**	**86**
50	**53**	**60**	**80**	**86**
60	**100**	**100**	**100**	**100**

The plant *O. gratissimum* contains a rich array of phytochemicals and essential oils (Akinmoladun *et al*, 2007) and any of these maybe implicated in the ability of the herb extract to affect the insects. Sharaby, 1988 and Dales, 1996 attributed the insecticidal activity of the herb to several things including mechanical action on insects. This agrees with observations from this study since morbidity of the WAA can be attributed to systemic damage in the insects. Apart from the results of this study presenting *O. gratissimum* as an insecticide against mosquitoes, Aguiyi *et al*, 2000, also recorded that the extract is an effective repellent against household insects beside having. The present results confirmed these assumptions. Ocimum was seen to compete favourably with the positive control, DDVP, which is a known organophosphate, whose mode of action is to target insects' nervous system and interferes with the enzymes autychlolinesterase, disrupting nerves impulses to kill or disable the insect. They are compared to warfare nerve agent such as a sarin, tabun, soman etc. because they work the same way and usually have a build up toxic effects in wildlife which causes the decline in their use, for better substitutes (WHO, 1989).

Conclusion

The mortality observed was time-dependent. This agrees with expected mortality pattern of potent insecticides as the toxin is expected to slowly penetrate the insect body via the respiratory system (Schreck *et al.,* 1989). Hence the 100% mortality observed at 60 mins., which is time enough for the active toxins to have fatal effect in the insects. According to the WHO guidelines, a range of 98-100% mortality indicates susceptibility to the extract. Thus, the results of this study present *O. gratissimum* as a possible bio-insecticide against the adult anopheles mosquitoes.

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