J. Entomol. Res. Soc., 19(1): 121-127, 2017 *ISSN:1302-0250*

Larvicidal and Pupicidal Potential of *Aframomum melegueta* K. Schum Extracts Against Mosquito, *Anopheles* Species

Kayode David ILEKE[1] Jacobs Mobolade ADESINA[2,3*]
Oladoyin Ganiyat OKUNOLA[1]

[1]Department of Environmental Biology and Fisheries, Adekunle Ajasin University, PMB 001, Akungba Akoko, Ondo State, NIGERIA
[2]Insect Chemical Ecology Laboratory, Institute of Bioresources and Sustainable Development, Takyelpat, Imphal, 795001, Manipur, INDIA
[3]Department of Crop, Soil and Pest Management Technology, Rufus Giwa Polytechnic, P. M. B. 1019, Owo, Ondo State, NIGERIA
e-mail: *mobolade72@gmail.com

ABSTRACT

The use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. The use of herbal products is one of the best alternatives for mosquito control. The present study was to screen the larvicidal and pupicidal potential of *Afromomum melegueta* leaf and seed extracts against larvae and pupae of *Anopheles* species in the laboratory at ambient temperature of 28±2°C and relative humidity of 75±5%. The extracts of the plant parts were extracted with methanol and they were prepared at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5. The larvae and pupae of *Anopheles* species were exposed to these concentrations of the plant extracts for 24 hours and mortality was recorded. At all levels of concentrations, larval and pupal mortalities of this insect increased with increase in the concentrations irrespective of the type of plant parts extract used. However, seed extract of *A. melegueta* showed more insecticidal effect on both the larvae and pupae of *Anopheles* species at rate 0.1% but its effect was not significantly ($p>0.05$) different from extract of *A. melegueta* at 0.2%, 0.3%, 0.4% to 0.5% concentrations. It was observed that the larvae of *Anopheles* species were more susceptible to the extracts of the plant parts tested. All the plant parts extracts used in this work showed high effectiveness to larval and pupal mortalities and could therefore be used to reduce occurrence of malaria incidence. Their adoption might prove to be an effective and eco-friendly herbal insecticides in ensuring maximum safety in any ecological condition.

Key words: Afromomum melegueta, *Anopheles,* concentrations, larvicidal, pupicidal.

INTRODUCTION

Malaria remains one of the most prevalent diseases in the tropical world and Nigeria alone accounts for over 40 percent of the estimated total malaria death globally. Mosquito-borne diseases have an economic impact, including loss in commercial and labour outputs, particularly in countries with tropical and subtropical climates (Srinivasan *et al*., 2014).

Anopheles stephensi Liston is one of the primary vectors of malaria in many tropical and subtropical countries. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. An obvious method for the control of mosquito borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. The use of conventional pesticides in the water sources for mosquito control introduces many risks to people and the environment. It has also provoked undesirable effects, including toxicity to non-target organisms, and fostered environmental and human health concerns (Muthusamy *et al*., 2014). These problems have necessitated the need for search and development of alternative strategies using eco-friendly, environmentally safe, biodegradable and low cost natural products.

In recent years, natural products of plant origin with insecticidal properties have been tried in the recent pest control of variety of insect pests and vectors around the world.

Aframomum melegueta (*Zingberaceae*) K. Schum., known as Alligator pepper, is an herbaceous tropical perennial West Africa spice plant which imparts a pungent peppery flavor with hints of citrus and used mainly as food, in brewing, and in both veterinary and traditional medicine (Igwe *et al.,* 1999). It is believed to have purgative and hemostatic properties and also to be very effective against schistomiasis (Alaje *et al.*, 2014). Various authors had reported the potential of *A. melegueta* for the management of stored products' insect pests (Ofuya, 1990; Adedire and Lajide, 1999; Adeyemo *et al.*, 2014; Onekutu *et al.*, 2015; Adesina *et al.*, 2015). However, there appears to be dearth of information on the empirical utilization of *A. melegueta* for the control of malaria vector.

Therefore this study was conceived to determine the insecticidal effect of *A. melegueta* leaf and seed extracts on larvae and pupae of *Anopheles* mosquito as potential bio-insecticide in the integrated management of malaria vectors.

MATERIALS AND METHODS

Collection and rearing of mosquito larva and pupa

Mosquitoes' baits in opaque coloured shallow containers with a large surface area were established in the Hatchery Laboratory, Department of Environmental Biology and Fisheries, Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria. The opaque coloured container was filled with rain water in order to mimic mosquito's natural breeding environment and also to attract adult mosquitoes for oviposition. Small quantity of industrial yeast was sprinkled on the water surface and allowed to decompose slowly to nourish the developing larva. Wild mosquitoes were allowed to freely visit the bait and to lay eggs. Thereafter, the containers bearing mosquitoes larvae and pupae were transferred to the laboratory, identified and maintained at temperature of 28 ± 2 °C and 75 ± 5 % relative humidity.

Collection of plant materials and preparation

Fresh leaves and seeds of *A. melegueta* were obtained free of pesticides from Kowode market in Ikare Akoko, Ondo State, Nigeria and authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State. The plant materials were rinsed in clean water to remove dirt and air dried in a well ventilated laboratory and ground into very fine powder using an electric blender. The powder was further sieved to pass through $1mm^2$ perforations, and packed in plastic containers with tight lids and then stored in a refrigerator at 4 °C prior to use.

Methanol extracts of *A. melegueta* were carried out using cold extraction method. About 150g of *A. melegueta* leaf and seed powders were soaked separately in an extraction bottle containing 99.5% methanol for 72 hours. The mixture was stirred occasionally with a glass rod and the resulting mixture was filtered using a double layer of Whatman No. 1 filter paper and the solvent was evaporated using a rotary evaporator at 30 to 40 °C with rotary speed of 3 to 6 rpm (Udo, 2011). The resulting materials were air dried in order to remove traces of solvents. The crude extracts were kept in a labeled amber specimen bottle and preserved in the refrigerator till further use.

Effect of *A. melegueta* extracts on larvae and pupae of *Anopheles* mosquito

Larvicidal and pupacidal activities of the plant extracts were carried out at different concentrations by preparing the required stock solutions following the standard procedure (WHO, 1996). The desired concentrations were achieved by adding 1.0 µg of the crude extract from leaves of as well as seeds to 100 ml of distilled water. From this, five concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% of the plant extracts were prepared. The treatments were separately added to 2.5l of water inside a bowl and yeast powder was added in order to provide source of food for the introduced larvae. Twenty larvae and pupae of *Anopheles* species were separately introduced into the treated water and untreated water was set as control. Each treatment was replicated three times. Mortality was observed over 24 hours after the introduction of larvae and pupae to notice recovery; a recovery time of 5 min was allowed. The larval mortality in treatments was corrected for the controls. Larvae and pupae were counted as dead when they were not coming to the surface for respiration and were insensitive to probe.

Phytochemical screening of *A. melegueta* leaves and seeds

Qualitative phytochemical constituents screening was carried out on the methanol extracts using standard laboratory procedures as described by Harborne (1973); Trease and Evans (1985); Sofowora (1993).

Analysis of data

Data collected were subjected to analysis of variance (ANOVA) using statistical package for social sciences (SPSS) 16.0 software (SPSS, inc., 2007) and significant treatment means were separated using the new Duncan's Multiple Range Test at 5% probability level.

RESULTS

Effects of *A. melegueta* on *Anopheles* species

Table 1 presented the mean percentage mortality of larvae and pupae of *Anopheles* species at 24 hours after exposure to different concentration levels of *A. melegueta*. The result indicated that mortality of *Anopheles* specie larvae and pupae is directly proportional to increase in the *A.melegueta* products concentration levels.

At 0.1% *A. melegueta* leaves extract caused 40% larval mortality and 35.37% pupal mortality while the seed extract caused 65.37% larvae mortality and 50% pupae mortality and at 0.2% leaves extracts of *A. melegueta* caused 70% larval mortality of mosquito and 60% pupal mortality while the seed extracts recorded 80% larval and 70% pupal mortality respectively. The developmental stages of *Anopheles* species exposed to 0.3% *A. melegueta* leaves extracts caused 90% larval mortality and 85.37% pupal mortality while the seed extracts caused 100% larval mortality and 95.37% pupal mortality; while the plant products evoked 100% mortalities on both larvae and pupae when exposed to 0.4% *A. melegueta* (Table 1), no mortality was observed in the control treatment.

Treatment means showed that the leaves and seeds extracts had significant difference on the larval and pupal mortalities compared to the control when applied 0.1% concentration and non-significant treatment means was observed on the developmental stages of *Anopheles* species exposed to both leaves and seeds extracts at 0.2-0.4% when compared statistically (Table 1).

Phytochemicals screening of *A. melegueta*

Table 2 presented the result of the phytochemical screening of leaves and seeds of *A. melegueta* methanol extracts. The phytochemicals of *A. melegueta* products revealed the presence of Alkaloids, Saponins, Tannins, Flavonoids and Cardiac glycosides and these may be responsible for the high insecticidal property for the control of mosquitoes.

Table 1. Percentage mortality of *Anopheles* species at 24 hours post treatment

Extract	Developmental stages							
	Larvae (0.1%)	Pupae (0.1%)	Larvae (0.2%)	Pupae (0.2%)	Larvae (0.3%)	Pupae (0.3%)	Larvae (0.4%)	Pupae (0.4%)
Leaf	40.00±0.9^{c}	35.37±0.3^{b}	70.00±0.9^{b}	60.00±0.9^{b}	90.00±0.93^{b}	85.37±0.33^{b}	100.00±0.00^{b}	100.00±0.00^{b}
Seed	65.37±0.3^{b}	50.00±0.3^{c}	80.00±0.9^{b}	70.00±0.9^{b}	100.00±0.0^{b}	95.37±0.33^{b}	100.00±0.00^{b}	100.00±0.00^{b}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Each value is a mean ± standard error of three replicates. Mean followed by the same letter along the column are not significantly different ($p>0.05$) using Duncan's Multiple Range Test.

DISCUSSION

Adverse environmental effects and the need to maintain a sustainable environment have created the need for environmental-safe, degradable and target specific

insecticides (Ezeonu *et al*., 2001). Extracts or essential oils from plants may be alternative sources of malaria vectors' control agents, since they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and are potentially suitable for use in control of mosquito larvae (Amer and Mehlhorn, 2006).

Table 2. Phytochemicals in methanol and aqueous extract of *A. melegueta* leaf and seeds.

Phytochemicals	Methanol extract of *A. melegueta* leaf	Methanol extract of *A. melegueta* seed
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Pholobatanins	-	-
Anthraquinines	-	-
Flavonoids	+	+
Cardiac glycosides	+	+

KEY: - Absent, + Present

The results from this study showed that the extracts of *A. melegueta* evoked high mortality of developmental stages of *Anopheles* mosquito compared to control. This confirmed earlier studies that reported the insecticidal activities of the plant against stored products' insect infestation management (Ofuya, 1990; Adedire and Lajide, 1999; Adeyemo *et al*., 2014; Onekutu *et al.,* 2015; Adesina *et al*., 2015). Also the result from this investigation are in accordance with the findings of Raji and Akinkurolere (2010); Akinkurolere *et al*., (2011); Ghosh *et al*. (2012); Yousaf and Zuharah, (2015); Ileke *et al.,* (2014); Dinesh *et al*., (2015) who reported the toxicity of some indigenous plants extracts on developmental stages of mosquitoes. However, at all the levels of concentration, larvae of *Anopheles* mosquito were more susceptible to the plant extracts than pupae. Also the seed extract exerted higher larvicidal activity compared to its effects on the pupal stage. This suggests that the bioactive constituents of the plant materials may be more available in the seeds which may be responsible for the higher mortality observed throughout the period of exposure (Ashamo and Akinnawonu, 2012; Ileke *et al*., 2014).

The active constituents in the plant materials appear to be responsible for their insecticidal properties against *Anopheles* mosquito (Ileke *et al*., 2014). Botanical source of insecticides have been noted to have a considerable effect on the normal respiration of insects as many of them have a knack to block the respiratory organ, i.e. spiracles of insect. Therefore, since the larvae of this insect depend solely on their spiracles for breathing, blockage of the spiracles by these plant extracts could lead to asphyxiation and subsequent death of the larvae (Akinkurolere *et al*., 2011; Ileke *et al.,* 2014; Ileke and Olotuah, 2012; Ileke and Oni, 2011; Ogungbite *et al*., 2014). Therefore, the ability of the tested plant to exert high mortality could be linked to the phytochemical constituents present in the plant parts tested.

The high larval mortality recorded in comparison to pupae could also be related to the larval active feeding activity since pupal stage is a resting (inactive) stage of the insect developmental cycle and do not feed. In this study, the larvicidal and pupacidal

effects of *A. melegueta* leaf and seed extracts on *Anopheles* could be as a result of respiratory, contact and stomach poisoning. These findings support the result of Ileke *et al.*, 2014; Al-Dakhil and Morsy, 1999; Amusan and Okorie, 2002; Nathan *et al.*, 2005; as well as Akinkurolere *et al.*, 2011 in which plant extracts were found effective against mosquito larvae and pupae. Ileke and Ogunbite (2015) opined that phytochemicals present in most plants can disrupt growth and reduce larval survival as well as disruption of life cycle of insects.

CONCLUSION

The study revealed the effectiveness of *A. melegueta* extracts in the reduction of the larval and pupal stages of *Anopheles* mosquito and therefore *A. melegueta* based compounds can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual, as well as at the community level in ensuring maximum safety in any ecological condition.

REFERENCES

Adedire, C. O., Lajide, L., 1999, Toxicity and oviposition deterrence of some plants extracts on cowpea storage bruchid, *Callosobruchus maculatus* (Fabricius). *Journal of Plant Disease and Protection*, 106: 647-653.

Adesina, J. M., Jose, A. R., Rajashekar, Y., Afolabi, L. A., 2015, Entomotoxicity of *Xylopia aethiopica* and *Aframomum melegueta* in suppressing oviposition and adult emergence of *Callasobruchus maculatus* (Fabricus) (Coleoptera: Chrysomelidae) infesting stored cowpea seeds. *Jordan Journal of Biological Sciences*, 8(4): 263-268.

Adeyemo, A. C., Ashamo, M. O., Odeyemi, O. O., 2014, *Aframomum melegueta*: A potential botanical pesticide against *Sitotroga cerealella* infestation on two paddy varieties. *Archives of Phytopathology and Plant Protection*, 47(15): 1841-1851.

Akinkurolere, R. O., Adedire, C. O., Odeyemi, O. O., Raji, J., Owoeye, J. A., 2011, Bioefficacy of extracts of some indigenous Nigerian plants on the developmental stages of mosquito (*Anopheles gambiae*). *Jordan Journal of Biological Science*, 4(4): 237-242.

Alaje, D. O., Owolabi, K. T., Olakunle, T. P., Oluoti, O. J., Adetuberu, I. A., 2014, Nutritional, minerals and phytochemicals composition of *Garcinia cola* (Bitter cola) and *Aframomum melegueta* (Alligator pepper). *Journal of Environmental Science Toxicology and Food Technology,* 8: 86-91.

Al-Dakhil, M. A., Morsy, T. A., 1999, The larvicidal activities of the peel oils of three citrus fruits against *C. pipiens*. *Journal of Egypt Social and Parasitology,* 29: 347-352.

Amer, A., Mehlhorn, H., 2006, Larvicidal effects of various essential oils against *Aedes, Anopheles*, and *Culex larvae* (Diptera, Culicidae). *Parasitology Research,* 99: 466-472.

Amusan, A. A. S., Okorie, T. G., 2002, The use of piper fruits oil as protectant of dried fish against *Dermestes maculates*, *Global Journal of Pure and Applied Science,* 8(2): 197-201.

Ashamo, M. O., Akinnawonu, O., 2012, Insecticidal efficacy of some plant powders and extracts against the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: *Gelechiidae*), *Archives Phytopathology and Plant Protection*, 45(9):1051-1058.

Dinesh, D. S., Kumari, S., Pandit, V., Kumari N., Kumar, P., Hassan, F., Kumar, V., Das, P., 2015, Insecticidal effect of plant extracts on *Phlebotomus argentipes* (Diptera: Psychodidae) in Bihar, India. *Indian Journal of Medical Research,* 142(Supplementary S1): 95-100.

Ezeonu, F. C., Chidume, G. I., Udedi, S. C., 2001, Insecticidal properties of volatile extracts of orange peels. *Bioresource Technology,* 76: 273-274.

Ghosh, A., Chowdhury, N., Chandra, G., 2012, Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research,* 135: 581-598.

Harborne, J. B., 1973, *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. Chapman and Hall, London, 279.

Igwe, E. A., Emeruwa, L. C., Modie, J. A., 1999, Ocular toxicity of *Afrornomium melegueta* (Alligator pepper) on healthy Igbo of Nigeria. *Journal of Ethnophamacology*, 65: 203-206.

Ileke, K. D., Oluotuah, O. F., 2012, Bioactivity of *Anacardium occidentale* (L) and *Allium sativum* (L) powders and oils extracts against cowpea bruchid, *Callosobruchus maculatus* (Fab). (Coleoptera: *Chrysomelidea*). *International Journal of Biology*, 4(1): 23-28.

Ileke, K. D., Afolabi, O. J., Ogungbite, O. C., Olagunju, J. O., Akanbi, O. M., 2014, Mosquitocidal activity of *Anacardium occidentale, Afromomum melegueta,Garcina kola* and *Citrus sinensis* against the developmental stages of mosquito, *Anopheles gambiae* Giles. *Journal of Mosquito Research,* 4(3): 21-26.

Ileke, K. D., Ogungbite, O. C., 2015, *Alstonia boonei* De Wild oil extract in the management of mosquito (*Anopheles gambiae*) a vector of malaria disease. *Journal of Coastal Life Medicine,* 3(7): 557-563.

Ileke, K. D., Oni, M. O., 2011, Toxicity of some plant powders to maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) on stored wheat grains*, African Journal of Agricultural Research*, 6(13): 3043-3048.

Muthusamy, R., Ramkumar, G., Karthi, S., Shivakumar, M. S., 2014, Biochemical mechanisms of insecticide resistance in field population of Dengue vector *Aedes aegypti* (Diptera: Culicidae). *International Journal of Mosquito Research*, 1(2): 1-4.

Nathan, S. S., Kalaivani, K., Murugan, K., 2005, Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae), *Acta Tropica*, 96: 47-55.

Ofuya, T. I., 1990, Oviposition deterrence and ovicidal properties of some plant powders against *Callosobruchus maculatus* in stored cowpea (*Vigna unguiculata*) seed during storage. *The Journal of Agricultural Science ,* 115(3): 343-345.

Ogungbite, O. C., Ileke, K. D., Akinneye, J. O., 2014, Bio-pesticide treated jute bags: potential alternative method of application of botanical insecticides against *Rhyzopertha dominica* (Fabricius) infesting stored wheat. *Molecular Entomology,* 5(4): 30-36.

Onekutu, A., Nwosu, L. C., Nnolim, N. C., 2015, Effect of seed powder of three pepper species on the bionomics of cowpea bruchid, *Callosobruchus maculatus* Fabricius. *International Journal of Science Research*, 5: 1-5.

Raji, J. I., Akinkurolere, R. O., 2010, The toxicity of some indigenous plant extracts on the developmental stages of Mosquito (*Anopheles gambiae*). *Nigerian Bioscientist*, 1-5.

Sofowora, A., 1993, *Screening plants for bioactive agents*. Medicinal Plants and Traditional Medicinal in Africa. 2nd. ed., Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, 134-156.

SPSS Inc., 2007, Statistical package for social sciences, Statistics 17.0 Brief Guide, SPSS, Inc., Chicago, IL., 181-185.

Srinivasan, R., Natarajan, D., Karthi, S., Shivakumar, M. S., 2014, Chemical composition and larvicidal activity of *Elaeagnus indica* Servett. (Elaeagnaceae) plant leaf extracts against dengue and malaria vectors. *International Journal of Mosquito Research,* 1(4): 66-71.

Trease, G. E., Evans, W. C., 2002, *Pharmacognosy*. 15th edn. Saunders Publishers, London, 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.

Udo, I. O., 2011, Potentials of *Zanthoxylum xanthoxyloides* as a model, *In:* Dr. Farzana Perveen (Eds.). Insecticides-Advances in Integrated Pest Management*,* 367-390.

WHO, 1996, *World Health Organization. Instruction for Determining the Susceptibility and Resistance of Mosquito Larvae to Insecticides*, Mimeographed document. Geneva, Switzerland WHO. WHO/VBC/75.583.

Yousaf, A., Zuharah, W. F. 2015, Lethal response of the dengue vectors to the plant extracts from family Anacardiaceae. *Asian Pacific Journal of Tropical Biomedicine,* 5(10): 812-818.

Received: May 12, 2016 *Accepted: August 20, 2016*