INSECTICIDAL ACTIVITY OF PETROLEUM ETHER EXTRACT AND ESSENTIAL OIL FROM GUINEA HEN WEED, *PETIVERIA ALLIACEAE* L. (PHYTOLACCACEAE) AGAINST *ANOPHELES GAMBIAE* L. (DIPTERA; CULICIDAE).

DENLOYE, A. A.*, AJELARA, O. K., ALAFIA, O. A., OLOWU, R. A.[1], MAKANJUOLA, W. A.[2]

Department of Zoology and Evironmental Biology, Faculty of Science,
Lagos State University, Lagos, Nigeria.
[1]Department of Chemistry, Lagos State University, Ojo, Lagos, Nigeria.
[2]Department of Zoology, University of Lagos, Akoka, Lagos, Nigeria.

***Corresponding author's e-mail: bio_denloye@yahoo.com**
Article received: 8th July, 2015; Article accepted: 27tht July, 2015.

ABSTRACT

Petroleum ether extract and essential oil from the roots of Petiveria alliacea (Phytolaccaceae) were evaluated for toxicity against 0-2 day old adult, 1st, 2nd, 3rd and 4th instar larvae of Anopheles gambiae (Diptera: Culicidae) under ambient laboratory conditions. In different experiments 30 larvae at each instar stage were exposed to extract with 50, 100, 250, 500, 750 and 1000 mg/L of extract and essential oil at 50, 100, 250, 500, 750 and 1000 ml/L in four replicates and dead larvae counted after 24 and 48 hours of exposure respectively. In another experiment 30 adults were also exposed to P. alliacea essential oil in air-tight rectangular glass cages with four replications. The results show that the essential oil was most toxic to the first instar larvae at 48 hours exposure time with LC_{50} of 8.83 ml/L and least toxic to fourth instar larvae at 24 hours exposure time. Petroleum ether extract was most toxic to the second instar larvae with LC_{50} of 30.01 mg/l value and least toxic to the first instar larvae at 24 hours exposure with LC_{50} value of 288.67 mg/l. The LC_{50} value of P. alliacea oil against the adult An. gambiae was 0.027mg/l. The study demonstrates the fumigant action and potency of P. alliacea as control agent against An. gambiae. It also supports the traditional use of the plant species for mosquito control.

Keywords: *Petiveria alliacea*, *Anopheles gambiae*, essential oil, Petroleum ether extract, toxicity, probit analysis, complete randomized block design, Lagos, fumigant action.

INTRODUCTION

Plants have been known over the years to possess noxious substances with which they repel or kill insects. These repellent and insecticidal constituents in plants include terpenoids, alkaloids, and flavonoids which may be exploited for the control of insect pests as practised for ages in African communities.

Don-Pedro (1996), Denloye *et al*. (2003, 2009) and other workers have reported the use of plants such as *Chenopodium ambroisioides*, *Citrus* sp, and *Allium sativum* for mosquitoes control. In many African homes some of these plants are hung on doorposts, by the bed, pulverized, burned or smouldered for mosquitocidal or repellent actions. One of such plants is *Petiveria alliacea*; which root is used in South-West Nigeria as a mild counter irritant for headache, fever and malaria. In the Philippines and some other countries outside Africa the plant is used for the treatment of convulsions or as an antipyretic, laxative and diuretic (Chopra *et al*., 1996; Soares *et al*., 2014). The locals in Badagry Local Government Area of Lagos State, Nigeria use it to repel mosquitoes by macerating the leaves and spraying living quarters with the water-based solution and recording high efficacy in repelling mosquitoes. This reported efficacy in local usage has therefore fired a die-hard belief in the use of this plant as mosquito control agent. There is therefore a strong need to establish and document the scientific bases for these believes. This underscores the purpose of the present study.

Malaria, is the number one killer disease in Africa south of the Sahara. Globally the disease afflicts 300-500 million people annually, Africa being worst hit with more than 90% of the infection (Coetzee, 1997). Unfortunately, the disease is increasingly more difficult to control owing to resistance of both the parasite and its vectors to drugs and insecticides respectively. In addition, prophylaxis and insecticdal chemicals are often out of reach for residents in local rural communities due to non-availability and their respective prohibitive costs.

The foregoing necessitates a search for alternative cheap, environmentally benign and readily available control measures. Such measures may include the strenghtening of the age-old practices which held sway in the rural communities and effectively kept malaria under check, for example the use of botanicals against the vectors. *Anopheles gambiae sensu stricto*, the African malaria vector is responsible for the transmission of the disease, especially in the coastal areas of Lagos State where its sibbling species have been found to be well established (Amajoh *et al*., 2002; Awolola *et al.,* 2007). Such botanical approach would however require a well established basis for the use of plants in traditional settings *ab initio*. Literature on the insecticidal uses of *P. alliacea* is scanty, the closest being the one that reported the acaricidal activity of the oil in Brazil (Neves *et al*., 2011). The objective of this study is therefore to determine the toxicity of *Petivera alliacea* petroleum ether extract and essential oil against larvae and adult *An. gambiae.*

MATERIALS AND METHODS

Source and preparation of test plant species.

The test plant species, *Petiveria alliacea* was collected from Ipara village, Badagry LGA, Lagos State Nigeria. Whole plants were collected and the roots severed for further processing to obtain the extract.

Preparation of Test Petroleum ether extract

The roots were chopped into small pieces, air dried in the

laboratory for 3 days at ambient room temperature and relative humidity, and then pulverized into powder using a Binatone® blender (BLG 400 model). The powder was screened through 0.1 mm mesh sized sieve to produce fine even-sized powder. For extraction, 500 g of the root powder was weighed out, soaked in 1 litre of petroleum ether for 48 hours and then filtered through Whatman no 1 filter paper (15 cm diameter). The filtrate was labeled as the stock solution and stored in Kilner® jar while the residue was re-extracted for another 48 hours after which the two filtrates were pooled together. The filtrates were then dried over water bath at 50 **ºC**. The residue was used as the test petroleum ether extract of *P. alliacea*. Graded concentrations of 50, 100, 250, 500,750 and 1000 mg/L of the extract were prepared by serial dilution using petroleum ether for exposures against the test *An. gambiae.*

Preparation of Test Essential Oil

A portion (500 g) of the roots of *P. alliacea* already air-dried as described earlier for preparation of Pettoleum ether extract was hydro-distilled in Clevenger apparatus for 6 h at 100 ºC (Kasali, *et al*., 2006). The essential oil (0.1 ml) was mixed with 0.3 ml of hexane to make it soluble in water. Graded concentrations of 50, 100, 250, 500, 750 and 1000 ml/L of the essential oil were prepared by serial dilutions.

Test mosquitoes and experimental design

The larvae and adults of *An. gambiae* used as experimental mosquitoes were obtained from stock maintained in the Applied Entomology Research Laboratory, Lagos State University, Lagos State Nigeria. The maintenace of this stock over several generations without contact with any insecticide has been described by Denloye *et al.* (2003). All experiments were carried out using Complete Randomized Block Design.

Acute toxicity of *P. alliacea* against *An. gambiae* larvae.

Petroleum ether extract: Thirty *An. gambiae* larvae in their 1st, 2nd, 3rd and 4th instar stages of development were transferred into 200 ml glass jars containing 10 ml of de-ionized water. From each of the graded concentrations of petroleum ether extract 3 ml was added to 90 ml of de-ionized water respectively. Each mixture was thoroughly agitated for 2 min and then transferred into the exposure cups. A control was prepared in the same way but using only 100 ml of de-ionized water. The larvae were then transferred into cups containing either *P. alliacea* extract or water (control) and maintained at 28 ± 1 ºC for 24 h or 48 h after treatment before survival was assessed. Each treatment and the control was replicated four times. After exposure, larvae were washed with de-ionized water and transferred into recovery cups. A recovery time of 5 min was allowed during which the water was agitated with a sterile stirrer to induce sinking of larvae to the bottom of the cups. Larvae that failed to swim to the water surface within 5 min were regarded as dead. Data obtained were used to compute percent mortality and median lethal concentration values.

Essential oil: Similar procedure was followed as described earlier using essential oil in place of petroleum ether extract of *P. alliacea* against *An. gambiae* larvae. The same graded concentrations were used for bioassay and consequent acute toxicity of test essential oil against the experimental mosquito larvae. Thirty 1st, 2nd, 3rd and 4th instar larvae were respectively exposed to each graded concentration in 200 ml plastic cups. A control was set up in each case exposing the same number of each larval stage to only deionized water mixed with hexane. All experiments and control were replicated four times; survival was assessed as described earlier every 24 hours for 2 days. Mortality data obtained were used to compute LC_{50} values to determine the toxicity of essential oil to the larvae.

Toxicity of *P. alliacea* essential oil vapour against adult *An. gambiae*.

Twenty 0-2 day old adult *An. gambiae* mosquitoes were transferred into four aluminum framed glass cages described earlier; which served as the exposure chamber and sealed with sticky tape to prevent vapor from escape; Whatman no 1 filter paper (15cm diameter) with attached thread was treated with different volume of *P. alliacea* oil and suspended immediately in each exposure chamber at treatment rates of 0.8, 1.6 and 2.4 µl/L respectively. A control was also set up using only dry filter paper. All treatments and controls were replicated four times. Mosquito mortality was assessed after 24 hours as earlier described and the data used to determine percent mortality and LC_{50} values of oil against adult *An. gambiae*.

Data Analyses

The number of dead mosquito larvae and adults at all treatments in acute toxicity experiments were corrected using Abbott (1925) formula after which Probit analyses were carried out following Finney (1971). Median lethal concentration (LC_{50}) values were computed using a computer program previously reported by Denloye *et al* (2009). Mean values of adult mosquito mortality in dry film experiment were compared by ANOVA and followed by Least Significant Difference (LSD) statistic ($P< 0.05$) using SPSS (version 17.0). All data were square root transferred before ANOVA.

RESULTS

Toxicity of *P. alliacea* root Petroleum ether extract to *An. gambiae*

The toxicity of Petroleum ether extract to the first, second, third and fourth instar larvae of *An. gambiae* are summarized in Tables 1 and 2. The Petroleum ether extract was more toxic to the second instar than any other larval stage with LC_{50} value of 77.42 mg/L during 24 h exposure period. The Petroleum ether extract was significantly more toxic to the second, third and fourth instars respectively as indicated by the 95 % Confidence Limits (CL) ($p = 0.05$). Similarly, the extract was more toxic to the second instar larvae than any other one (LC_{50} = 3.07 mg/L), followed by the third instar during 48 h exposure period. Comparison of 95 % CL ($p = 0.05$) shows that the test extract was significantly more toxic to the second instar larvae than either the first or fourth but not the third instar (Table 1).

Toxicity of *P. alliacea* root oil to *An. gambiae* larvae and adults

Exposure for 24 h showed that the oil was toxic to the second instar with LC_{50} value of 63.35 mg/L followed by the first instar larvae with LC_{50} of 119.40 mg/L. The essential oil was not significantly more toxic to the first instar when the 95 % CL **was** compared (Table 2). Extension of exposure time to 48 h shows that the oil was more toxic to the first instar than any other larval stage with LC_{50} values of 8,13 mg/l. The toxicity of test oil to the first instar was however not significantly higher than the second instar (95 % CL). The *P. alliacea* root oil was toxic to adult *An. gambiae* with a computed 24 h LC_{50} value of 0.027 mg/L (Table 3).

Table 1: Toxicity of *Petiveria alliacea* Root Petroleum Ether Extracts to Larvae of *Anopheles gambiae*

Larval Stage	Exposure	LC_{50} (mg/L)	95 % Confidence Limits	Regression Equation	DF	Slope (SE)
First Instar	24 h	288.67	225.7 – 369.22	Y= 3.708+401x	4	1.101 ± 0.018
Second Instar		77.42	55.23 – 108.08	Y = 2.299+1.217x	4	1.217 ± 0.020
Third Instar		169.40	102.42 – 278.47	Y = 1.287+0.5777x	4	0.577 ± 0.016
Fourth Instar		147.98	126.99 – 172. 57	Y = 4.964+2.260x	4	2.260 ± 0.029
First Instar	48 h	153.30	113.62 – 206.35	Y = 2.234+1.022x	4	1.022 ± 0.017
Second Instar		30.01	15.31 – 58.03	Y = 1.568+0.870x	4	0.870 ± 0.018
Third Instar		43.23	22.34 – 90.20	Y = 1.226+0.741x	4	0.741 ± 0.017
Fourth Instar		87.12	73.62 – 103.03	Y = 4.676+2.410x	4	2.410 ± 0.045

Key: DF = Degree of Freedon, SE = Standard Error

Table 2: Toxicity of *Petiveria alliacea* Root Oil to Larvae of *Anopheles gambiae*

Larval Stage	Exposure	LC_{50} (ml/L)	95 % Confidence Limits	Regression Equation	DF	Slope (SE)
First Instar	24 h	119.40	79.39 – 178.64	Y= 1.709+0.832x	4	0.823 ± 0.016
Second Instar		63.35	37.87 – 105.17	Y = 1.568+0.870x	4	0.870 ± 0.018
Third Instar		412.86	309.09 – 552.25	Y = 2.53+0.967x	4	0.967 ± 0.017
Fourth Instar		20544.83	202629.50 - 2220.95	Y = 2.706+0.627x	4	0.627 ± 0.030
First Instar	48 h	8.83	1.96 - 38.01	Y = 0.622+0.658x	4	0.658 ± 0.021
Second Instar		19.09	4.76 – 73. 20	Y = 0.676+0.528x	4	0.528 ± 0.017
Third Instar		147.35	108.55 - 199.49	Y = 2.203+1.016x	4	1.016 ± 0.017
Fourth Instar		380.42	315.37 - 459.03	Y = 3.928+1.522x	4	1.522 ± 0.022

Key: DF = Degree of Freedon, SE = Standard Error

Table 3: Toxicity of *Petiveria alliacea* Root Oil Vapour To 0 – 3 Day Old Adult *Anopheles gambiae*

Exposure	LC_{50} (µl/L)	95 % Confidence Limits	Regression Equation	DF	Slope (SE)
24 h	0.027	0.05 - 0.654	Y= 3.418+2.187x	3	2.187 ± 0.460

Key: DF = Degree of Freedon, SE = Standard Error

DISCUSSION

This study demonstrates the toxic effects of the essential oil and crude petroleum ether extract of *Petivera alliacea* root on the larvae and adult of *An. gambiae* in the laboratory. The results showed that the test essential oil was most potent to 1st instar larvae at 48 hours exposure period and least potent against the 4th instar larvae. This may be because the 4th instar larvae were about to pupate hence the extract had little effect on them. This reduced mortality of *An. gambiae* 4th instar larvae may be explained in terms of behavioural avoidance shown by the larvae. As a self-preservation strategy, 4th instar larvae of mosquitoes transform into pupae which are less susceptible to insecticides in their environment (Shaalan *et al*., 2005). This avoidance behaviour may be responsible for the low number of 4th instar larvae recorded in the present study.

The test essential oil vapour used in this study resulted in initial knockdown of adult *A. gambiae*, which eventually led to mortality. This result demonstrates the fumigant efficacy of the *P. alliacea* essential oil against malaria vector, *A. gambiae*.

The observed toxicity of the test plant crude extracts and essential oil may be explained in terms of the noxious chemical constituents. The toxicity of the essential oil reported here is attributable to its chemical constituents. Analysis using Gas Chromatography coupled with Mass Spectrometery showed that the major constituent of *P. alliaceae* essential oil is Phytol and others are 4-vinyl-2-methoxy-phenol, hexadecanoic acid, benzenecarboxylic acid, 5,6,7,7a-Tetrahydro-6-hydroxy-4,4,7a-trimethyl-2(4H)-benzofuranone, benzenemethanethiol, 4-vinyl-2-methoxy-phenol (Medina-Santos *et al*., 2009). Another independent analysis has shown that the test plant species has (*Z*)-thiobenzaldehyde *S*-oxide, a lachrymatory principle (Kubec *et al*., 2002). It has been established over time that some of these constituents are insecticides (Ansari *et al.*, 1999; Soares *et al*., 2014**).** The demonstrated toxic effects of essential oil and petroleum ether crude extract of *P. alliacea* to all the four larval and adult stages of *An. gambiae* presents a likelihood of its being used in insecticide preparations.

Earlier workers screening plants for toxicity against insects have stated that the use of certain plant materials for insecticidal purposes is not for their proven activity but rather out of allegiance to tradition (Delobel and Malonga, 1987). The result of this study has shown that the use of *P. alliacea* is not just an allegiance to tradition, but rather that it has insecticidal efficacy just as Omolo *et al* (2005) reported while bioprospecting for insecticidal plants in Kenya. The results of the present study goes a long way to support the practice by local folks in Badagry Local Government Area of Lagos State in using this plant species to avoid mosquito bites and stem malaria infection. In this area, the plant is hung on door posts, bed head and main entrances into buildings. The mosquitoes may therefore avoid such houses having percieved the noxious principles in the essential oils from the plants through fumigant action as shown in the study. Essential oils are generally known to have low boiling points and consequently with high volatility, even at room temperature. This property may be responsible for the presence of the toxic oil constituents in the indoor atmosphere of the buildings where this plant is hung and consequent mortality or avoidance of mosquitoes.

This study reports the fumigant toxicity of *P. alliacea* essential oil to *An. gambiae* with an LC_{50} value of 0.027 µl/L. This further supports the use of the plant locally as fumigant in homesteads.

In view of these practices it will be worthwhile to further research into the potentials of these plant products as control agents for malaria vectors and incorporate it into integrated management of malaria vectors already in existence.

REFERENCES

Abbott, W.S. (1925). A Method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265 - 267.

Amajoh, C.N., Oduoko, J.B and Mosanya, M.E. (2002). Preliminary investigations on malaria in Atlantic Coastal Margin of Ibeju-Lekki, Lagos State, Nigeria. *Journal of Malaria in Africa and Tropics* 1: 17 - 19.

Ansari, M.A., Vasudevan, P., Tandon, M and Razdan, R.K. (1999). Larvicidal and Mosquito repellent action of peppermint, *Mentha piperita* oil. *Bioresource Technology* 72: 267.

Awolola, T. S., A. O., Oduola, J. B. Obansa, N. J. Chukwura and K. G. Unyimadu (2007). *Anopheles gambiae* breeding in polluted water bodies in urban Lagos. *Journal of Vector Borne Diseases*, 44: 241 – 244.

Chopra, R N; Nayar, S. L and Chopra. I. C. (1996). *Glossary of Indian medicinal plants* (CSR, New Delhi). 330 pp.

Coetzee, M. (1997). Malaria in Africa. Proceedings of the joint congress of the Entomological Society of Southern Africa and the African Association of Insect Scientists, 30 June – 4 July 1997. Stllenbosch, South Africa.

Delobel, A and Malonga, P. (1987). Insecticidal properties of six plant materials against *Caryedon serratus* (OL). *Journal of Stored Product Research*, 23 (3): 173 – 176.

Denloye, A.A.B., Makanjuola,W.A and Babalola, O.O. (2003). Toxicity and repellent effects of Crude aqueous extracts of garlic (*Allium sativum*) on larval adult *Anopheles* mosquito. *African Entomology*, 11: 287 - 290.

Denloye, A. A., O. K. Ajelara, R. A. Olowu, A. Eshilokun and W. A. Makanjuola. (2009). Insecticidal activity of petroleum ether extract and essential oil of *Chenopodium ambrosioides* L. (Chenopodiaceae) against *Anopheles gambiae* (Diptera; Culicidae). *Acta Entomologica Sinica* 52 (8): 923 – 928.

Don-Pedro, K. N. (1996). Fumigant toxicity is the major route of insecticidal activity of citruspeel essential oils. *Pesticide Science* 46: 71 – 78.

Finney, D. J. (1971). *Probit Analysis*. Cambridge University Press, 333 pp.

Kasali, A. A., Ekundayo, O., Paul C., Konig, W. A., Eshilokun, A.O., Ige, B. (2006). 1,2,3,4-Diepoxy-p-menthane and 1,4-Epoxy-pmenth-2-ene: rare monoterpenoids from the essential oil of *Chenopodium ambrosioides* L. var ambrosioides leaves. *Journal of Essential Oil Research,* 18 (1): 13 – 15.

Kubec R, Kim S, Musah RA. (2002). S-Substituted cysteine derivatives and thiosulfinate formation in *Petiveria alliacea* -part II. *Phytochemistry*, 61: 675 – 680.

Medina-Santos, E. O., Viggiano-Beltrocco, M. V., De Jesus-Echevarría, M. (2009). Essential Oil Constituents of *Petiveria alliacea* L. by Soxhlet Extraction and GC-MS. Proceedings of the 2009 Annual Meeting of the American Institute of Chemical Engineers.

Neves, I de A, C. A. G. da Camara, J. C. S. de Oliviera & A. V. de Almeida (2011). Acaricidal Activity and Essential Oil Composition of *Petiveria alliacea* L. from Pernambuco (Northeast Brazil). *Journal of Essential Oil Research* 23 (1): 23 – 26.

Omolo, M. O., Okinyo, D., Ndiege, I. O., Lwande, W. and Hassanali, A. (2004). Repellency of some essential oils of Kenyan plants against *Anopheles gambiae*. *Phytochemistry.* 65*:* 2797 - 2802.

Shaalan, E. A., D. V Canyon, M. F. W. Yonnes, H., and A. Mansour (2005). Effects of sub-lethal concentrations of synthetic insecticides and *Callitri glaucophylla* extracts in the development of *Aedes aegypti*. *Journal of Vector Ecology,* 30 (2): 295 – 298.

Soares, B. O; Oliveira, M. B. N; Mansur, E.; Dantas, F. J. S; De Mattos, J. C. P; Caldeira-de-Araujo, A. and Gagliardi, R. F. (2014). Effect of extracts from field and in vitro plants of *Petiveria alliacea* L. on plasmidial DNA. *Journal of Medicinal Plant Research,* 8 (35): 1101 - 1109.