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SUSCEPTIBILITY STATUS OF *ANOPHELES* MOSQUITO EXPOSED TO EXTRACTS OF (*MORINGA OLEIFERA*) AND DELTAMETHRIN INSECTICIDE WITHIN NASARAWA STATE UNIVERSITY COMMUNITY KEFFI

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ABSTRACT
This study was carried out to determine the susceptibility status of *Anopheles* mosquitoes exposed to extracts of (*Moringa oleifera*) and Deltamethrin insecticide within Nasarawa State University Community Keffi. Anopheline mosquito larvae were collected from some selected breeding site within Nasarawa State University community Keffi between the month of February, March and April (2022). The mosquitoes were reared at Insectary. 100g of blended leave extracts (*Moringa oleifera*) were mixed with 70% ethanol in separate jar and allowed it to stay for 24 hours .The suspensions were filtered using the whatman's filter paper. The solution was impregnated with filter paper for WHO bioassay and the larvicidal assay. The highest number of adult female *Anopheles* mosquitoes exposed to Moringa extract with mortality of 73% in the month of March and lowest mortality of 34% in the month of February. The larval stage of *Anopheles* mosquitoes exposed to *Moringa* extract having highest mortality of 72% and lowest mortality of 43% in the month March. The Moringa extract used in this study showed a promising level of larvicidal activity against the larvae of *A. gambiae s.l* than the adult stages. *Anopheles* mosquitoes exposed to Deltamethrin were susceptible, 98%-100% mortality. Mosquitoes exposed to Deltamethrin were susceptible and Moringa extract were resistance. Statistically there was significant difference in the comparison as $p>0.005$.The results obtained showed that Deltamethrin is more effective in controlling mosquitoes than Moringa extract.

Keywords: *Moringa oleifera, Susceptibility, Deltamethrin, Anopheline*

INTRODUCTION
Mosquitoes are responsible for the spread and transmission of several harmful diseases such as malaria and lymphatic filariasis. It is known to infect over 700 million people causing 1 million deaths each year especially in developing regions of the world including sub-Saharan Africa (WHO, 2016). Despite years of control efforts, malaria continues to be a major threat to public health in parts of sub-Saharan Africa, Nigeria inclusive. About 97% of Nigeria's population is at risk of malaria where 60% of hospital outpatient visits and 30% of hospitalization among children under five years and pregnant women occur due to malaria (Minakawa *et al.,* 2012). Entomological studies focused on the diversity, density, behavioral patterns and temporal variations of *Anopheles* species have long been found to be beneficial in the identification and monitoring of malarial vectors (Tajebe *et al.,* 2014). A combination of factors that determine the capacity of a vector to transmit malaria include; abundance, anthropophily, zoophily, susceptibility to infection by the malaria parasite, infection rates and female longevity (Kolade *et al.,* 2013).
The relative importance of mosquitoes in disease transmission has made them the target of several life cycle control activities including chemical, non-chemical and biological control (WHO, 2017).
Vector-borne diseases remain a major public health issue in the tropical and subtropical regions of the world (WHO, 2014). Anopheline vector of malaria consists of various species with unique behaviour associated with their biting activities and transmission dynamics, Human malarial protozoa are transmitted by mosquitoes of the genus *Anopheles*. Mosquitoes of the family Culicidae are considered a nuisance and a

major public health problem, because their females feeds on human blood and thus transmit extremely harmful diseases, such as malaria, yellow fever and filariasis (Wikipedia, 2014). Malaria leads to a lot of social and economic problems, such as school absenteeism, lower agricultural production among others; consequently, more control efforts are required in order to reduce the rates of disease incidences and mortality.

Insecticide resistance is now a pervasive phenomenon that has been reported in approximately two-thirds of countries with ongoing malaria transmission (Corbel *et al.*, 2013). In addition, many vector populations are resistant to multiple insecticides from different chemical classes; of the 73 countries that provided monitoring data from 2010 onwards, 50 reported resistance to two or more insecticide classes (WHO, 2016). The continued spread of resistance could threaten malaria control progress achieved thus far and ultimately lead to operational failure of prevailing control measures (WHO, 2016).

Presently, there are 12 insecticides recommended by the WHO insecticide evaluation scheme for indoor residual spraying (IRS) against mosquitoes, out of this only dichlorodiphenyltrichloroethane (DDT) which has the longest residual effect (> 6 months) is not yet used in Nigeria, because of environmental concerns (Coetzee 2004). The re-introduction of DDT into the mosquito control is expected to produce mosaic defense against the development of resistance (Awolola *et al.,* 2007).

Resistance to LLIN exposure increases mosquito survival, which may lead to rising malaria incidence and fatality in Africa (Kawada *et al.*, 2011). However, insecticide resistance of malaria vectors is not limited to PYs only but also exists to the other three classes of insecticides used in public health, such as CAs, OCs and, to a lesser extent, OPs. However, some differences have been observed in the distribution of resistance among regions across the continent. For example, resistance to DDT, the most common OC used in IRS, has been reported in *A. gambiae* and *A. funestus* in western, central and eastern Africa (Kawada *et al.,* 2011), whereas it is practically absent in southern Africa, with the exception of an *A. funestus* population in southern Malawi (Riveron *et al.*, 2016). DDT resistance has been also reported in *A. arabiensis* in southern Africa, specifically in Madagascar, Mozambique and South Africa (Clements,2006).

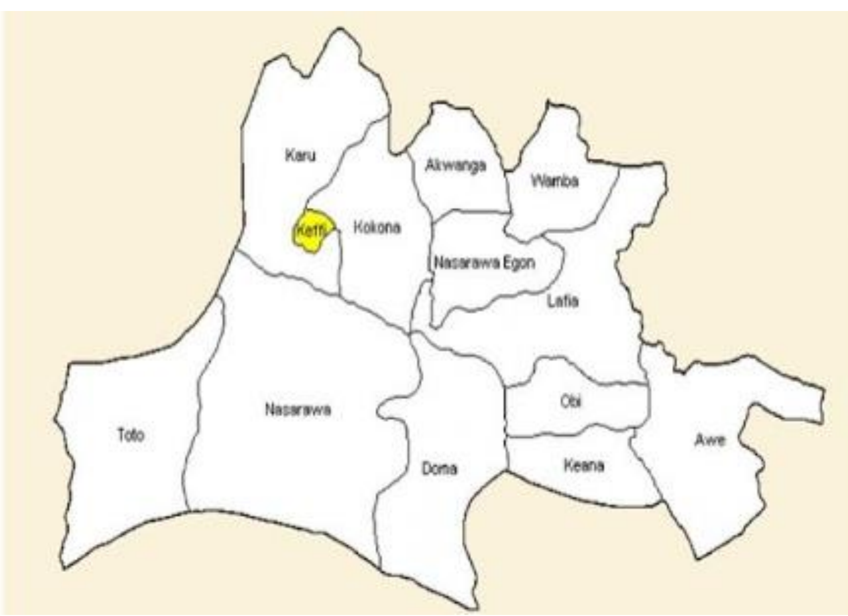
After Africa, Southeast Asia is the area with a higher incidence of malaria, with 7% of the cases reported. A good number of vectors (belonging to complexes or groups of species that are difficult to distinguish) are involved in transmission, presenting an extraordinary biodiversity, heterogeneity in distribution, linked with a high variety in host feeding and ecological habitat preferences, as well as high differences in vector competence (Quiñones *et al.*, 2015). Currently in Southeast Asia, PY resistance has been detected in An. epiroticus in Vietnam (Van *et al.*, 2008), *An. minimus* in Thailand and Vietnam, *An. sinensis* in China and Vietnam (Cui, Raymond and Qiao, 2006) and *An. vagus* in Cambodia and Vietnam (Van *et al.*, 2008).

Alarming, high level of multiple resistances to all classes of insecticides used in public health has been reported recently in *An. sinensis* in malaria endemic areas of China, including permethrin, deltamethrin, bendiocarb, DDT, malathion and fenitrothion, among others (Zhang *et al.*, 2017). In South Asia, represented mainly by India, *An. baimaii* and *An. minimus* are also present but geographically restricted to East and Northeast regions and are fully susceptible to all classes of insecticides (Dev and Sharma 2013). *An. stephensi*, *An. culicifacies* species E and *An. fluviatilis* species S are the other predominant vectors responsible for malaria transmission in mainland India. *An. stephensi*, prime urban vector in India, has shown resistance to PY, DDT and OPs in Goa State (Mishra *et al.*, 2016).

Botanical pesticides are promising in that they are effective, environment – friendly, easily biodegradable and also inexpensive. Botanical pesticides have been used traditionally by human communities in many parts of the world against pest species of insects (Jacobson, 2008).Some botanicals such as neem (*Azadirachta indica*) seed extract and scent leaf (*Ocimum gratissimum*) extract are used as insect-repellent liquids (Silva *et al.,* 2010). *Moringa oleifera* leaves have been found to possess some antibacterial and antifungal characteristics (Rao *et al.,* 2007; Arya *et al.*, 2010). Moringa is an all-purpose plant. It is a native of India but is widely cultivated in some sub-Saharan African countries like Zimbabwe, Madagascar, Zanzibar, South Africa, Tanzania, Malawi, Benin, Burkina Faso, Cameroon, Chad, Gambia, Ghana, Guinea, Kenya, Liberia, Mali, Mauritania, Nigeria, Niger, Sierra Leone, Sudan, Ethiopia, Somalia, Zaire, Togo, Uganda and Senegal (Amaglo, 2010; Fuglie and Sreeja, 2011). Every part of the plant can be used for one thing or the other. The

leaves have very high nutritional value. They are good sources of protein, minerals, vitamins, beta-carotene, amino acids and various phenolic compounds. They provide a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeoylquinic acid and kaempferol (Moyo *et al.,* 2011).
Moringa is very important for its many impressive ranges of medicinal uses. Various parts of this plant such as the leaves, roots, seeds, fruits, flowers and immature pods act as cardiac and circulatory stimulants. They possess antitumor, antipyretic, antiepileptic, anti-inflam-matory, antiulcer, antispasmodic, diuretic, antihypertensive, antidiabetic, hepatoprotective, antibacterial and anti-fungal, cholesterol lowering properties and some antioxidants (Fuglie and Sreeja, 2011; Moyo *et al.*, 2011; Oz, 2014). The leaves are ground and used for scrubbing utensils and for cleaning walls. Its seeds yield about 40% of non-drying oil, known as Ben or Oleic oil, used for cooking, lubricating watches and other delicate machinery, soap and cream making etc. The oil is clear, sweet and odorless, and it is useful in the manufacture of perfumes and weave-on oil in hairdressing. The oil compares favorably with olive oil (Moyo *et al.*, 2011; Oz, 2013). Moringa wood yields a blue dye. The leaves and young branches are eaten by livestock. It is planted as a living fence tree. The bark can serve for tanning; its mature seeds can also be used to purify water. The flowers which are present throughout the year, are good sources of nectar for honey producing bees, thus its presence enhances production in other crops due to increase in pollination activities by bees (Fuglie and Sreeja, 2011).
Resistance in mosquitoes has led to the development of a wide variety of conventional insecticide which has posed a serious problem in the control of mosquitoes (WHO, 2015). Since 2010, a total of 60 countries have reported resistance to at least one class of insecticide, with 49 of those countries reporting *Anopheles* resistance to two or more classes (WHO, 2017).
In addition, many vector populations are resistant to multiple insecticides from different chemical classes; of the 73 countries that provided monitoring data from 2010 onwards, 50 reported resistance to two or more insecticide classes (WHO, 2016). The continued spread of resistance on synthetic insecticides could threaten malaria control progress achieved thus far and ultimately lead to operational failure of prevailing control measures (WHO, 2016).This study was aimed at determining the susceptibility status of *Anopheles* mosquito exposed to extracts of (*Moringa oleifera*) and deltamethrin insecticide within Nasarawa State University Community Keffi.

MATERIALS AND METHODS
STUDY AREA



This study was conducted within Nasarawa State University community Keffi, Nasarawa State. Keffi is about 58Km from Abuja (the Federal Capital Territory) and 128Km from Lafia, the Nasarawa State Capital.

The town is situated on latitude 80 5" North and longitude 70 50" East and about 850 meters above the sea level (Awka*et al*., 2007). Keffi has population of 92,664 (National population census of 2006) making it the second populated city in Nasarawa State. Map of the study area is shown in figure 1

Samples Collection.

Anopheline mosquito larvae were collected from some selected breeding site within Nasarawa State University community Keffi, Nasarawa state. The mosquitoes were reared in USAID/PMI-AIRs Insectary and Entomological Laboratory located at the Nasarawa State University Keffi, Nasarawa state.

Adult Mosquito Susceptibility Bioassays with Deltamethrin.

CDC bottle bioassays method was used to conduct the test according to (Centre for Disease Control, 2012) guidelines. Each Wheaton 250ml bottle and its caps were coated with 1 ml of insecticide solution by rolling and inverting the bottles. In parallel, a control bottle were coated with 1 ml of acetone, and all bottles were covered with a sheet and left to dry in the dark. 25-Female *Anopheles* mosquitoes were introduced into the coated bottles for 30min in four replicates with two control bottles.

Ethanol Extraction Preparation.

The leave extracts (*Moringa oleifera*) were blended and allow it to dry at room temperature (keep away from sunlight penetration). 200g of blended leave extracts (*Moringa oleifera*) were mixed with100ml of 70% ethanol in separate jar and allow it to stay for an hour. The mixture was separated with filter paper into conical flasks using Whatman's filter paper.

Larvicidal Bioassay.

The larvicidal activity of the moringa crude extracts were evaluated as per the method recommended by World Health Organization (2005). Batches of 25 third instar larvae were transferred to small disposable test beakers, each containing 100 ml of water. The 1ml moringa extract dilution was added to 100 ml water in the beakers .Four replicates were set up and an equal number of controls were set up simultaneously using tap water. To this, 1 ml of appropriate acetone will be added The control mortalities were corrected by using Abbott's formula. The LC50 was calculated after 24 and 48 h by Probit analysis (Finney 1979).

$$Corrected\ mortality = \frac{\text{observed motarlity in treatmenat} - \text{observed mortality in control}}{100 - \text{control mortality}} \times 100$$

Data Analysis.

The data obtained was interpreted as mortality rate between 98 and 100% indicate susceptibility; knockdown rate between 90% and 97 % suggest possible resistance and further examination was required; knockdown rate below 90%, indicates resistance (WHO, 2013). Analysis of variance (ANOVA) was used to compare the data at 5% significance level.

RESULTS

4.1 Susceptibility status of Adult female Anopheline on Moringa extract base on locations and months.

The results show that in all the location, the mosquitoes were resistance to Moringa extract at 24 hours of exposure using WHO Tube method. Highest mortality was observed on mosquitoes collected behind boys' hostel of Nasarawa state university keffi with mortality of 73% in the month of March and lowest mortality of 34% in zoological garden in February as shown in figure1

4.2 Susceptibility Status of larva stage of Anopheline using Moringa extract base of location and month.

The larval stage of Anopheline mosquitoes exposed to Moringa extract show resistance in all locations at different months. The highest mortality of 72% was observed on mosquitoes collected behind boys hostel in the month of February having 72% and the lowest mortality of was observed on mosquitoes collected from Entrepreneurship Center with rate of 43% in the month of March as shown in figure 2.

4.3 Resistance status of Adult female Anopheline mosquitoes exposed to Deltamethrine insecticides in the month of February to April in three locations.

Figure 3 shows that mosquitoes exposed to Deltamethrin shown that all the mosquitoes were susceptible to Deltamethrin insecticide with 100% mortality recorded in the month of February in all the three locations and lowest mortality of 98% in March at zoological garden and Esp center.

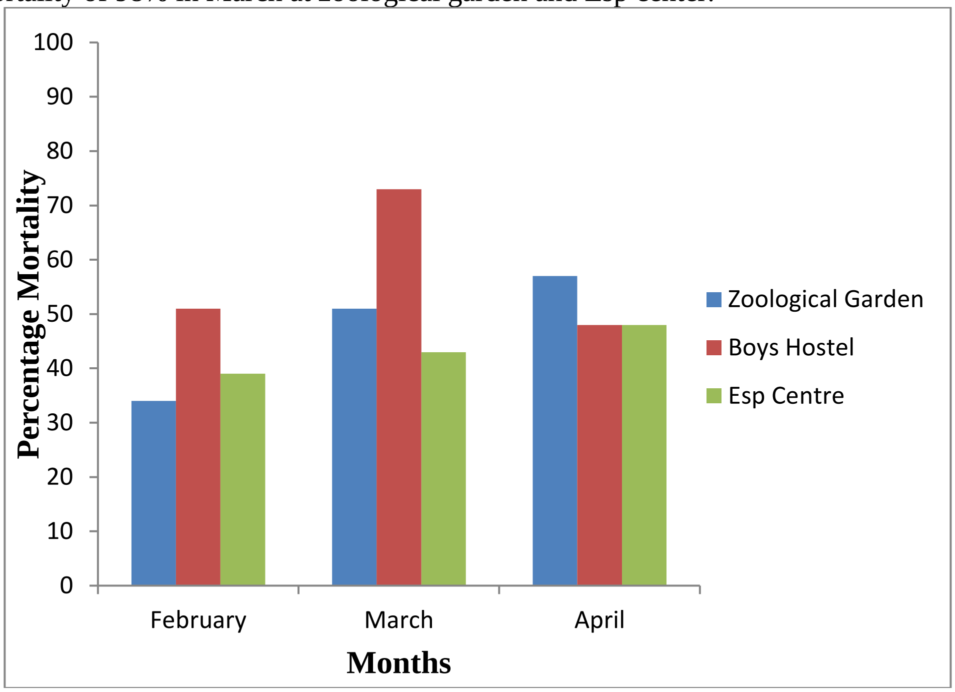


Figure 1: Susceptibility status of Adult female Anopheline mosquitoes to Moringa extract base on locations and months.

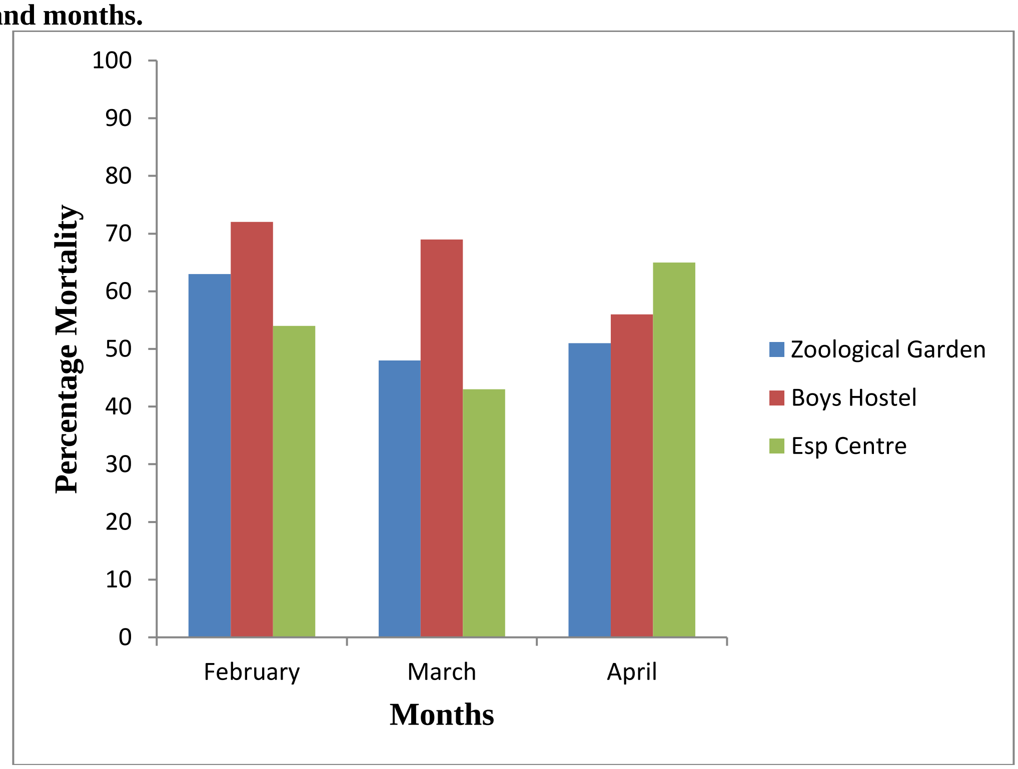


Figure 2: Susceptibility status of Anopheline mosquito Larvae to Moringa extract base on locations and months.

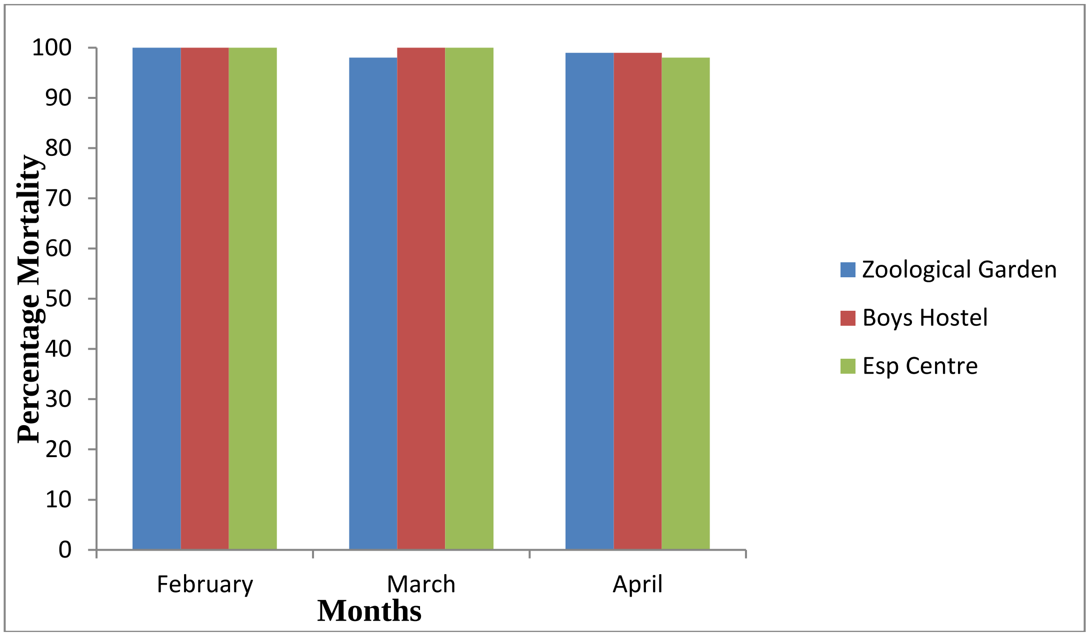


Figure 3: Resistance status of Adult female Anopheline mosquitoes exposed to Deltamethrine insecticides in the months of February to April in three locations.

4.4 Comparison of Susceptibility status of Adult female Anopheline mosquitoes exposed to Moringa plant extract and Deltamethrin pyrethroid insecticides

The results obtained showed that Deltamethrin is More effective in controlling mosquitoes than Moringa extract as all the Mosquitoes exposed to Deltamethrin were susceptible and Moringa extract were resistance . Therefore, there's significant difference in the comparison as p>0.005.

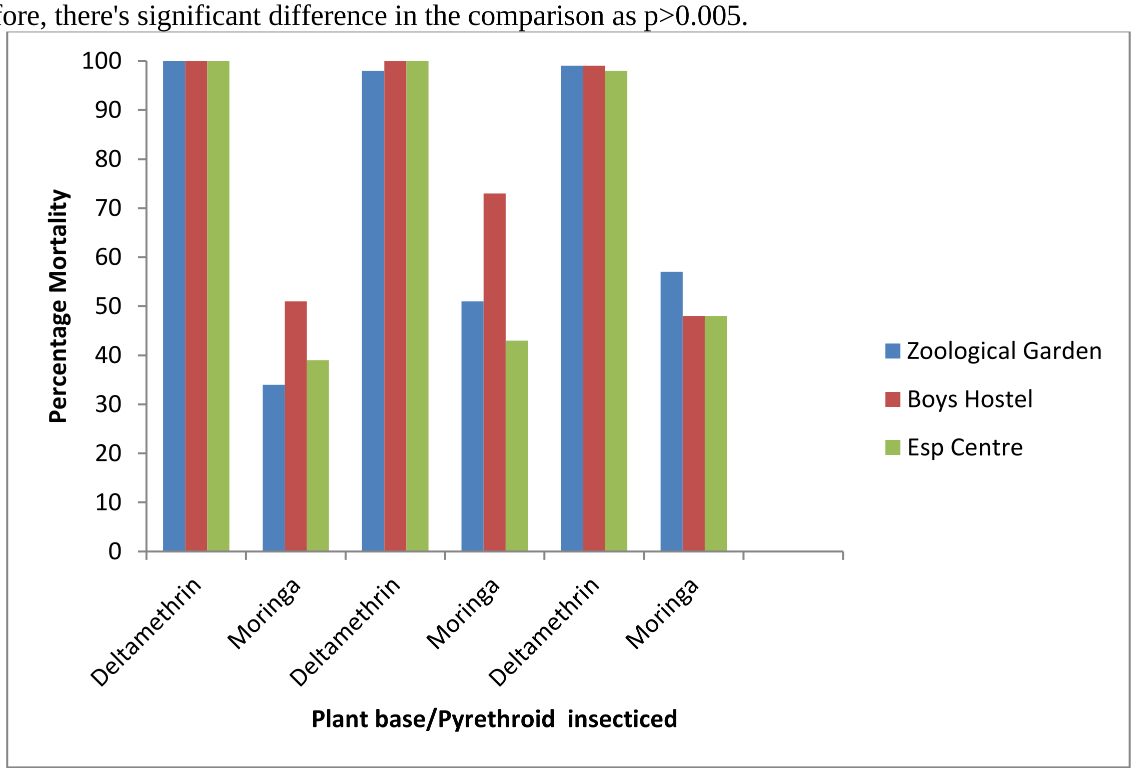


Figure 4: Comparison of Susceptibility status of Adult female Anopheline mosquitoes exposed to *Moringa* plant extract and Deltamethrin pyrethroid insecticides.

DISCUSSION

The control of *Anopheles* mosquito is essential as it is the major and primary vector of malaria infections and many other arthropod-vector related diseases in sub-Saharan Africa; and they also constitute an intolerable biting nuisance (Collins and Paskewitz, 2009). A survey of literature on control of different species of mosquitoes reveal that assessment of the efficacy of different phyto-chemicals obtained from various plants have been carried out by a number of researchers in the field of vector control (Njom and Eze, 2011).

The Moringa extract used in this study showed high larvicidal activity against the larvae of *A. gambiae* s.s and the adult stages. Early reports on the use of plant extracts against mosquito larvae shows that chemicals from plant extracts have effective larvicidal, pupicidal or adulticidal activities on various species of mosquitoes and also at different stages of their life cycles (Njom and Eze, 2011). Ajayi (2008) screened 48 medicinal plants in Nigeria for their antimicrobial activity and 23 of these plants (47.9 %) caused over 70% mortality of the test organism including Anopheline and Culicine larvae. Similarly, Nath *et al.*, (2006) indicated that root extract of *M. oleifera* showed larvicidal activity against *Aedes albopictus* and *Culex quinquefasciatus* at higher doses. In this study, Moringa ethanol extract account for above 70% mortality in some of the study locations. The aqueous extract of M. oleifera leave was also found very effective on *A. gambiae s.s.* to minimize its role in malaria transmission as larval mortalities were observed with the use of the respective concentration doses within the exposure periods in the study of Ohia *et. al.*, (2013).

This study confirmed that the Moringa extract is an effective larvicide since the control had minimal effect (i.e. less than 20% mortality) on mosquitoes according to WHO standard for testing potential larvicide effectiveness (WHO, 2013) and it is certain that the larvicidal effects observed were due to the Aqueous extracts of *Moringa oleifera* seeds(AEMOS). Ferreira *et al.* (2019) also reported that Water extracts of *Moringa oleifera* seeds (WEMOS) were larvicidal against 3rd instar larvae of *Aedes aegypti*, while Ohia *et al.* (2013) found that aqueous extract of *Moringa oleifera* leaves were larvicidal against 3rd instar larvae of *A. gambiae* s.s. The present study showed that extract of *Moringa oleifera* leaves was highly effective as a larvicidal against *A. gambiae* s.s., as it recorded more than 20% mortality this is encouraging and the effect may be due to the active chemical compounds present in the plant. Phytochemicals derived from Moringa plant have been suggested as effective for mosquito vector control agents and plant extracts maybe used for future integrated pest management programs (Prabhu *et al*, 2011).

The toxicity results of *M. oleifera* extracts on *Anopheles* mosquitoes show that its extracts influenced adult survival. This could be explained by the presence of secondary compounds in the extracts, such as Salicylic acid, Quinic acid, Hesperidin, Fumaric acid etc. According to Boulogne and Sciences du Vivant (2011), almost 116 molecules are identified to have insecticidal activity in plant extracts and the molecules most often responsible for this are terpenoids, alkaloids and phenolic compounds. However, the insecticide activity of organic extracts of *M. oleifera* is due to the biological activity of the compounds present in these extracts, which have an anti-nutritional effect and cause respiratory disorders. They inhibit nutrition and cause death and malformations in future generations of phytophagous insects (Carpinella *et al.*, 2003).

Anopheles mosquitoes exposed to Deltamethrin were susceptible 98%-100% mortality was observed at 24 hours. This is because Pyrethroid insecticides works by disrupting the nervous system of *Anopheles* mosquitoes by weakening the insect leading to death. These findings support Aizoun *et al.*, (2013) who observed *Anopheles* mosquitoes' susceptibility to Deltamethrin (100%) mortality. Vatandoost *et al.*, (2019) also reported susceptibility of *Anopheles* mosquitoes to Deltamethrin using CDCbottle bioassay with 100% mortality in Iran. Though, this study is in contrast with the study of Michae *et al.*, (2018) who observed that *Anopheles gambiae* were resistance to Deltamethrin with only 87% mortality. Diagnostic dose is the concentration of insecticide that kills 100% of susceptible mosquitoes within a given time (Brogdon and Chan, 2010). During the determination of diagnostic doses of deltermethrin insecticides against *Anopheles gambiae* s. s showed fast mode of action and this is in line with this study.

Resistance to malaria vectors to the major classes of insecticides currently in use is a potential threat that soon may contribute to absolute failure of the control interventions being employed. This already evident with the reversal of gains made in the fight against malaria as already presented in the latest WHO reported increase of malaria cases (WHO, 2018). Pyrethroids (deltamethrin) susceptibility test in two study sites showed high level of resistance. This results confirms the previous findings in other studies by (Ochomo *et al.*, 2013).This is a clear indication of potential threat to the efficacy of pyrethroids which is used intensively in controlling malaria vector for LLINs treatments as well as in IRS. A proactive approach should be adopted so as to delay the spread or arrest resistance in areas with pyrethroids resistance deterring the effectiveness of the already available insecticides.

CONCLUSION

The report from this study provides clue(s) to what could be expected from a more in-depth investigation of Moringa-based extracts on the malarial vector *A. gambiae.* Based on its activity the Moringa leave extract may be used to control the malaria vector, *A. gambiae s.s.* and will not be toxic to non-target organisms if used within the dosages lethal to the mosquito larvae. Hence, Moringa leave extract can be used in controlling the mosquito larvae and adults in order to reduce the distribution of malaria vectors and also the prevalence of malaria in endemic areas. The phytochemical screening of the plants has also shown that the plants are relatively safe, inexpensive and readily available in many parts of the state. Also the continual usage of this plant base insecticide will be problem caused by the synthetic insecticide already in use that has been causing a lot of resistance due to the continual exposure of the vectors to the insecticides
Deltamethrin also is active insecticides which should be recommended for control of mosquitoes and could be used as one of the intervention by National Malaria Vector Control Programme (NMVCP).

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