Original Research *Spatula DD. 2016; 6(3):77-83*

Larvicidal activity of essential oils from spices sold at Kariakoo market in Dar es Salaam, Tanzania, against *Anopheles gambiae* Giles ss and *Culex quinquefasciatus* Say

Dar es Selam, Tanzanya'daki Kariakoo piyasasında satılan baharatlardaki uçucu yağların *Anopheles gambiae* Giles ss and *Culex quinquefasciatus* Say tülerine karşı larvisidial aktivitesi

Deborah KB Runyoro[1,*], Olipa D Ngassapa[1], Ester Innocent[2], Raphael Z Sangeda[3], Thomas Lukuba[1]

[1]Department of Pharmacognosy, School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania
[2]Institute of Traditional Medicine, MUHAS, Dar es Salaam, Tanzania
[3]Department of Pharmaceutical Microbiology, School of Pharmacy, MUHAS, Dar es Salaam, Tanzania

ABSTRACT

Aim: Vector borne diseases are a threat to human lives throughout the world. Synthetic insecticides used to control these vectors have been associated with a number of negative effects and also the vectors have developed resistance to them, triggering the search for alternatives. Essential oils are natural products which have previously shown promising larvicidal activity; hence this study aimed at determining mosquito larvicidal activity of essential oils hydrodistilled from spices sold at Kariakoo market, in Dar es salaam, Tanzania.

Methods: Essential oils were hydro-distilled from eight spices purchased from Kariakoo market in Dar es Salaam, Tanzania. The spices included cloves, cinnamon, coriander, cumin, dill, fennel and ginger. The oils were screened for larvicidal activity, in 2012, against third instar larva of *Cu lex quinquefasciatus* and *Anopheles gambiae,* based on WHO protocol. The TLC profiles of the oils were also determined.

Results: Among the tested samples, essential oils from cumin, cinnamon, ginger and fennel were the most active against *Culex quinquefasciatus* larvae with LC_{50} values of 54.60, 59.25, 66.98 and 70.85 µg/ml, respectively. For *Anopheles gambiae* larvae, LC_{50} values of 44.7, 47.2, and 68.8 µg/ml were obtained for fennel, cinnamon and dill essential oils, respectively. These essential oils were chemically different as suggested by their TLC profiles.

Conclusion: The potential of spice essential oils in the control of mosquito vectors has been substantiated by the low LC_{50} values attained by most of the studied oils. Tanzania is rich in spices; hence further studies are necessary for effective exploitation of this avenue.

Keywords: Larvicidal activity; Spices; *Cinnamomum zeylanicum*; *Cuminum cyminum*; *Anethum graveolens*; *Foeniculum vulgare;* Tanzania; *Culex quinquefasciatus; Anopheles gambiae.*

ÖZET

Amaç: Vektör kaynaklı hastalıklar bütün Dünya'da insan yaşamı için bir tehdit oluşturmaktadır. Bu vektörleri kontrol altına almak için olumsuz yan etkileri olan sentetik insektisitler kullanılmaktadır. Sentetik insektisitlere karşı vektörler direnç geliştirmekte ve alternatif arayışını arttırmaktadır. Uçucu yağlar doğal ürünlerdir ve larvisidial aktiviteleri gösterilmiştir. Bundan dolayı, bu çalışmada, Dar es Selam, Tanzanya'daki Kariakoo piyasasında satılan baharatlardaki uçucu yağların sineklere karşı larvisidal aktivitelerinin ölçülmesi amaçlandı.

Yöntemler: Karanfil, tarçın, kişniş, kimyon, dereotu, rezene ve zencefilden elde edilen uçucu yağlar kullanıldı. Yağların üçüncü evre *Culex quinquefasciatus* ve *Anopheles gambiae* larvalarına karşı etkinliği WHO protokolü kullanılarak tarandı. Yağların TLC profilleride ölçüldü.

Bulgular: Kimyon, tarçın, zencefil ve rezeneden elde edilen uçucu yağlar sırasıyla 54.60, 59.25, 66.98 ve 70.85 µg/ml LC_{50} değerleriyle *Culex quinquefasciatus* larvalarına karşı en etkili ajanlar idi. *Anopheles gambiae* larvasına karşı rezene, tarçın ve dereotundan elde edilen uçucu yağların LC_{50} değerleri sırasıyla 44.7, 47.2 ve 68.8 µg/ml idi. Daha önceden belirtildiği üzere TLC profilleri farklı olan yağların kimyasal yapılarıda farklı idi.

Sonuç: Baharatlardan elde edilen uçucu yağların sivrisinek vektörlerin kontrol altına alınmasında etkili olduğu yapılan çalışmalardan elde edilen IC_{50} değerleriyle gösterildi. Tanzanya baharat açısından zengindir ve sivrisinek larvalarına karşı etkinliğin ortaya konulması adına daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Larvisidal aktivite; Baharatlar; *Cinnamomum zeylanicum*; *Cuminum cyminum*; *Anethum graveolens*; *Foeniculum*

vulgare; Tanzanya; *Culex quinquefasciatus; Anopheles gambiae.*

Corresponding Author:
*Dr. Deborah KB Runyoro,
Department of Pharmacognosy, School of Pharmacy, MUHAS, P.O Box 65013, Dar es Salaam, Tanzania.
drunyoro@yahoo.co.uk, /drunyoro@gmail.com

Received January 22, 2016; **Accepted** May 04, 2016
DOI 10.5455/spatula.20160613034220
Published online in ScopeMed (www.scopemed.org).
Spatula DD..

INTRODUCTION

Mosquitoes constitute a major public health problem as vectors of serious human diseases such as malaria, filariasis and dengue fever which cause substantial morbidity and mortality among people living in tropical and subtropical countries [1-3]. Malaria and filariasis alone cause about one million deaths every year [4]. In Tanzania, malaria and other vector-borne diseases contribute to the major disease burden; for instance malaria constitutes 38% and 32% of children and adults admissions in health facilities [5, 6]. Furthermore, households in malaria endemic regions in Tanzania face substantial amounts of direct cash expenses on malaria prevention and treatment [7].

By the year 2008 the number of mosquito–borne diseases in Europe stood at 10 [8]. Also, *Aedes albopictus,* a vector for dengue virus can be found in all continents of the world [9]. Furthermore, this mosquito species has been associated with the spread of chikunganya in Europe. This indicates that mosquito- borne diseases are not confined to tropical and subtropical climates only since all parts of the world are at risk.

Synthetic insecticides have been extensively used for mosquito control as adulticides, repellents and larvicides [10-12]. However, development of insect resistance to synthetic insecticides and bio-pesticides [13-15], toxicity of synthetic insecticides [16], high operational cost [17] and environmental pollution [18] call for alternative approaches to control vector-borne diseases [19].

Essential oils are associated with a number of biological activities including antimicrobial, antiepileptic, antioxidant, mosquito larvicidal, adulticidal, and repellent activities [20-26]. These liquids have a very complex chemistry, being made up mostly, of lower terpenes and their oxygenated derivatives. Some of the essential oil constituents have been reported to interfere with octapaminergic nervous system in insects, a site absent in mammals, which makes these chemicals relatively safe in mammals and fish [23]. Moreover, being synthesized within living organisms they are perceived to be associated with fewer unwanted effects seen with synthetic insecticides and/or repellents [23, 27].

Larvicidal activity of spice essential oils on a number of mosquito larvae, has been done elsewhere [28-31] demonstrating good activities. This created a need to determine the potential of essential oils from readily available spices growing in Tanzania. Moreover, promising results have been reported for other Tanzanian plant products, including essential oils [32-34]. Therefore, the current study aimed at determining mosquito larvicidal activity of essential oils hydro-distilled from spices sold at Kariakoo market, in Dar es salaam, Tanzania.

METHODS

Collection of spices and extraction of essential oils

The spices were purchased in 2012 from Kariakoo market, Dar es Salaam; the largest market in the country where most of the spices can be obtained. Selection of spices was based on their ready availability. The spices included fresh ginger (*Zingiber officinale* Roscoe) and seven other dried spices including cardamom (*Elettaria cardamomum* (L.) Maton), cloves (*Syzygium aromaticum* (L.) Merrill & Perry*)*, cinnamon *(Cinnamomum zeylanicum* Blume), coriander (*Coriandrum sativum* L.), cumin (*Cuminum cyminum* L.), Dill (*Anethum graveolens* L) and fennel (*Foeniculum vulgare* Mill). They were identified by a botanist, in the Department of Pharmacognosy, at the School of Pharmacy, MUHAS. The spices were separately subjected to hydro-distillation for 4 hours in a Clevenger-type apparatus. The resultant essential oils were dried over anhydrous sodium sulphate and stored in air-tight screw-cap vials in a refrigerator until required for testing.

Screening for larvicidal activity

Test larvae

Third-instar larvae of the two mosquito species of *Anopheles gambiae* Giles ss and *Culex quinquefasciatus* Say served as the test organisms. The larvae were reared in the mosquito insectary unit at the Institute of Traditional Medicine (ITM), MUHAS. Colonies were maintained at 26 ± 2ºC, with a photoperiod of 12 hours with light and 12

hours in the dark. Yeast powder, tetramin fish food and basic flakes were used as food source for the reared larvae.

Mosquito larvicidal tests

Mosquito larvicidal tests were carried out in 2012, based on the WHO protocol [35] with slight modifications. Stock solutions of the oils in Dimethyl Sulfoxide (DMSO) were prepared at an initial conce fishof 50mg/ml. Then two-fold dilutions were prepared in 1ml of DMSO in each of the test cups to get a total of six different concentrations ranging from 50, 25, 12.5, 6.25, 3.125 to 1.5625 mg/ml of the essential oils. Ten late third-instar mosquito larvae were placed in 19.8 ml of distilled water, followed by addition 0.2ml of essential oil solutions in DMSO in a plastic cup using a micro-pipette with disposable tips, followed by gentle shaking to ensure a homogeneous test solution. This resulted into test solutions with concentrations ranging from 500 to 15.625µg/ml of essential oil. Each test comprised three replicates with the six concentrations (500, 250, 125, 62.5, 31.25, 15.625 µg/ml). The tests were repeated on three different days. The negative control consisting of 1% DMSO was carried out in parallel.

The experiments were carried out under laboratory conditions, at 26-28ºC and a photoperiod of 12 h of light followed by 12 h of darkness, against laboratory reared third-instar larvae of *Anopheles gambiae* and *Culex quinquefasciatus* as per WHO standardized procedures and guidelines on larvicidal test method [35]. Mortality was recorded after 24 h of exposure during which larval food (tetramin for *Anopheles gambiae* and fish food for *Culex quinquefasciatus*) was added. The larvae which died were counted and the average percentage mortality was calculated.

Data analysis

The average percentage mortality was plotted against the logarithm of concentrations using the Fig P computer program. Regression equations obtained from the graphs were used to obtain LC_{50} and the 95% CI values [36].

Determination of TLC profile of the essential oils

One microlitre of a 10% solution of the essential oil in ethyl acetate was applied on the silica gel 60 F254 plate followed by developing for 10 cm in petroleum ether: ethyl acetate (48:1). The developed plates were visualized by using UV lamp (254 and 365nm), after which they were sprayed with vanillin-sulphuric acid reagent, followed by heating with a hand hair drier until coloured spots were evident.

RESULTS

The spices yielded essential oils ranging from 0.1 to 3.8% V/W (Table 1). The average percentage mortality of third-instar larvae of *Culex quinquefasciatus* mosquito induced by 24h exposure to various concentrations of essential oils were as shown in Table 2 and Fig. 1. At a concentration of 500µg/ml, 50% of the investigated essential oils including cinnamon, cumin, dill and fennel induced 100% larval mortality against *Culex quinquefasciatus* larvae. No mortality was noted in the control tests containing 1% DMSO indicating that the mortality observed was exclusively due to the spice essential oils.

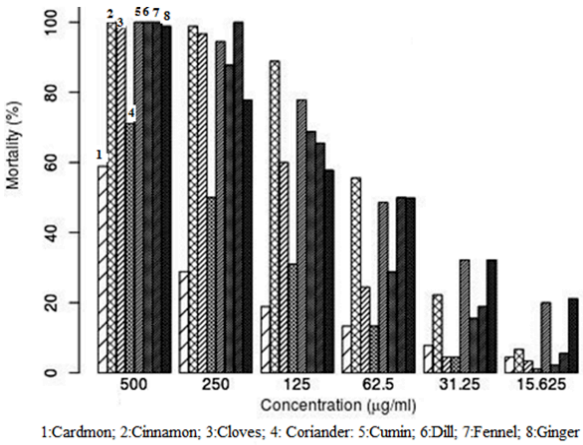

Figure 1. Percentage mortality of third- instar larvae of *Culex quinquefasciatus* after 24 h and exposure to spice essential oils

Table 1. Essential oils yields and previously reported major components

S/N	Spice	Species	Family	Yield (%V/W)	Official values (%V/W)	Major essential.oil constituents [38]
1	Cardamom	*Elletaria cardamomum*	Zingiberaceae	3.8	≥4 [37], 2.8-6.2 [38]	Terpinyl acetate and cineole
2	Cinnamon	*Cinnamomum zeylanicum*	Lauraceae	0.5	≥1.2 [37]	Cinnamic aldehyde
3	Clove	*Syzygium aromaticum*	Myrtaceae	3.3	≥15 [37], 14-21 [38]	Eugenol

4	Coriander	*Coriandrum sativum*	Apiaceae	0.2	≥0.3% [37], 1.8 [38]	(+)-Linalool
5	Cumin	*Cuminum cyminum*	Apiaceae	0.9	2.5-4 [38]	Cumic aldehyde, α-pinene, -terpinene *p*-cymene and α-terpineol
6	Dill	*Anethum graveolens*	Apiaceae	1.5	3-4 [38]	Carvone and limonene
7	Fennel	*Foeniculum vulgare*	Apiaceae	0.6	1-4 [38]	Anethole , estragole and fenchone
8	Ginger	*Zingiber officinale*	Zingiberaceae	0.1	1-2 [38]	Monoterpenes, sesquiterpnes and sesquiterpene alcohol, zingiberol

Table 2. Percentage mortality of third instar larvae of *Culex quinquefasciatus* 24 h after treatment with spice essential oils

Spice	Concentrations (µg/ml) /percentage mortality					
	500	**250**	**125**	**62.5**	**31.25**	**15.625**
Cardamon	58.9	28.9	18.9	13.3	7.8	4.4
Cinnamon	100	98.9	88.9	55.6	22.2	6.7
Clove	98.9	96.7	60.0	24.4	4.4	3.3
Coriander	71.1	50.0	31.1	13.3	4.4	1.1
Cumin	100	94.4	77.8	48.6	32.2	20
Dill	100	87.8	68.9	28.9	15.6	2.2
Fennel	100	100	65.6	50.0	18.9	5.6
Ginger	98.9	77.8	57.8	49.9	32.2	21.1

The average percentage mortality of the third instar larvae of *Anopheles gambiae* caused by 24h exposure to various concentrations of essential oils of dill, fennel and cinnamon was as displayed in Table 3 and Figure 2. At a concentration of 250 µg/ml all three essential oils exhibited 100% larval mortality after 24 h of exposure. Cinnamon essential oil was found to be the most toxic, inducing 100% larval mortality at a concentration as low as 125µg/ml.

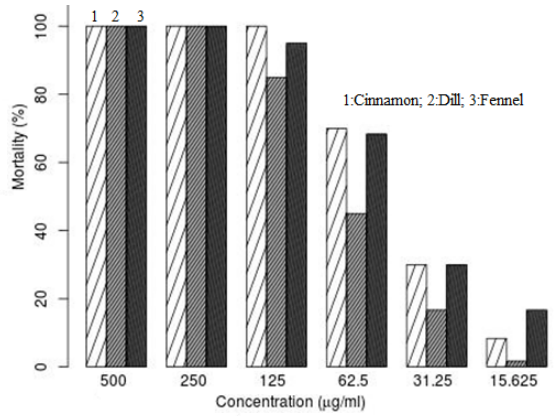

Figure 2. Percentage mortality of third- instar larvae of *Anopheles gambiae* after 24 h exposure to spice essential oils

The LC_{50} and 95% CI values (Table 4) were calculated from the regression equations. The $LC_{50,}$ for the studied essential oils against *Culex quinquefasciatus* ranged from 54.6 µg/ml (cumin) to 645.77 µg/ml (coriander). Also, the LC_{50} values for the three essential oils from cinnamon, dill and fennel against the third instar larvae of *Anopheles gambiae* ranged from 44.7µg/ml for fennel, through 47.2 µg/ml for cinnamon, to 68.3µg/ml for dill (Table 5). The TLC chromatograms (Fig 3) of the spice essential oils showed at least two separated spots as in the case of cloves; for some oils the spots were as many as seven. Several components were fluorescent under UV light. Also, the chromatogram displayed several colours when the plates were sprayed with vanillin-sulphuric acid. The TLC profiles were quite distinguishable.

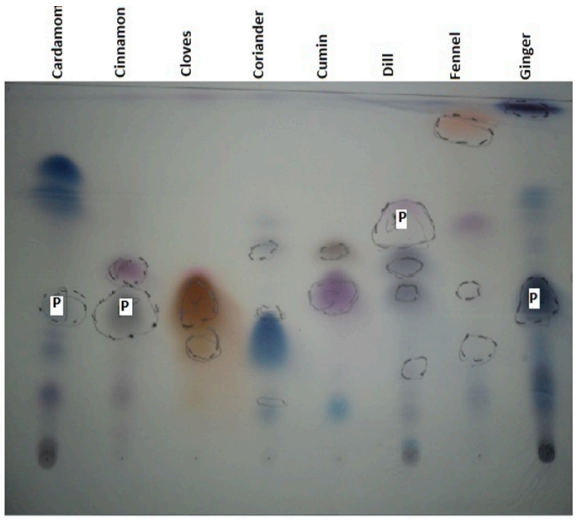

Figure 3. TLC Profiles of the essential Oils

Table 3. Percentage mortality of third- instar larvae of *Anopheles gambiae* in 24 h after exposure to spice essential oils

Spice	Concentrations (µg/ml) and % mortality					
	500	250	125	62.5	31.25	15.625
Cinnamon	100	100	100	70	30	8.3
Dill	100	100	85	45	16.7	1.7
Fennel	100	100	95	68.3	30	167

Table 4. Median Lethal concentrations of essential oils against *Culex quinquefasciatus larvae*

Spice	LC_{50} (µg/ml)	Regression equation	95% CI
Cardamom	645.77	Y= 32.376*log*X- 40.979	(237.92-1752.75)
Cinnamon	59.25	Y= 69.286*log*X- 72.821	(37.16-94.5)
Clove	94.09	Y= 74.981*log*X- 97.979	(61.14- 144.80)
Coriander	248.35	Y= 47.878*log*X- 64.671	(126.42- 487.76)
Cumin	54.60	Y= 58.424*log*X- 51.493	(31.40-94.95)
Dill	86.81	Y= 70.762*log*X- 87.176	(54.97-137.07)
Fennel	70.85	Y= 69.391*log*X- 78.396	(44.47- 112.86)
Ginger	66.98	Y= 50.725*log*X- 42.620	(35.41- 126.65)

Table 5. Median Lethal concentrations of essential oils against A*nopheles gambiae larvae*

Spice	LC_{50} (µg/ml)	Regression equation	95% CI
Cinnamon	47.21	Y= 66.28*log*X- 60.952	(28.99- 76.91)
Dill	68.83	Y= 74.19*log*X- 86.347	(44.52- 106.41)
Fennel	44.74	Y= 62.01*log*X- 52.361	(26.56-75.34)

DISCUSSION

Among the spices studied cardamom was found to have the highest yield of essential oils when compared to other spices while ginger gave the lowest yield. However, essential oil yields were very much lower than standard values [37, 38]. A number of factors including improper cultivation, collection, preparation and storage could have led to decreased essential oil yields. Also, deliberate adulteration with exhausted drug could have the same effect. However, in the case of cinnamon the spice at Kariakoo market

is normally unpeeled hence it contains cork and cortex structures which are supposed to be absent from the official drug [37]. Cork and cortex are devoid of essential oils and this could have largely contributed to the decreased yield. Furthermore, fresh Ginger was used and since the % yield is based on dry weight of plant materials this led to a low essential oil yield for this spice.

This study showed that all essential oils, except the essential oils of coriander and cardamom, exhibited good LC_{50} values. Moreover, the test larvae were exposed to the essential oils for only 24 h implying that the LC_{50} values are likely to decrease further with increased exposure time [34, 39, 40]. In a previous study done in India clove essential oil exhibited LC_{50} and LC_{90} values of 17.84 and 23.99 mg/ml respectively against *Aedes albopictus* [29]. These values are relatively higher when compared with LC_{50} of 94.09µg/ml obtained in this study for *Culex quinquefasciatus*. Cinnamon oil was observed to be one of the most active essential oils in this study with LC_{50} of 47.21 and 59.25µg/ml for *Anopheles gambiae* and *Culex quinquefasciatus,* respectively. However, the commercial cinnamon oil in Delhi, India, was comparatively more active exhibiting a LC_{50} of 0.63159 mg/L against fourth instar larvae of *Aedes aegypti* [41]. Moreover, it has been reported that lower LC_{50} values for cinnamon could be associated with cinnamic aldehyde, the major component of this essential oil, which has been reported earlier, to possess larvicidal activity [23]. The *Anopheles gambiae* larvae were more susceptible to the essential oils when compared to *Culex quinquefasciatus;* this trend is similar to that observed in previous studies [36, 39]. The observed LC_{50} values for fennel and cinnamon essential oils does not differ much from those previously reported for a Tanzanian plant *Cryptomeria japonica* leaf essential oil [34]. Differences seen in the larvicidal activity of the various studied essential oils could be attributed to the difference in their chemical composition as indicated in the literature (Table 1) [38] and also confirmed by their TLC profiles (Fig 3). Since the types of reported constituents are influenced by a number of factors including climate and collection time, there is a need to determine the actual constituents of these essential oils by GC-MS, especially for essential oils which showed marked larvicidal activity. The determined TLC profile (Fig.3), however, provides a cheap tool for tracking active essential oils for further study in resource limited settings.

CONCLUSION

Seventy five percent (75%) of the studied spice essential oils demonstrated a good larvicidal activity. These promising results, in concurrence with the increased problems linked with usage of synthetic insecticides and management of mosquito borne diseases, call for more studies including semi-field and field studies, toxicity to non-targeted organisms aiming at developing effective and safe larvicides from essential oils. In addition, it is also important to determine the chemical constituents for the observed larvicidal activity.

ACKNOWLEDGEMENT

We appreciate the technical support granted by technicians of the School of Pharmacy and the Institute of Traditional Medicine, at Muhimbili University of Health and Allied Sciences.

REFERENCES

1. Nauen R. Perspective insecticide resistance in disease vectors of public health importance *Pest Manag Sc* 2007;63:628–633 .
2. Makundi EA, Mboera LGE, Malebo HM, and Kitua AY. Priority setting on malaria interventions in Tanzania: Strategies and challenges to mitigate against the intolerable burden. Am J Trop Med Hyg 2007; 77(Suppl 6), 106–111.
3. Tolle MA. Mosquito-borne Diseases. Cur Probl Pediatr Adolesc Health Care 2009; 39 (4): 87-140.
4. Slater HC, Gambhir M, Parham PE, Michael E. Modelling co-infection with malaria and lymphatic filariasis. PLoS Comput Biol 2013; 9(6): e1003096. doi:10.1371/journal.pcbi.1003096
5. Jowett M, Miller NJ. The Financial burden of malaria in Tanzania: Implications for future government policy. Int J Hlth Plan Manag 2005; 20(1):67-84.
6. Magesa SM, Lengeler C, de Savigny D, Miller JE, Njau RJA, Kramer K, Kitua A, Mwita A. Creating an "enabling environment" for taking insecticide treated nets to national scale: the Tanzanian experience. Malar J 2005, 4:34 doi:10.1186/1475-2875-4-34.
7. Chamwali LA. The economic Burden of malaria in Tanzania. A Post Field Report Submitted to AERC in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy (Economics) of the University of Dar es Salaam, November, 2013.
8. Hubálek Z. Mosquito-borne viruses in Europe. Parasitol Res 2008, 103: 1. Supplement 1, 29-43.
9. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. *Aedes albopictus*, an arbovirus vector: From the darkness to the light. Microbes Infect 2009; 11 (14–15): 1177–85.
10. Joseph CC, Ndosi MM, Malima RC, Nkunya NHM. Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and petrocarpans from *Neorautanenia mitis*. Trans R Soc Trop Med Hyg 2004; 98(8):451-5.
11. Tiwary M, Naik SN, Tewary DK, Mittal PK, Yadav S. Chemical composition and larvicidal activity of the essential oils of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. J Vect Borne Dis 2007; 44(3):198-204.
12. Okumu OF, Moore SJ. Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for

the future. Malar J 2011, 10:208 http://www.malaria journal.com/content/10/1/208 [Access date: 21.08.2015].
13. Kumar PM, Kovendan K, Murugan K. Integration of botanical and bacterial insecticide against *Aedes aegypti* and *Anopheles stephensi*. Parasitol Res 2013. 112:761–771.
14. Protopopoff N, Matowo J, Malima R, Kavishe R, Kaaya R, Wright A, West PA, Kleinschmidt I, Kisinza W, Mosha FW, Rowland M. High level of resistance in the mosquito *Anopheles gambiae* to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania. Malaria J 2013; 12:149. http://www.malariajournal.com/content/12/1/149 [Access date: 08.04.2016].
15. Thanspong K, Sathantriphop S, Chareonviriyaphap T. Insecticide resistance of *Aedes aegypti* and *Culex quinquefasciatus* in Thailand. J. Pestic. Sci., 2008; 33(4), 351–356 DOI: 10.1584/jpestics.G08-12
16. Rogan WJ, Chen A. Health risks and benefits of bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT). The Lancet 2009; 9487:763-773.
17. Yukich JO, Zerom M, Ghebremeskel T, Tedios F, Lengeler C. Costs and cost-effectiveness of vector control in Eritrea using insecticide-treated bed nets. Malar J 2009; 8:51 doi:10.1186/1475-2875-8-51.
18. DeMicco A, Cooper KR, Richardson JR, White LA. Developmental neurotoxicity of pyrethroid insecticides in Zebrafish embryos. Toxicol Sci 2010;113(1), 177–86.
19. Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya PR. A review on prospects of essential oils as biopesticide in insect-pest management. J Pharmacognosy Phytother 2009; 1(5) 052-063.
20. Liu C, Liu Q, Zhou L, Liu ZL. Evaluation of larvicidal activity of the essential oil of *Allium macrostemon* Bunge and its selected major constituent compounds against *Aedes albopictus* (Diptera: Culicidae). Parasites & Vectors 2014, 7:184. http://www.parasites and vectors.com/content/7/1/184. [Access date: 08.07.2015]
21. Park H-M, Kim J, Chang K-S, Kim B-S, Yang Y-J, Kim G-H, Shin S-C, Park I-K. Larvicidal activity of Myrtaceae essential oils and their components against *Aedes aegypti*, acute toxicity on *Daphnia magna*, and aqueous residue. J Med Entomol 2011; 48(2): 405- 10.
22. Govindarajan M, Sivaku S. Mosquito adulticidal and repellent activities of botanical extracts against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Asian Pac J Trop Dis. 2011: 4(12): 941-7.
23. Kaoul O, Walia S, Dhaliwal GS. essential oils as green pesticides: Potential and constraints. Biopestic Int 4(1): 63–84 (2008).
24. Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of Rosemary and Sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) Essential Oils J Agric Food Chem. 2007, 55:7879–85.
25. Quintans- Junior LL, Souza TT, Leite BS, Lessa NMN, Bonjardim LR, Santos MRV, Alves PB, Blank AF, Antoniolli AR. Phythochemical screening and anticonvulsant activity of *Cymbopogon winterianus* Jowitt (Poaceae) leaf essential oil in rodents. Phytomedicine 2008; 15(8): 619–24.
26. Nóbrega de Almeida R, Agra M de F, Maior FNS, Pergentino de Sousa D. Essential oils and their constituents: Anticonvulsant activity. *Molecules* 2011; 16 (3): 2726-42.
27. Lahlou M. The success of natural products in drug discovery. Pharmacology & Pharmacy 2013; 4: 17-31 http://dx.doi.org/10.4236/pp.2013.43A003. [Access date:15.07.2015].
28. He W, Huang B. A review of chemistry and bioactivities of a medicinal spice: *Foeniculum vulgare.* J Med Plant Res. 2011; 5(16): 3595-3600.
29. Bhat SK, Kempraj V. Biocidal potential of clove oils against *Aedes albopictus* – A comparative study. Afr J Biotechnol 2009; 8 (24): 6933-7.
30. Sedaghat MM, Dehkordi AS, Abai MR, Khanavi M, Mohtarami F, Vatandoost Abadi SY, Rafi F, Vatandoost H. Larvicidal activity of essential oils of Apiaceae plants against malaria vector, *Anopheles stephensi.* Iran J Arthropod-Borne Dis, 2011, 5(2): 51–9.
31. Youssif RS, Shaalan EA. Mosquitocidal activity of some volatile oils against *Aedes caspius* mosquitoes J Vector Borne Dis 2011; 48: 113–5
32. Kweka E J, Nyindo M, Mosha F, Silva AG. Insecticidal activity of the essential oil from fruits and seeds of *Schinus terebinthifolia* Raddi against African malaria vectors. Parasit Vectors 2011; 4: 129 http://www.parasitesandvectors.com/content/4/1/129 [Access date: 09.07.2015].
33. Runyoro DKB, Ngassapa O, Kachali L, Obare V, Lyamuya EF. Biological activities of essential oils from plants growing in Tanzania. East Cent Afr J of Pharm Sci 2010; 13:85-91
34. Mdoe FP, Cheng S-S, Lyaruu L, Nkwengulila G, Chang S-T 4and Kweka EJ. Larvicidal efficacy of *Cryptomeria japonica* leaf essential oils against *Anopheles gambiae*. Parasit Vectors 2014; 7:426 http://www.parasitesandvectors.com/content/7/1/426. [Access date:17.07.2015].
35. World Health Organisation, Guidelines for Laboratory and field testing of mosquito larvicides. http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf. [Access date:22.06. 2012]
36. Nkya JW, Erasto P, Chacha M. Larvicidal against mosquito vectors and brine shrimp activities of extracts from the flowers of *Moringa oleifera.* Lam. Amer J Res Comm. 2014; 2(8):15-29.
37. British Pharmacopoeia Commission, *British Pharmacopoeia,* Vol. IV. London: TSO. 2015. p. 127,141,146 & 151.
38. Evans WC. Trease and Evans Pharmacognosy. 16th edition. Edinburgh, London New, York, Philadelphia, St Louis, Sydney, Toronto: Elsevier Limited; 2009. p. 263-303.
39. Ohaga SO, Ndiege IO, Kubasu SS, Beier JC, Mbogo CM. Larvicidal activity of *Piper guineense* and *Spilanthes mauritiana* crude powder against *Anopheles gambiae* and *Culex quinquefasciatus* in Kilifi District, Kenya. J Biol Sci 2007; 7(7): 1215-20.
40. Obomanu FG, Ogbalu OK, Gabriel UU, Fekarurhobo GK, Adediran BI. Larvicidal properties of *Lepidagathis alopecuroides* and *Azadirachta indica* on *Anopheles gambiae* and *Culex quinquefasciatus*. Afr J Biotechnol. 5: 761-5
41. Warikoo R, Wahab N, Kumar S. Larvicidal potential of commercially available pine (*Pinus longifolia*) and cinnamon (*Cinnamomum zeylanicum*) oils against dengue fever mosquito, *Aedes aegypti* L. (Diptera Culicidae). Acta Entomol Sin 2011; 54(7): 793-9.