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Mosquitocidal activity of *Anacardium occidentale*, *Afromomum melegueta*, *Garcinia kola* and *Citrus sinensis* against the developmental stages of mosquito, *Anopheles gambiae* Giles

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Abstract

The insecticidal potential of *Anacardium occidentale*, *Afromomum melegueta*, *Garcinia kola* and *Citrus sinensis* plants were tested against malaria vector, mosquito, *Anopheles gambiae* in the laboratory at ambient temperature of (28±2)°C and relative humidity of (75±5)%. The oils of the four plants were extracted with hexane and they were prepared at concentration of 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. The larvae and pupae of *An. gambiae* Giles were exposed to these concentrations of the plant oils for 24 hours and mortality was recorded at this periods. At all levels of concentrations, mortality of both the larvae and pupae of this insect increased with increase in the concentrations regardless the type of plant extract used. However, *A. occidentale* oil extract showed more insecticidal effect on both the larvae and pupae of *An. gambiae* at all concentrations but its effect was not significantly ($p>0.05$) different from oil of *A. melegueta* and *C. sinensis* at 0.1% to 0.3% concentrations. The oil extract of *G. kola* showed the lowest mortality effect on both the larvae and pupae of the insect at all concentrations but the effect was significantly ($p<0.05$) different from the control. It was observed that the larvae of *An. gambiae* were more susceptible to the oil extracts of all the plants tested. All the plants extracts used in this work showed high potency to larval and pupal mortality and could consequently be used to reduce prevalence of malaria in the endemic areas.

Keywords Mosquito; Malaria; *Anacardium occidentale*; *Afromomum melegueta*; *Citrus sinensis*; *Garcinia kola*

Introduction

Many developing countries of the world including Nigeria have been noted for high prevalence of malaria disease among their citizens. Nigeria being the most populous country in Africa, more than 50% of her citizens is suffering from at least one episode of malaria every year. Ogungbamigbe et al. (2005) reported that in Nigeria, malaria disease kills more than 25% of her young once and thereby imposing great burden on the country in terms of loss in outputs and cost of treatments. Malaria disease is caused by protozoan parasites of the genus *Plasmodium* transmitted by the female anopheles mosquitoes. Mosquitoes transmit disease to more than 700 million people annually in Africa, South America, Central America, and much of Asia, with Africa being the most

affected continent (WHO, 2010; Akinkulolere et al., 2011). WHO (2010) reported that the primary public health intervention for reducing malaria transmission at the community level is through vector control. It is the only intervention that can reduce malaria transmission from very high levels to close to zero (Akinkulolere et al., 2011).

Over the years, the control of this infamous vector insect has overwhelmingly relied on the use of synthetic chemical insecticides such as organophosphates, organochlorines and carbamates. In spite of the success of many of these synthetic chemical insecticides, they have many cons that are associated with their use; these include pest resurgence and resistance, lethal effects on non-target organisms,

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adverse effect on both human and environmental health (Akinkulere et al., 2011; Ileke and Olotuah, 2012; Ashamo et al., 2013). The public alertness of these adverse effects of synthetic chemical insecticides is impeding their widespread use in the control of insects. Therefore, to make an improvisation to the use of these chancy synthetic chemical insecticides, a lot of research activities have been concentrated on the plant kingdom as new thoroughfare of controlling insect pests. Prior to the discovery and commercial success of the synthetic chemical insecticides in the late 1930s and early 1940s, botanical base insecticides have remained important weapon in the farmers armory in managing insect pest of their farm produce (Forim et al., 2012).

Many botanicals have proved their efficacy as insecticides against a wide range of insects and they are believed to be ecofriendly because they are readily biodegradable and target specific. Tropical countries of the world including Nigeria are well endowed with plants having insecticidal properties (Akinkulere et al., 2006). Until now, despite the effectiveness of many botanical powders and extracts, their insecticidal activity is yet to be comparable to many synthetic chemical insecticides and the once that are believed to be comparable with chemical insecticides have not commanded more than 1% of the global insecticide market (Isman, 2000; Begum et al., 2013) because of their loss of potency over time. Therefore, there is a need to search for other plants that could potentially contend with chemical insecticides in their action. In responding to Nigerian government's quest to fight against malaria disease in the country, this study investigated the potential of *A. occidentale*, *C. sinensis*, *A. melegueta* and *G. kola* against the developmental stages of malaria vector, *Anopheles* mosquitoes.

Discussion

Plant materials have received more attention since government of some countries have started restricting the use of many synthetic chemical insecticides because of their adverse effect on both human and their environment (Isman, 2000). However, plant base insecticides have been noted to have different effects on insects based on the type of botanical used, part of

the plant used, solvent used for the extraction and types of phytochemical compounds present in the plant (Jeyabalan et al., 2003). Jenson et al. (2006.) reported that crude plant extracts are highly efficient for the control of mosquitoes, rather than the purified compounds.

The result obtained in this work showed that plant extracts have significant effect on the mortality of the *An. gambiae* larvae and pupae compared to the controls. Oil extract of *A. occidentale* showed the highest mortality of the insect larvae and pupae throughout the period of exposure. The high mortality of the insect larvae and pupae may be attributed to the active compounds presents in this plant. *A. occidentale* have been reported to contain anacardic acid and cardinal, quercetin, kaempferol, glycosides, triacylglycerols, fatty acids, several unsaponifiable compounds, triterpene, alcohols, sterols and tocopherols (Oliver-Bever, 1986; Rehm and Espig, 1991; Ileke and Olotuah, 2012). All these compounds have high mortality effect against insect larvae (Philipson and Wright, 1991). The oil extract of *C. sinensis* and *A. melegueta* also had a high mortality against both larvae and pupae of *An. gambiae* and this could also be associated with the type of secondary metabolite present in these plants. Toxicity of *Citrus sinensis* can be attributed to strong choky odour disrupting respiratory activity of the insect (Ileke et al., 2013).

At all levels of concentrations and periods of exposure, the larvae of *An. gambiae* were more susceptible to all the plant extracts than their pupae. The high mortality effect by these oil extracts could be related to their ability to block the spiracles through which the larvae of this insect breathe (Kaufmann and Briegel, 2004). Therefore, since the larvae of this insect depend solely on their spiracles for breathing, blockage of the spiracle by these oil extracts could lead to asphyxiation and subsequent death of the larvae (Akinkulere et al., 2006, Ileke and Oni, 2011; Ileke and Olotuah, 2012). The high mortality recorded for the larvae of this insect than its pupae could also be related to the active feeding of the larvae since pupae stages of the insect are not feeding. During feeding, the larvae must have ingested some active compounds in the oil extract of the plants, thereby leading to

stomach poisoning. The findings of this work were in accordance with the result of Al-Dakhil and Morsy (1999), Amusan and Okorie (2002), El-Bokl (2003), Nathan et al. (2005) as well as (Akinkulolere et al. (2011) in which plant oils were found effective against mosquito larvae and pupae. From the results obtained in this research, the effectiveness of the plant extracts could be graded as *A. occidentale*>*S. citirus*>*A. melegueta*>*G. kola*.

Treatment of diseases is not as important as controlling the vector of the disease. In many developing countries like Nigeria where malaria disease is still responsible for the morbidity and mortality of about three million of both their old and young citizens, high level of mosquito breeding has be the major concern (Olasehinde et al., 2010). Also, high level of illiteracy and financial problem had also been a major obstacle in screening out mosquito because many Nigerians could not afford good accommodation (Akinkulolere et al., 2011).

Conclusion

In joining the Nigeria government in their combat against malaria disease and bearing in mind the importance of botanical base insecticides, high level of illiteracy and financial problem of many Nigerians, this research has been able to show the high efficacy of oil extracts of *A. occidentale*, *S. citirus*, *A. melegueta* and *G. kola* against *An. gambiae* larvae and pupae. The problem of finance may not be a major obstacle since these plants are readily available to both poor and the riches.

Results

Tables 1-5 present the mortality effect of different plant extracts on *Anopheles gambiae* at different concentrations. The mortality of *An. gambiae* larvae and pupae increased with increase in plants extracts concentration. At all concentrations, oil extract of *A. occidentale* recorded the highest larvae and pupae mortality and its effect was not significantly ($p > 0.05$) different from other plant extracts. The effect of oil extract of *A. occidentale* was significantly ($p < 0.05$) different from the extract of *G. kola* which recorded the lowest larvae and pupae mortality of *An. gambiae* at all the concentrations used. However, at 0.4% concentration effect of *A. occidentale* on *An. gambiae*

larvae was significantly ($p < 0.05$) different from all other plants extracts. None of the plants extracts was able to cause 100% larvae and pupae mortality except at 0.5% concentration at which the oil extract of *A. melegueta*, *A. occidentale* and *S. citirus* were able to cause complete mortality of *An. gambiae* larvae mortality.

Table 1 Percentage mortality of *Anopheles gambiae* at 24 hours post treatment with 0.1% of plant extracts

Plant extracts	Development stages	
	Larvae	Pupae
<i>A. melegueta</i>	32.00±2.89 ^c	20.00±4.08 ^{cd}
<i>G. kola</i>	17.50±2.50 ^b	10.00±2.13 ^b
<i>A. occidentale</i>	32.50±7.50 ^c	25.00±2.89 ^d
<i>S. citirus</i>	27.50±2.50 ^c	15.00±2.89 ^{bc}
Control	0.00 ±0.00 ^a	0.00 ±0.00 ^a

Note: Each value is a mean ± standard error of four replicates; Means followed by the same letter along the column are not significantly different ($P > 0.005$) using New Duncan's Multiple Range Test

Table 2 Percentage mortality of *Anopheles gambiae* at 24 hours post treatment with 0.2% of plant extracts

Plant extracts	Development stages	
	Larvae	Pupae
<i>A. melegueta</i>	57.50±2.50 ^c	32.50 ±7.50 ^c
<i>G. kola</i>	30.00±4.08 ^b	17.50 ±2.50 ^b
<i>A. occidentale</i>	58.00±2.89 ^c	37.50±2.50 ^c
<i>S. citirus</i>	50.00±5.77 ^c	35.00±2.89 ^c
Control	0.00 ±0.00 ^a	0.00 ±0.00 ^a

Note: Each value is a mean ± standard error of four replicates; Means followed by the same letter along the column are not significantly different ($P > 0.005$) using New Duncan's Multiple Range Test

Table 3 Percentage mortality of *Anopheles gambiae* at 24 hours post treatment with 0.3% of plant extracts

Plant extracts	Development stages	
	Larvae	Pupae
<i>A. melegueta</i>	70.00±0.00 ^c	47.50±2.50 ^c
<i>G. kola</i>	42.50±7.50 ^b	25.00±2.80 ^b
<i>A. occidentale</i>	75.00±2.89 ^c	50.00±5.77 ^c
<i>S. citirus</i>	67.50±2.50 ^c	45.00±2.89 ^c
Control	0.00 ±0.00 ^a	0.00 ±0.00 ^a

Note: Each value is a mean ± standard error of four replicates; Means followed by the same letter along the column are not significantly different ($P > 0.005$) using New Duncan's Multiple Range Test

Table 4 Percentage mortality of *Anopheles gambiae* at 24 hours post treatment with 0.4% of plant extracts

Plant extracts	Development stages	
	Larvae	Pupae
<i>A. melegueta</i>	82.50 ± 7.50 ^c	60.00 ± 4.08 ^c
<i>G. kola</i>	55.00 ± 2.89 ^b	32.50 ± 2.50 ^b
<i>A. occidentale</i>	90.00 ± 4.08 ^d	75.00 ± 2.89 ^d
<i>S. citirus</i>	82.50 ± 7.50 ^c	70.00 ± 4.08 ^d
Control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a

Note: Each value is a mean ± standard error of four replicates; Means followed by the same letter along the column are not significantly different ($P > 0.005$) using New Duncan's Multiple Range Test

Table 5 Percentage mortality of *Anopheles gambiae* at 24 hours post treatment with 0.5% of plant extracts

Plant extracts	Development stages	
	Larvae	Pupae
<i>A. melegueta</i>	100.00 ± 0.00 ^c	72.50 ± 7.50 ^c
<i>G. kola</i>	65.00 ± 2.89 ^b	47.50 ± 2.50 ^b
<i>A. occidentale</i>	100.00 ± 0.00 ^c	90.00 ± 4.08 ^d
<i>S. citirus</i>	100.00 ± 7.50 ^c	85.00 ± 2.89 ^d
Control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a

Note: Each value is a mean ± standard error of four replicates; Means followed by the same letter along the column are not significantly different ($P > 0.005$) using New Duncan's Multiple Range Test

Materials and Methods

Collection of plant materials

The nuts of *A. occidentale*, seeds of *A. melegueta*, *G. kola* and peels of *C. sinensis* were bought at Oba market, Akure, Ondo State, Nigeria. The collected plant materials were taken to Research Laboratory, Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo state for authentication. The plant materials were air dried in the laboratory and milled into fine powders and were kept inside different covered plant containers until further use.

Extraction of plant materials

The oil extracts of the plant materials were made by soaking 200g of each powder in 2litres of n-hexane and heated in water bath at 60°C for 1h and decanted. The mixtures were stirred occasionally with a glass rod. The resulting mixture was filtered using a double

layer of Whatman No. 1 filter paper and the solvent evaporated using a rotary evaporator at 30 to 40°C with rotary speed of 3 to 6 rpm for 8 hours (Udo 2011). Each of the extracted oils was kept inside covered containers for subsequent use.

Mosquito baits, consisting of shallow containers with a large surface area were established under a partial shade in an open field. The container was filled with rain water to mimic mosquito natural breeding environment and to attract adult female for oviposition. Small quantity of industrial yeast was sprinkled on the surface of the water and allowed to decompose slowly to nourish the developing larvae. Wild mosquitoes were allowed to freely visit the baits and to lay eggs and the baits were later transferred to the laboratory where larvae and pupae were identified using stand methods and maintained at temperature of (28 ± 2)°C, (75 ± 5)% and 14:10 L:D relative humidity.

Effect of plant extracts on larvae and pupae of *An. gambiae*

Larvicidal and pupacidal activity of the plant extracts was carried out at different concentrations by preparing the required stock solutions following the standard procedure described by WHO (1996). The desired concentrations were achieved by adding 1.0 µg of crude extract of any of the four plant extracts to 100 ml of de-chlorinated water. From this, five concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% of each plant extracts were prepared. The extracts were mixed with water in a beaker at the desired concentration in the presence of small amount of yeast powder to serve as food source for the larvae. Then 10 larvae and pupae of *An. gambiae* were introduced into separate beaker. Beakers containing only water were set as control and were also infested with larvae and pupae of the insect. There were three replicates for each concentration and the control. Mortality was observed over 24 h, after which the larvae and pupae were introduced into distilled water to notice recovery. A recovery time of 5 minutes was allowed (WHO, 1996). The larval mortality in treatments was corrected for the controls (Abbott, 1925). Larvae and pupae were counted as dead when they were not coming to the surface for respiration and were insensitive to probe (Sivagnaname and Kalyanasundaram, 2004;

Akinkulore et al., 2011).

Data analysis

Data were subjected to analysis of variance (ANOVA), and means were separated using the New Duncan's Multiple Range test performed with statistical package for Social Sciences (SPSS) 16.0 Software (SPSS, Inc. 2007).

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