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Research Article

MANAGEMENT OF *ANOPHELES* MOSQUITO LARVAE AND ADULT WITH *ZINGIBER OFFICINALE* POWDER AND OIL EXTRACT

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ABSTRACT

This research evaluated the effectiveness of powder and oil extracts of *Zingiber officinale* against larva and adult stages of *Anopheles* mosquito. Water sample was obtained from stagnant pool of water within the environment of University of Lagos and adult female *Anopheles* mosquitoes were allowed to lay eggs after which was transported to the laboratory where the larva and the adult stages of the mosquito emerged. Rhizoids of *Z. officinale*s was obtained from farm in Ondo town, Ondo West Local Government Areas of Ondo State and transported to the Entomology Research Laboratory Federal University of Technology, Akure Ondo State where they were processed into fine powder. Oil extraction was carried out using absolute ethanol in a soxhlet extractor and redistilled using rotary evaporator. Different concentrations of the oil extract (2 %, 4 %, 6 %, 8 % and 10 %) and plant powder (1 g-5 g) of *Z. officinale* were prepared after which larval and adult stages of *Anopheles* mosquito were exposed to the treatment for the period of 30 min, 1 hour, 1 hour 30 min and 2 hours. The result obtained showed that the oil extract of *Z. officinale* at all concentrations had significant effect on larvae and adult mosquito at both contact and fumigant toxicity with percentage mortality range of 70-100% within 120 min of exposure period ($P<0.05$). The aqueous extract of *Z. officinale* was relatively ineffective against the larval stage of the mosquito at all levels of concentration however, the fumigant effect of the powder was also effective against the adult stage of the mosquito which invoked 73-90% mortality at 4g and 5g concentrations ($P<0.05$). The result of this study revealed that oil extract of *Z. officinale* as both contact and fumigant were very effective against both larvae and adult stages of *Anopheles* mosquito, thus can serve as an to synthetic insecticides which could pose serious health hazard to human.

Keywords: *Anopheles* mosquito; *Z. officinale*; Powder; Oil extract; Toxicity

INTRODUCTION

Most insects have been incriminated as vectors of different parasitic diseases. Amongst all insects, mosquitoes (Diptera: Culicidae) have been reported to vector lots of these parasitic diseases which are very deadly to populace in most tropical and subtropical region of the world. Mosquitoes are vectors of parasitic diseases such malaria, dengue fever, Lymphatic filariasis, encephalitis, west nile virus, yellow fever, zika virus etc. (Becker et al., 2003). Worldwide, about 3000 species of mosquitoes known, of all these species, only about 100 have been reported to have the ability of transmitting parasitic diseases. Globally, it has been reported that mosquito borne diseases infect over 700 million people every year being prevalent in more than 100 countries (Taubes, 2000). In 2010, the World Health Organization (WHO) estimated that about 216 million cases of malaria (vector by *Anopheles* mosquito) resulting to 655,000 deaths. About 91 % of these deaths occurred in sub-Saharan Africa, and were mostly children under 5 years of age (WHO, 2011). Nigeria having a very large population size is always at risk of malaria infection causing about 1 million deaths annually (WHO, 2010). Also, Lymphatic filariasis has been reported to affect at least 120 million people globally, including Africa, India, Southeast Asia and Pacific Islands (Bagavan and Rahuman, 2010). In areas where mosquito borne parasitic diseases are endemic, these diseases does not cause high level of mortality and morbidity alone but also cause great economic loss and social disruption (Ciccia et al., 2000).

Awad and Shimaila (2003) stated that the principal objective of vector control is the reduction in morbidity and mortality due to malaria and other diseases transmitted by mosquitoes; this is achieved by reducing level of transmission. The use of synthetic insecticides for vector control has been in use for decades but the toxicity effect of the synthetic chemicals to human and the environment have been a major public health concern (Poopathi and Archana, 2010). Synthetic insecticides are very effective in their use, in addition to their effectiveness, they pose serious health problem to human. Apart from this health issue, the cost of these chemicals is very expensive (Cartilla and Dela, 2012). However, the intensive use of synthetic insecticides for very long period of time has made insects developed resistance

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against their use thus reducing the effectiveness of these pesticides for vector control (Charles and Nielsen-Leroux, 2000).

Haven't been aware of this hazardous effect of synthetic insecticides on human, plants and the environment, this has prompted scientists to develop other effective means that are environmental friendly through which vectors can be controlled (Montasser et al., 2011). However, this has brought about the development of alternatives such as botanicals to produce insecticides that are very effective for vector control.

MATERIALS AND METHODS

Study Area

The research was carried out at the University of Lagos, Akoka, Lagos State, Nigeria. The University is located in Lagos State, a Southwestern region of Nigeria. Lagos State has two seasons, which includes the wet season which ranges from March to October and the dry season which ranges from November to February. The research was conducted during the wet season from April to July, 2017.

Collection and Culture of Mosquito

Water sample was obtained from stagnant pool of water within the University environment and transferred into a transparent plastic bowls and transported to the Zoological garden of University of Lagos where it was placed in a shady area. After few days (about 7 days) it was observed that there was emergence of mosquito larvae from egg that has been laid by adult Mosquitoes.

The plastic containing the larvae was transported to the Entomology Research Laboratory after which the larvae were identified and separated into different Genera. The larvae were first separated using their resting habit on waster surface. *Anopheles* larvae, lying parallel to the water surface were separated after which morphological keys of Gillies and De Meillon for *Anopheles* mosquito larvae and adults was used for proper identification (Gillies and De Meillon, 1968). Only *Anopheles* mosquito larvae were transferred into another container and placed in a cage designed with mosquito net to avoid the escape of the adult mosquitoes during emergence. During the larva stage, the larvae were fed with yeast until the adult mosquitoes emerged. The adult *Anopheles* mosquitoes fed on the blood of a live albino rat that was placed in the cage. The culture was maintained at a temperature of 28 ± 20C.

Preparation of Plant Materials

Plant Powder: Rhizoids of *Zingiber officinale* was obtained at Akure, Ondo State and transported to the Entomology Research Laboratory where they were cut into smaller pieces and air dried. After air drying, the pieces of the rhizoids were pounded using pestle and mortar after which was finally pulverized into fine powder with the use of electric blender. The powder was stored in an air tight plastic container at ambient temperature of 28 ± 20C until use.

Plant Oil Extraction: 150 g of the pulverized plant material was weighed into thimble containing 500 ml of absolute ethanol for 72 hours. The mixture was agitated occasionally each day for 30 min with a glass rod and extraction was terminated after 72 hrs. The mixture was filtered with the use of muslin bag, the ethanolic extract was obtained while the solvent was evaporated using a rotary evaporator (Resona, model: SW 200) with rotary speed of 3 to 6 rpm for 8 hours. The oil was stored in a plastic plate and kept in a refrigerator until use.

Contact effect of Aqueous Extract of *Zingiber officinale* on *Anopheles* Mosquito Larvae

The following concentrations; 1 g, 2 g, 3 g, 4 g and 5 g of *Z. officinale* powder were soaked into a 100 ml of distilled water for 24 hrs. and then filtered separated using whatman's No 1 filter paper. The filtrate was collected into a flask and corked. With the use of syringe, 10 ml of each concentration prepared was transferred into petri dishes of 9 cm diameter and 3 cm depth. 10 third instar larvae of *Anopheles* mosquito was introduced into the treated water. Mortality was recorded after 30 min, 1 hour, 1 hour 30 min and 2 hours. Each concentration was replicated three times, also, the larvae was introduced into untreated control water.

Larvae mortality rate was calculated using the formula, (Number of dead larvae)/(Total number of larvae introduced) × 100.

Contact effect of Oil of *Zingiber officinale* on *Anopheles* Mosquito Larvae

Concentrations 2 %, 4 %, 6 %, 8 % and 10 % of the oil were measured into petri dishes of 9 cm diameter and 3 cm depth containing 10ml of distilled water. Ten (10) third instar larvae of *Anopheles* mosquito were introduced into the treated water. Mortality was recorded after 30 min, 1 hour, 1 hour 30 min and 2 hours. Each concentration was replicated three times, also, the larvae was introduced into untreated water.

Larvae mortality rate was calculated using the formula, (Number of dead larvae)/(Total number of larvae introduced) × 100.

Fumigant effect of Powder of *Zingiber officinale* on Mortality of Adult *Anopheles* Mosquito

Different dosage (1 g-5 g) of the plant powder were weighed in muslin cloths sown into sack of 3 cm by 2 cm dimension and suspended with the aid of thread at a distance of 6 cm from the lid of plastic containers of dimension 13 cm depth and 12 cm diameter. Ten adult *Anopheles* mosquitoes (about 48 hours old) were introduced into the plastic containers, each containing the suspended bags of plant powders. The experiment was replicated three times and adult mortality was recorded after 30 min, 60 min, 90 min and 120 mins. The control experiment was also setup and replicated.

Fumigant effect of Oil Extract of *Zingiber officinale* on Mortality of Adult *Anopheles* Mosquito

The fumigant effect of the oil extract on adult *Anopheles* mosquitoes was tested by measured 1 %, 2 %, 3 %, 4 % and

5 % of the oil extract and poured into 10 ml of ethanol inside a petri dish of 9 cm diameter and 3 cm depth. Whatman's No. 1 filter paper of dimension 2 cm by 2 cm were formed into strip and then soaked into each concentration, after which was allowed to air dried for 2 hours. The treated filter papers were then place inside a plastic container of dimension 13 cm diameter and 12 cm depth. Ten adult mosquitoes (about 48 hours old) were introduced into the container with the treated filter paper and then covered with lid. Each experiment was replicated three times after which adult mortality was recorded after 30 min, 60 min, 90 min and 120 min. The control experiment was also replicated.

Statistical Analysis

Data generated from the research were analysed using Statistical Package for Social Sciences (SPSS) for windows version 20.0. The statistical parameters used were Analysis of Variance (ANOVA) and Duncan Multiple Range Test was used to separate the means at 95 % confidence level.

RESULTS

Contact Toxicity effects of Oil Extract on Mortality of *Anopheles* Mosquito Larvae

Table 1 shows the result of the contact toxicity of oil extract of *Zingiber officinale* on larvae mortality of *Anopheles* mosquitoes. At 2 % oil concentration, 16.67 %, 33.33 %, 46.67 %, 63.33 % percentage mortality was obtained at 30 min, 60 min, 90 min and 120 min exposure period respectively. At concentrations 6 %, 8 % and 10 % oil extract, 93-100 % larval mortality were obtained at 120 min exposure period. It was observed that no concentration of the oil extract attained 90 % larval mortality except 10 % rate. At all levels concentration and exposure periods the value obtained were significantly different from the control ($P<0.05$).

Contact Toxicity of Aqueous Extract on Mortality of *Anopheles* Mosquito Larvae

The result of the contact toxicity of *Z. officinale* aqueous extract on *Anopheles* mosquito larvae showed that 1 % of the aqueous extract powder caused no mortality against the mosquito larvae, however, the highest mortality of 86.87 % was recorded for 5 % aqueous extract powder concentration. The 2 % and 3 % rate of the aqueous extract powder concentration significantly recorded the least larval mortality at 30 min and 60 min exposure periods. Values obtained were significantly different from the control samples ($p<0.05$) (Table 2).

Each value is the mean ± Standard error of 3 replicates. Mean followed by the same letters are not significantly different from each other using New Duncan's Multiple Range Test ($p<0.05$).

Fumigant Toxicity of *Z. officinale* powder on mortality of Adult *Anopheles* Mosquito

Table 3 shows the fumigant effect of *Z. officinale* powder on mortality against adult *Anopheles* mosquito. Result obtained indicated that at 1 g powder concentration, percentage mortality obtained at 60-120 min exposure period ranges from 6.67–36.67 %. At 2 g and 3 g concentration, 53.33-66.67 % adult mortality was obtained at 120 min exposure period. 4 g and 5 g concentration rates of the powder caused 73.33-90.00 % adult mortality of the mosquito and were significantly different from the control($P<0.05$) and other treated samples.

Table 1: Contact Toxicity of Oil Extract on Larval Mortality of *Anopheles* Mosquito.

		Percentage Mortality after			
Concentration (%)	**No of Mosquitoes**	**30**	**60**	**90**	**120**
2	10	16.67 ± 3.33^{b}	33.33 ± 8.82^{b}	46.67 ± 6.67^{b}	63.33 ± 3.33^{b}
4	10	33.33 ± 3.33^{c}	60.00 ± 5.77^{c}	76.67 ± 12.02^{c}	90.00 ± 5.77^{c}
6	10	36.67 ± 8.82^{cd}	53.33 ± 8.82^{c}	73.33 ± 6.67^{c}	93.33 ± 0.00^{c}
8	10	43.33 ± 3.33^{cd}	63.33 ± 3.33^{c}	80.00 ± 0.00^{c}	100.00 ± 0.00^{c}
10	10	50.00 ± 5.77^{d}	93.33 ± 3.33^{d}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
Control	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is the mean ± Standard error of 3 replicates. Mean followed by the same letters are not significantly different from each other using New Duncan's Multiple Range Test ($p<0.05$).

Table 2: Contact Toxicity of Aqueous Extract powder on Larval Mortality of *Anopheles* Mosquito.

		Percentage Mortality after			
Concentration (%)	**No of Mosquitoes**	**30**	**60**	**90**	**120**
1	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
2	10	16.67 ± 6.67^{bc}	16.67 ± 6.67^{bc}	16.67 ± 6.67^{ab}	26.67 ± 6.67^{b}
3	10	6.67 ± 3.33^{ab}	6.67 ± 3.33^{ab}	20.00 ± 0.00^{ab}	33.33 ± 3.33^{b}
4	10	23.33 ± 6.67^{c}	23.33 ± 6.67^{c}	30.00 ± 10.00^{b}	40.00 ± 5.77^{b}
5	10	23.33 ± 6.67^{c}	43.33 ± 6.67^{d}	70.00 ± 11.55^{c}	86.87 ± 8.83^{c}
Control	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is the mean ± Standard error of 3 replicates. Mean followed by the same letters are not significantly different from each other using New Duncan's Multiple Range Test ($p<0.05$).

Table 3: Fumigant Toxicity of *Z. officinale* powder on mortality of Adult *Anopheles* Mosquito.

	Percentage Mortality per Minute				
Concentration (g)	**No of Mosquitoes**	**30**	**60**	**90**	
1	10	0.00 ± 0.00^{a}	6.67 ± 3.33^{ab}	20.00 ± 5.77^{b}	
2	10	0.00 ± 0.00^{a}	13.33 ± 3.33^{bc}	30.00 ± 0.00^{bc}	66.67 ± 6.67^{c}
3	10	0.00 ± 0.00^{a}	10.00 ± 0.00^{ab}	30.00 ± 5.77^{bc}	53.33 ± 6.67^{bc}
4	10	0.00 ± 0.00^{a}	23.33 ± 6.67^{c}	43.33 ± 8.82^{cd}	73.33 ± 8.82^{cd}
5	10	0.00 ± 0.00^{a}	23.33 ± 6.67^{c}	53.33 ± 3.33^{d}	90.00 ± 0.00^{d}
Control	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	

Each value is the mean ± Standard error of 3 replicates. Mean followed by the same letters are not significantly different from each other using New Duncan's Multiple Range Test ($p<0.05$).

Table 4: Fumigant Toxicity of *Z. officinale* oil extract on mortality of Adult *Anopheles* Mosquito.

	Percentage Mortality after				
Concentration (%)	**No of Mosquitoes**	**30**	**60**	**90**	
1	10	6.67 ± 3.33^{ab}	23.33 ± 6.67^{b}	46.67 ± 3.33^{b}	
2	10	10.00 ± 0.00^{bc}	30.00 ± 0.00^{b}	50.00 ± 5.77^{b}	80.00 ± 0.00^{b}
3	10	13.33 ± 3.33^{bc}	30.00 ± 5.77^{b}	50.00 ± 0.00^{b}	80.00 ± 0.00^{b}
4	10	16.67 ± 3.33^{a}	36.67 ± 3.33^{b}	63.33 ± 3.33^{d}	96.67 ± 3.33^{c}
5	10	23.33 ± 3.33^{a}	56.67 ± 6.67^{c}	96.67 ± 3.33^{d}	100.00 ± 0.00^{c}
Control	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	

Each value is the mean ± Standard error of 3 replicates. Mean followed by the same letters are not significantly different from each other using New Duncan's Multiple Range Test ($p<0.05$).

Fumigant Toxicity of *Z. officinale* oil extract on mortality of Adult *Anopheles* Mosquito

The result of the fumigant effect of *Z. officinale* oil extract against adult *Anopheles* mosquito is shown in Table 4. At 1 % concentration of the oil extract, 6.67-46.67 % adult mortality of the mosquito was achieved at 300 min to 90 min exposure period. At 3 %, 4 %, and 55 concentrations of the oil extract, 50.00-96.67 % mortality rate were recorded at 90 min post treatment period. At 4 % and 5 % concentrations of the oil extract 96.67-100.00 % mortality were obtained at 120 min period of exposure. All the values obtained at all rate were significantly higher than the control ($p<0.05$).

DISCUSSION

The toxicity effect of oil extract and powder of *Zingiber officinale* on larva and adult *Anopheles* mosquito were evaluated in this study. In this present study, the assessment of oil extract of *Z. officinale* shows that at all concentrations (2 %, 4 %, 6 %, 8 % and 10 %) achieved 80-100 % larvae mortality within 120 min period of exposure. This result is similar to the result obtained by Oke et al., (2011) who reported that hexanolic extract of *Piper guineense* achieved both 77 % and 95 % mortality for *Aedes aegypti* larvae within 1-24 hours period of exposure. The mortality of the larvae in this study could be as a result of the pungent odour of the plant oil. Early study on *Z. officinale* dried rhizoid contains bioactive ingredients such as; shogaols, dehydrated gingerol derivatives as its constituents which gives it a pungent odour. Also, it has been reported that plants with pungent odour have high bioactivity against insects (Dupriez and De-Leener, 1998). Plants oil is commonly used for insect control because they are relatively bioactive against virtually all stages of the life cycle of insects (Aranilewa et al., 2006).

The contact insecticidal activity of the aqueous extract of *Z. officinale* was relatively ineffective against the larvae of *Anopheles* mosquito. There was no mortality recorded during the larvae exposure to 1 g concentration for 30 min, 60 min, 90 min and 120 min post treatment, which could be as a result of the water used in the extraction as a polar solvent (Akinneye et al., 2014).The fumigant effect of the ethanolic oil extract against the adult *Anopheles* mosquito in this study achieved high mortality within 120 min post-treatment. This observation is in agreement with the report of Akinkurolere et al., (2011) who opined that oil extract of *Xylopia aethiopica* is highly toxic to the larvae and adults of *Anopheles gambiae* mosquito due to the strong pungent smell of the plant. Also, Fafioye et al., (2004) reported that the ethanolic oil extract of *Parkia biglobasa* and *Raphia vinifera* were more potent against the juvenile of *Clarias garapinus* than the aqueous form. This could be as a result of the polarity, volatility and power of the ethanol to dissolve more of the active ingredients of the plant than the aqueous solution. The result of this current research revealed that the powder of *Z. officinale* has slight effect on the mortality of the adult mosquito; the slight effect of the powder could be attributed to the chemical constituents of the plant powder which might have caused neurological disorder in the adult insect.

In general, the result obtained from this research revealed that the powder and oil as fumigants possess insecticidal properties as they caused the mortality of adult stages of *Anopleles* mosquito and on the other hand the aqueous extract of the powder and oil extract were also found effective against larvae and adult of *Anopheles* mosquito. However, the oil extract from *Z. officinale* compare to its powder extract has very high larvicidal as well as insecticidal effects on both

stages of *Anopleles* mosquito. It has also been reported in previous researches that *Z. officinale* has both lavicidal and insecticidal effects on other insects. Pushpanathan et al., (2008) who reported on the essential oil of *Z. officinalis* as mosquito larvicidal and repellant agent against Culex quinquefasciatus that *Z. officinalis* oil has significant lavicidal effect on the mosquito larvae. Pushpanathan et al., (2008) comparing their result with earlier reports of Singh et al., (2003) who worked on lavicidal activity of *Ocimum canum* against vector mosquito and Traboulsi et al., (2005) who worked on lavicidal activity of *Citrus sinensis*, *Eucalyptus* spp, *Ferrula hermonis*, *Laurus nobilis* and *Pinus pinea* against *C. pipiens* revealed that *Z. officinalis* oil has more lavicidal effect than the reported plants. *Z. officinale* is likely to have almost the same bioactive constituents as *Z. officinalis* since they belong to the same genus. Although the aim of this work does not involve the identification of various bioactive constituents of *Z. officinale* but previous studies on this plant revealed that it contains β-sesquiphellandrene, cis-caryophyllene, zingiberene, α-farnesene and ar-curcumine and geranial as its main constituent (Singh et al.,2008; El-Baroty et al., 2010; Nampoothiri et al., 2012; Abdurahman et al.,2013). Therefore, *Z. officinale* having high toxicity effect of oil extract and powder on larva and adult *Anopheles* mosquito could be as a result of the synergistic effect of different bioactive constituents of the plant on the different stages of the mosquito.

CONCLUSION

The result of this study revealed that oil extract of *Z. officinale* as both contact and fumigant were very effective against both larvae and adult stages of *Anopheles* mosquito compared to the powder extract. Therefore, the use of oil extract of *Z. officinale* as larvicide and fumigant insecticide against larvae and adult stages respectively should be highly encouraged than the use of synthetic insecticides as products from plant origin are eco-friendly than chemicals whose constituents could pose serious health problem to human.

CONFLICTS OF INTEREST

Authors declare that there exit no conflict of interest.

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