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The Insecticidal Efficacy of the Extracts of *Piper nigrum* (Black Pepper) and *Curcuma longa* (Turmeric) in the Control of *Anopheles gambiae* Giles (Dip., Culicidae)

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Abstract

The use of botanicals as alternatives to synthetic insecticides offers a more environmentally friendly method of insect control. The current study evaluated the toxic effects of the essential oils, aqueous and methanolic extracts of *Piper nigrum* (black pepper) and *Curcuma longa* (turmeric) extracts on the larvae, pupae and adults of *Anopheles gambiae*. The moulded insecticidal coils and essential oils of the plants were used to determine adults' repellence in the laboratory. The essential oils were most effective against *A. gambiae* larvae showing 100% mortality for both *P. nigrum* and *C. longa* with LC_{50} values of 15 and 149 ppm for *P. nigrum* and *C. longa*, respectively. The methanolic extracts had LC_{50} value of 27 and 214 ppm for *P. nigrum* and *C. longa*, respectively. Maximum (100%) concentration of the essential oils of *C. longa* and *P. nigrum* oils gave 95.7% and 95.3% protection, respectively. The insecticidal coil (100% concentration) of *P. nigrum* and *C. longa*, showed 76.7% and 70% knockdown action on the adult mosquitoes respectively. Therefore, *P. nigrum* and *C. longa* can serve as repellents against *A. gambiae*, and can be used in integrated vector management control programs.

Key words: *Piper nigrum* extracts, *Curcuma longa* extracts, Botanicals, *Anopheles gambiae,* Plant extracts, Insecticidal coils.

1. Introduction

Malaria, the leading cause of morbidity and mortality in Nigeria, causes about 1-2 million deaths (mostly amongst pregnant women and children under five years) annually in Africa (Breman, 2001; Muriu *et al*., 2013). In south-eastern Nigeria, *Anopheles gambiae* Giles (Dip., Culicidae) is the major malaria transmitting vector, followed by *A. funestus* (Uneke and Ibeh, 2009).

Vector control is an essential component of malaria prevention. Such control has been proven to successfully reduce or interrupt malaria transmission when coverage is sufficiently high (Innocent *et al*., 2014). The two cores, broadly applicable measures for malaria vector control are long-lasting insecticidal nets and indoor residual spraying (WHO, 2006). The burden of malaria is worsened by mosquito resistance to synthetic insecticides (Guinovart *et al*., 2006; Ranson and Lissenden, 2016), and malaria parasites' resistance to antimalarial drugs (Muriu *et al*., 2013; Raj *et al*., 2014; Ashley *et al*., 2014)

The problem of high cost and development of resistance in many mosquito species to several synthetic insecticides has revived interest in the use of botanicals as insecticides (Grainge and Ahmed, 1988). These bioactive chemicals may act as insecticides, antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones, and attractants (Adewoyin *et al*., 2006).

Botanicals as an alternative to synthetic insecticides in controlling mosquitoes, offer a more environmentally friendly method of insect control (Irungu and Mwangi, 1995). Apart from being of low mammalian toxicity and degradability, most insects find it difficult to resist the toxic effects of botanicals. Various plant species have been exploited, throughout the world, to control the mosquito populations (Muthukrishnan *et al*., 1997). *Piper nigrum* L. (black pepper) of the family Piperaceae, often called the 'King of Spices', is a universal table condiment used to flavour all types of cuisines worldwide (Mathew *et al*., 2001). The spicy taste can be attributed mainly to the presence of the compound Piperine which is a pungent alkaloid (Tripathi *et al*., 1996) that enhances the bioavailability of various structurally and therapeutically diverse drugs (Khajuria *et al*., 2002). It also contains small amounts of safrol, pinene, sabinene, limonene, caryophyllene and linaonol compounds. Black pepper is also an important traditional medicine used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion and fever, intermittent fever, cold extremities, colic, gastric ailments and diarrhoea (Balasubramanian *et al*., 2016). The plant has been credited with interesting pesticidal properties against insect pests (Maenthaisong *et al*., 2014; Custódio *et al*., 2016; Samuel *et al*., 2016).

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Curcuma longa (Turmeric), of the family Zingiberaceae, is one of most essential spices used all over the world particularly in the countries of the east (Ravindran, 2007). It is a deep yellow-to-orange powder that comes from the underground stems of the tropical perennial herb. Primarily, phenolic compounds and terpenoids have been identified from this spice (Li *et al*., 2011). The plant has been credited with interesting pesticidal properties against insects and fungi of agricultural significance, including repellent properties against mosquito species (Ali *et al*., 2015; Tisgratog *et al*., 2016).

The aim of this study is to look for a more environmental friendly way, and a cheap, and readily-available effective method to control *A. gambiae.* This research was, therefore, carried out to investigate the efficacy of the essential oils, crude aqueous extracts and methanolic extracts of *P. nigrum* and *C. longa* against larvae, pupae and adult stages of *A. gambiae.*

2. Materials and Methods

2.1. Collection and Preparation of Test Materials

The seeds of *P. nigrum* and fresh rhizomes of *C. longa* were purchased from local markets in Lagos Nigeria. The *P. nigrum* seeds were dried for ten days at room temperature, then powdered using a Binatone blender. The *C. longa* rhizomes were cut into smaller pieces and dried for ten days before being grinded with blenders. The powders were sieved using a mesh size of 0.05 mm^2 to get a fine powder stored in amber bottles on the laboratory shelf for later use.

2.2. Preparation of Extracts

Crude aqueous extracts were obtained by separately dissolving fifty g of *P. nigrum* and *C. longa* powder in 500 ml of distilled water leaving them for twenty-eight hours. The liquid extracts were carefully collected by manually squeezing the soaked powder out. Then they were kept in amber bottles in a refrigerator to retain the potency of its active components for future use. The same method was adopted in the preparation of methanolic extract only that methanol was used in the place of distilled water. Essential oil was obtained by the hydro-distillation process using the Clevenger apparatus of the Department of Chemistry at Lagos State University in Ojo Lagos, Nigeria. Three replicates of 200 g of powdered *P. nigrum* and *C. longa* were separately introduced into the apparatus. The concentration of the *P. nigrum* was 1.75%, while that of the *C. longa* was 1.50%. The process lasted for three hours each. Hexane was added in the process in order to make the oil insoluble in water, and to demarcate the water from the oil, and avoid evaporation. Oil was collected in amber bottles and kept in the refrigerator to retain its potency until needed. One ml of essential oil was dissolved in 0.3 ml of hexane, which was mixed in 100 ml of distilled water to form a stock solution from which serial dilutions were made. The larvae, pupae and adult mosquitoes were obtained from a laboratory culture maintained in the Department of Zoology and Environmental Biology of Lagos State University, Lagos.

2.3. Bioassays and Test Procedures

Ten larvae of the second and third instar of *A. gambiae* were introduced. Mortality rate was recorded after twenty-four hours. 50 ppm to 450 ppm serial dilutions (50, 100, 150, 200, 250, 300, 350, 400 and 450 ppm) were used for turmeric, while 100 mL of distilled water served as a control. Three replicates were set for each concentration. The same procedure was carried out using a methanolic extract.

A larva was classified dead if it did not move when gently touched with the tip of moistened camel hairbrush, or when found lying immobile at the bottom of the container or not showing the characteristic diving reaction when water is disturbed. Same procedures were used for essential oils of the test plants on *A. gambiae* larvae.

Ten *A. gambiae* pupae were introduced into a transparent plastic cup containing 100 mL of serial dilution made from stock solution of aqueous extracts at various concentrations ranging from 10 ppm to 90 ppm for black pepper, and from 50 ppm to 450 ppm for turmeric extracts. Mortality rates were recorded after twenty-four hours using slandered units. Ten pupae in 100 mL of distilled water in plastic cup served as control. Three replicates were also set for each concentration. The same procedure was carried out using a methanolic extract.

2.4. Preparation and Tests Using Insecticidal Coils

Ground fine plant powder and wood shavings were used to mould coils of various concentrations ranging from 0% (control) to 100% (absolute plant powder mould). The insecticidal coil is a smoke test used to check for the repellent action of burnt plant extracts on mosquitoes. Coils moulded into different concentrations were burnt in a rectangular cage (measuring 59×44×45) containing twenty adult mosquitoes. Three replicates were set for each concentration with the wood shavings alone serving as the control. The dried coil was lit and the number of dead mosquitoes or those knocked down were counted and recorded for thirty minutes.

2.5. Data Analysis

The data of different treatments and different concentration of the same treatment were statistically analyzed using Finney (1947) formula to correct for mortality. The LC50, LC99, parameter estimates and chi-square values were calculated by using probit analysis (Finney, 1947). Data obtained from larval mortality and repellent activity were subjected to the analysis of variance (ANOVA). The statistical software SPSS was used for data analysis. Means were compared using Duncan’s Multiple Range Test at 0.05% level of significance.

3. Results and Discussion

3.1. The Effect of Aqueous, Methanolic Extracts and Essential Oil on Anopheles gambiae Larvae

The concentration of the extracts was positively correlated with the mortality of the larvae. The correlation was 0.84, 0.94 and 0.96 for essential oils, aqueous and methanolic extracts of *P. nigrum*, respectively (Figure 1). As for the *C. longa*, the correlation was 0.94, 0.97 and 0.98, respectively (Figure 2). After a twenty-four-hour treatment, using 90 ppm concentration of *P. nigrum*, the mortality rate of *A. gambiae* larvae was 100%, 90% and

83.3% using essential oil, methanol and aqueous extracts, respectively (Table 1). On the other hand, 450 ppm concentration of *C. longa* gave 100, 86.7% and 76.7% mortality rates, respectively (Table 2). There was no mortality in the control treatment after twenty-four hours.

In almost all the concentrations of the botanicals used, the essential oil was significantly more toxic and effective on the mortality of larvae than the methanolic extract, which was significantly (P<0.05) more toxic than the aqueous extract (Tables 1 and 2). Moreover, 90 ppm of the essential oils of *P. nigrum* gave 100% larval mortality after twenty-four hours, while 450 ppm of *C. longa* gave the same mortality rate using the same time. The LC_{50} for the essential oils of *P. nigrum* on *A. gambiae* larvae was 15.4 ppm, while that for *C. longa* was 148 ppm. Amer and Mehlhorn (2006) reported larvicidal activity of *P. nigrum* with LC_{50} values between 10 and 105 ppm against *A. stephensi* Liston, *Ae. aegypti* and *Culex quinquefasciatus* after a twenty four-hour exposure. On the other hand, Kalaivani *et al*. (2012) found the LC_{50} of *C. longa* extract to be effective against the larvae of *Ae. aegypti* at 115.6 ppm. Similar results by Srivastava *et al*. (2003) of the aqueous and methanolic extract of *Nerium indium* lattices against the *C. quinquefasciatus* showed that different dilutions of the lattices delay the post embryonic development of *Culex* larvae, and was 1.8 times more toxic than the aqueous extract. Moreover, Akinneye and Afolabi (2014) showed that the aqueous extract of *Cleisthopholis patens* was relatively ineffective against the larvae of *Anopheles gambiae*e. They concluded that the ineffectiveness of aqueous extract may be attributed to the fact that water was used for the extraction, and water is a polar solvent. Fafioye *et al*. (2004) reported that the ethanolic oil extracts of *Parkia biglobosa* and *Raphia vinifera* were more potent against the juvenile of *Clarias gariepinus* than the aqueous forms. This is due to the polarity, volatility and ethanol's power to dissolve more of the active ingredients.

The crude extracts and essential oils of plant species have a complex mix of chemical elements. These secondary metabolites have been used empirically in vector control and causal agents of the disease (Granados-Echegoyen *et al*., 2014). The developmental stage of the mosquito species determines the effectiveness of the plant extract (Ebe *et al*., 2015). The mosquito larvae stage is the most susceptible to any treatment, and is restricted to the common aquatic habitats (Bisset *et al*., 2014)

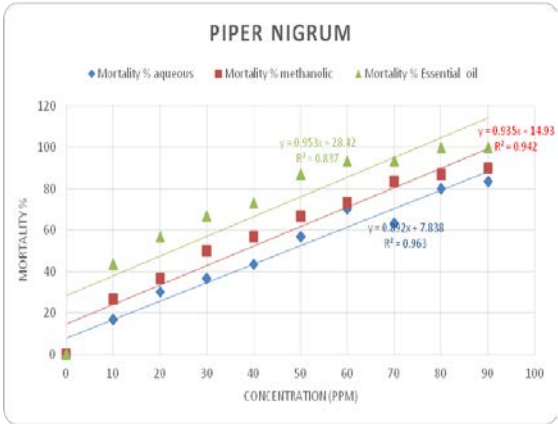

Figure 1. Effect of aqueous, methanolic and essential oils of *Piper nigrum* on larvae of *Anopheles gambiae* after 24 hours.

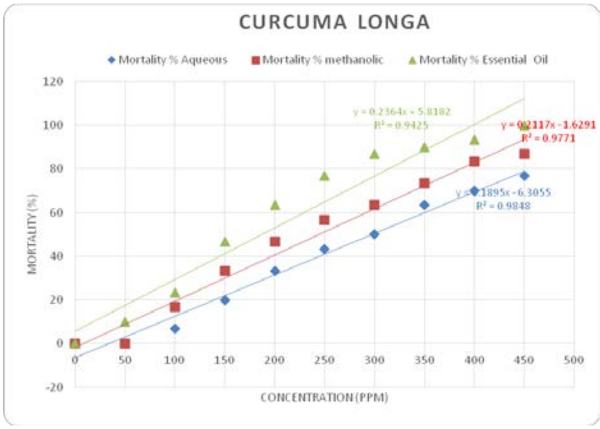

Figure 2. Effect of aqueous, methanolic and essential oils of *Curcuma longa* on *Anopheles gambiae* after 24 hours of treatment.

Table 1. Effect of the different extracts of *Piper nigrum (*Black pepper) on *Anopheles gambiae* larvae after 24hours of treatment.

Conc. (ppm)	Mean mortality			Mortality %		
	Aqueous	Methanolic	Essential oil	Aqueous	Methanolic	Essential oil
Control	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0	0	0
10	1.67±0.58[a]	2.67±0.58[b]	4.33±0.58[c]	16.7	26.7	43.3
20	3.00±0.00[a]	3.67±0.58[a]	5.67±0.58[b]	30	36.7	56.7
30	3.67±0.58[b]	5.00±0.00[b]	6.67±0.58[c]	36.7	50	66.7
40	4.33±0.58[a]	5.67±0.58[b]	7.33±0.58[c]	43.3	56.7	73.3
50	5.67±0.58[a]	6.67±0.58[b]	8.67±0.58[c]	56.7	66.7	86.7
60	7.00±1.00[a]	7.33±0.58[a]	9.33±2.15[b]	70	73.3	93.3
70	7.33±0.58[a]	8.33±0.58[b]	9.33±1.15[c]	63.3	83.3	93.3
80	8.00±1.00[a]	8.67±0.58[a]	10.00±0.00[b]	80	86.7	100
90	8.33±1.15[a]	9.00±1.00[b]	10.00±0.00[c]	83.3	90	100

A total of three replicates for each concentration and 10 larvae in each replicate were used

Mean mortality with different superscripts in the same row are significantly different from each other at P<0.05.

Table 2. Effect of the different extracts of *Curcuma longa* (turmeric) on *Anopheles gambiae* larvae after 24hours of treatment.

Conc. (ppm)	Mean mortality			Mortality %		
	Aqueous	Methanolic	Essential Oil	Aqueous	Methanolic	Essential Oil
0	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.0	0.0	0.0
50	0.00±0.00[a]	0.00±0.00[a]	1.00±1.00 [a]	0.0	0.0	10.0
100	0.67±0.58[a]	1.67±0.58[b]	2.33±0.58 [c]	6.7	16.7	23.3
150	2.00±0.00[a]	3.33±0.58[b]	4.67±0.58 [c]	20.0	33.3	46.7
200	3.33±1.53[a]	4.67±0.58[b]	6.33±1.53 [c]	33.3	46.7	63.3
250	4.33±0.58[a]	5.67±1.15[b]	7.66±1.15 [c]	43.3	56.7	76.7
300	5.00±1.00[a]	6.33±1.53 [b]	8.67±1.53 [c]	50.0	63.3	86.7
350	6.33±1.15[a]	7.67±0.58 [b]	9.00±1.00 [c]	63.3	73.3	90.0
400	7.00±1.00[a]	8.33±1.15 [b]	9.33 ±1.15 [c]	70.0	83.3	93.3
450	7.67 ±0.58[a]	8.67±1.53 [b]	10.00±0.00 [c]	76.7	86.7	100.0

A total of three replicates for each concentration and 10 larvae per replicate

Mean mortality with different superscripts in the same row are significantly different from each other at $P<0.05$.

3.2. The Effects of Extracts on Anopheles gambiae Pupae

There was no mortality observed in the pupae test in both aqueous and methanolic extracts. After a forty-eight-hour treatment, most pupae had metamorphosed to adult in both *P. nigrum* and *C. longa* treatments. Murty *et al*. (1997) reported that when treated with a leaf extract of *Polyalthia longifolia* adults of *Culex quinquefasciatus* emerged deformed from pupae being caught in the outer shell of the insect. However, Candido *et al*. (2013) evaluated the effects of extracts from *Cnidosculos phyllacanthus, Ricinus communis,* and *Coutarea hexandra* on the developmental periods of *A. aegypti* larvae and pupae. They reported that the promising effects of these products on the pupal stage of *A. aegypti* are related to the morphological differences between the pupae and larvae, indicating that the mode of action of these products occurs through contact or choking, but not swallowing, because the pupal stage does not involve ingestion.

3.3. The Effect of Insecticidal Coils from Extracts on Adult Anopheles gambiae

The insecticidal coil repellency increased with the increase in the concentration of extracts and time of exposure. *A. gambiae* adult mortality rate of 76.7% and 70% was observed with *P. nigrum* and *C. longa* plants respectively. The LC_{50} and LC_{95} for *P. nigrum* coils were 52 and 499 while that for *C. longa,* was 63 and 468, respectively (Table 4). Similar results were obtained by Pavitha and Poornima, (2014) on the repellent potential of *Tagetes erecta* and *Callistemon brachyandrus* against *Anopheles stephensi*, *Culex infulus* and *Aedes Aegypti*. This is in accordance with Pavitha and Poornima (2014) who found that the repellent activity test for the cream formulation showed 89.87%, 87.5% and 90% protection while the smoke toxicity test for the incense coil showed 66.25%, 70% and 67.5% protection against *An. stephensi*, *Cx. infulus* and *Ae. aegypti*, respectively.

3.4. Toxicity Tests

The LC_{50} of the *P. nigrum* for the larvae of *A. gambiae* was the least and highest in essential oils (15ppm), and the aqueous extracts (52.4 ppm), respectively. The same trend was observed with *C. longa.* It was 148 ppm and 276 ppm for the larvae of *A. gambiae,* respectively. Anyaele *et al*. (2002) found that the methanolic extract *Piper guineense* (Schum) showed high toxicity on the third instar larvae of *Aedes aegypti* with LC_{50} of 1.7±0.84g/mL and had a mortality rate of 77% of the larvae. The result was also in agreement with the study of Karunamoorthi *et al*. (2014) on the mosquito repellent activity of the essential oil of *Juniperus procera* against *Anopheles arabiensis* at 1 and 5 mg/ cm^2 concentrations. The result showed repellency and protection (80.60% in 311 min) against *A. arabiensis*.

Table 4. Toxicity of *Piper nigrum* and Curcuma longa extracts on *Anopheles gambiae*.

	LC_{50} (95% C.L)	LC_{95}(95% C.L)	SLOPE±S.E	PROBIT LINE EQUATION
Piper nigrum				
Aqueous extract on larvae	36.9 (30.08-43.89)	222.9 (150.1-441.9)	2.10±0.30	Y=0.89x+7.8
Methanolic extract on larvae	26.8 (20.49-32.59)	177.7 (122.4-337.7)	2.00±0.29	Y=0.94x+14.9
Essential oil on larvae	15.4 (10.85-19.51)	79.5 (61.5-118.5)	2.31±0.32	Y=0.95x+28.4
Coil on adult mosquitoes	52.4 (45.37-61.07)	499.1 (317.6-1013.8)	1.68±0.19	Y=0.43x+4.1
Curcuma longa				
Aqueous extract on larvae	276.4 (246.9-213.6)	827.5 (640.9-1255.2)	3.45±0.45	Y=0.19x-6.3
Methanolic extract on larvae	214.1 (188.1-240.9)	659.5 (528.8-925.1)	3.38±0.40	Y=0.21x-1.63
Essential oil on larvae	148.5 (127.2-168.6)	441.5 (368.4-571.8)	3.48±0.38	Y=0.24x+5.8
Coil on adult mosquitoes	63.2 (53.9-74.7)	468.1(299.8-984.4)	1.89±0.25	Y=0.41x+3.0

4. Conclusion

The present study showed the acute toxicity and repellency of *P. nigrum* and *C. longa* against larvae and adult *A. gambiae* at varied concentrations. Extracts of *P. nigrum* and *C. longa* have shown strong toxicity and repellent activities against *A. gambiae* larvae and adults. Since these botanicals safe, cheap, abundant, and biodegradable, they can be used in the control of malaria vectors in rural areas.

More studies should, however, be carried out especially on *P. nigrun* that has a lower LC_{50} to discover, identify, and isolate the insecticidal compounds that can control the malaria vector. The government should encourage, empower and establish research institutes, give financial support for further research into the bioactivity of these plants to be able to formulate insecticidal coils that can be used to repel and prevent mosquito bites, which may eventually reduce the use of synthetic insecticides.

5. References

Adewoyin FB, Odaibo AB and Adewunmi CO. 2006. Mosquito repellent activity of *Piper guineense* and *Xylopia aethiopica* fruits oils on *Aedes aegypti*. *Afr Trad J CAM*, **3(2)**: 79-83.

Akinneye, JO and Afolabi, OJ. 2014. Toxicity and fumigant effect of powder and oil extracts of *Cleisthopholis patens* (Benth) against larvae and adults *Anopheleles* mosquito. *J. Mosq Res.*, ***4*(11)**: 1-6.

Ali A, Wang YH and Khan IA. 2015. Larvicidal and biting deterrent activity of essential oils of *Curcuma longa*, Ar-turmerone, and Curcuminoids against *Aedes aegypti* and *Anopheles quadrimaculatus* (Culicidae: Diptera). *J Med Ent.*, **52(5)**: 979-986.

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

Anyaele O, Amusan AS, Okorie TG and Oke OA. 2002. Toxicity of methanolic extract of *P. guineense* Schum and Thonn seed oil to larvae of *Aedes aegypti*. *Nig J Ent.*, **15**: 15-21.

Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B and Sopha C. 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *New England J Med.,* **371(5)**: 411-423.

Balasubramanian, S, Roselin P, Singh, KK, Zachariah, J and Saxena, SN 2016. Postharvest processing and benefits of black pepper, coriander, cinnamon, fenugreek, and turmeric spices. *Crit Rev Food Sci and Nutri.*, ***56*(10)**: 1585-1607.

Bisset JA., Rodríguez MM, French L, Severson DW, Gutiérrez G, Hurtado D and Fuentes I. 2014. Insecticide resistance and metabolic mechanisms involved in larval and adult stages of *Aedes aegypti* insecticide-resistant reference strains from Cuba. *J Am Mosq Cont Ass., **30***(**4**): 298-304.

Breman J. 2001. The ears of the hippopotamus: manifestations, determinants and malaria burden. *Am J Trop Med Hyg.*, **64(1–2**): 1-11.

Candido LP, Cavalcanti MT and Beserra EB. (2013). Bioactivity of plant extracts on the larval and pupal stages of *Aedes aegypti* (Diptera Culucidae). *Revis da Soc. Brasil. de Med. Trop.,* **4**6(4): 420-425.

Custódio KM, Oliveira JGD, Moterle D, Zepon KM, Prophiro JS and Kanis LA. 2016. A biodegradable device for the controlled release of *Piper nigrum* (Piperaceae) standardized extract to control *Aedes aegypti* (Diptera, Culicidae) larvae. *Revista da Sociedade Brasil. de Med. Trop.,* **49(6)**: 687-692.

Ebe TE, Mgbemena IC, Njoku-Tony RF, Njoku JD and Emereibeole E. 2015. Comparative analysis of petroleum ether and aqueous extracts of neem leaf and neem stem on different stages of *Anopheles gambiae. J. of Nat. Sci. Res.*, **5(12)**: 95-98.

Fafioye OO, Adebisi AA and Fayode SO. 2004. Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. *Afr. J. Biotech*., **3(10)**: 627-630

Finney D. 1947. The Estimation from Individual Records of the Relationship Between Dose and Quantal Response. *Biometrika,* **34 (3/4)**: 320-334.

Grainge M and Ahmed S. 1988. **Handbook of Plants with Pest Control Properties**. John Wiley and Sons, New York, pp 41 and 470.

Granados-Echegoyen C, Pérez-Pacheco R., Soto-Hernández M., Ruiz-Vega J, Lagunez-Rivera L, Alonso-Hernandez N and Gato-Armas R. (2014). Inhibition of the growth and development of mosquito larvae of *Culex quinquefasciatus* (Diptera: Culicidae) treated with extract from leaves of *Pseudocalymma alliaceum* (Bignonaceae). *Asian Pac J of Trop Med*., **7(8)**: 594-601.

Guinovart C, Navia MM, Tanner M and Alonso PL. 2006. Malaria: burden of disease. *Currt Mol Med.,* **6(2**):137-140

.Innocent E, Hassanali A, Kisinza WN, Mutalemwa PP, Magesa S and Kayombo E. 2014. Anti-mosquito plants as an alternative or incremental method for malaria vector control among rural communities of Bagamoyo District, Tanzania. *J Ethnobiol Ethnomed.,* **10(1)**: 56.

Irungu LW and Mwangi RW. 1995. Effects of a biologically active fraction from *Melia volkensii* on *Culex quinquefasciatus*. *Inst Appl Sci*, **2:** 159-162.

Kalaivani K, Senthil-Nathan S and Murugesan AG. 2012. Biological activity of selected Lamiaceae and Zingiberaceae plant essential oils against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Parasitol Res.,* **110**: 1261–1268

Karunamoorthi K., Girmay A and Fekadu S. 2014. Larvicidal efficacy of Ethiopian ethnomedicinal plant *Juniperus procera* essential oil against Afrotropical malaria vector *Anopheles arabiensis* (Diptera: Culicidae). *Asian Pac J Trop Biomed.,* **4**: S99-S106.

Khajuria A, Thusu N and Zutshi U. 2002. Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics: influence on brush border membrane fluidity, ultra-structure and enzyme kinetics. *Phytomed*., **9(3)**: 224-231.

Li SY, Yuan W, Deng GR and Wang P. 2011. Chemical composition and product quality control of turmeric (*Curcuma longa* L.). *Pharm Crops,* **2**: 28-54.

Maenthaisong R, Chaiyakunapruk N, Tiyaboonchai W, Tawatsin A, Rojanawiwat A and Thavara U. 2014. Efficacy and safety of topical Trikatu preparation in relieving mosquito bite reactions: A randomized controlled trial. *Comp. Therap Med.,* **22(1)**: 34-39.

Mathew PJ, Mathew PM and Kumar V. 2001. Graph clustering of *Piper nigrum* L. (Black pepper). *Euphytica*, **118**: 257-264.

Muriu SM, Coulson T, Mbogo CM and Godfray HCJ. 2013, Larval density dependence in *Anopheles gambiae* s.s., the major African vector of malaria. *J Anim Ecol.,* **82**: 166-174.

Murty US., Sriram K and Jamil K. 1997. Effect of leaf extract of *Polyalthia longifolia* (Family: Annonaceae) on mosquito larvae and puape of *Culex quinquefasciatus* (Diptera: Culicidae) Say of different habitats. *Intern Pest Cont.,* **39**: 52-53.

Muthukrishnan J, Pushpalatha E and Kasthuribhai A. 1997. Biological effects of four plant extracts on *Culex quinquefasciatus* larval stages. *Insect Sci Appl.,* **17:** 389-394.

Pavitha P and Poornima S. 2014. Repellent potential of *Tagetes erecta* L. and *Callistemon brachyandrus* Lindl. against mosquito larvae for formulation of herbal repellent compounds. *Intern J Innov Res Sci Eng Technol.*, **3(5)**:12030-12037.

Raj R, Biot C, Carrère-Kremer S, Kremer L, Guérardel Y, Gut J, Rosenthal PJ and Kumar V. 2014. 4-Aminoquinoline -β-Lactam conjugates: Synthesis, antimalarial, and anti -tubercular evaluation. *Chem Biol Drug Design*, **83(2)**:191-197.

Ranson H and Lissenden N. 2016. Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends in Parasitol.*, **32(3):**187-196.

Ravindran PN. 2007. Turmeric: The golden spice of life. *In:* **Turmeric: The Genus Curcuma**, Ravindran PN, Nirmal Babu K, Sivaraman K (Eds.). CRC Press, Boca Raton, FL, USA, pp 1-14.

Samuel M, Oliver SV, Coetzee M and Brooke BD. 2016. The larvicidal effects of black pepper (*Piper nigrum* L.) and piperine against insecticide resistant and susceptible strains of Anopheles malaria vector mosquitoes. *Parasit Vec*., **9(1)**: 238 – 245

Srivastava VK., Singh SK., Rai M and Singh A. 2003. Toxicity of *Nerium indicum* and *Euphorbia royleana* lattices against *Culex quinquefasciatus* mosquito larvae. *Nig J Nat Prod Med*., **7**:61-4.

Tisgratog R, Sanguanpong U, Grieco JP, Ngoen-Kluan R and Chareonviriyaphap T. 2016. Plants traditionally used as mosquito repellents and the implication for their use in vector control. *Acta Tropica*, **157**:136-144.

Tripathi AK, Jain DC and Kumar S. 1996. Secondary metabolites and their biological and medical activities of Piper species plants. *J Med Aroma. Plant Sci.*, **18:** 302-321.

Uneke C and Ibeh L. 2009. Impact of deforestation on malaria in South-eastern Nigeria: the epidemiology, socio-economic and ecological implication. *Internet J Third World Med*, **8**(1): 1-2.

WHO/CDS/NTD/WHOPES/GCDPP. 2006.1. Department of Control of neglected Tropical Diseases viii, 114 p