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Repellent Activities of the Essential Oils of Four Sudanese Accessions of Basil (*Ocimum basilicum* L.) Against *Anopheles* Mosquito

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Abstract: Basil (*Ocimum basilicum* L.) of the Labiatae is an important culinary herb and essential oil source widely recognized worldwide, though much less in Sudan. The essential oil of 4 basil accessions were assessed for mosquito repellency were suggested. Basil seed accessions were collected as seeds and grown at the University of Gezira farm, Wad Medani, Sudan. All four essential oil conferred complete mosquito repellency (assayed by the human-bait technique) lasting for 1.5 to 2.5 h per one application of 0.1 mL to a volunteer's arm. Repellency generally decreased with bioassay time. The experimental results obtained from this study suggest that essential oil of basil are promising as repellents at 0.1% concentration against *Anopheles* mosquito and could be useful in the search for new natural repellent compounds.

Key words: *Ocimum basilicum*, essential oil, extraction, *Aopheles* mosquito, repellency

INTRODUCTION

Mosquitoes are important vectors of diseases and nuisance pests. A mosquito's principal food is nectar or similar sugar source. Mosquitoes can be an annoying, serious problem in man's domain. They interfere with work and spoil hours of leisure time. Their attacks on farm animals can cause loss of weight and decreased milk production. Some mosquitoes are capable of transmitting diseases such as malaria, yellow fever, dengue, etc. Malaria is transmitted by different *Anopheles* species, depending on the region and the environment (Burfield and Reekie, 2005). Mosquitoes are a major threat for over 2 billion people in the tropics (Odalo *et al*., 2005).

To control mosquitoes, any type of control should involve careful consideration of the biology of the mosquitoes and based on scientific surveillance. A response to control nuisance mosquitoes may look very different from a response to control disease-vectoring mosquitoes (Lee, 2000). In all cases, larval mosquito control should be considered as the first option for abatement. This involves location of larval habitats, followed by their modification or treatment in such a way that the integrity of the habitat is preserved but the mosquito larvae are reduced in numbers.

By controlling larval mosquitoes, the adults may never become problem. Larviciding has the greatest control impact on mosquito populations because the larvae are concentrated, immobile and accessible. Integration of disease treatment with vector control (the latter, comprising of insect population control and personal protection from mosquito bites) is considered the most effective means for diseases control. Currently, repellents and Insecticide-Treated Bed (ITBs) nets represent the most practical and economic methods of controlling vectors, the most common mosquito repellent products available in the market contain DEET (N,N-diethyle-3-toluamide) (Odalo *et al*., 2005). The search for effective vaccines against malaria and other mosquito responsible diseases are still in progress. Several studies have on focused natural products for controlling some mosquitoes as insecticides and larvicides, but with varied results (Cavalcanti *et al*., 2004).

Essential oil are natural volatile substances obtained from a variety of plants. Commercially, essential oil have many uses such as pharmaceuticals, flavor in many food products, odorants in fragrances and as insecticides. Also, particular emphasis has been placed on their antibacterial, antifungal and insecticidal activities.

Recently, various plants extracts, such as neem (*Azadirachta indica,* A. jass), basil oil (*Ocimum basilicum* L., *O. gratissimum* L. and *O.americanum* L.), citronella grass (*Cymbopogon nardus* Rendle), galingale (*Alpinia galangal* L.), clove (*Syzygium aromatium* L.) and thyme

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(*Thymus vulgaris* L.) have been studied as possible mosquito repellents (Odalo *et al*., 2005).

Therefore, the purpose of this study is to determine the repellent activity of four accessions of basil (*Ocimum basilicum* L.) essential oil against *Anopheles* mosquito.

MATERIALS AND METHODS

Basil seeds used in these studies were obtained from 20 different parts of Sudan; seeds of basil accessions were directly sown on 60 cm wide ridges at the Demonstration Farm (Nishishiba), Fac. Agri. Sciences, University of Gezira, Wad Madani, Sudan. Sowing was done on Feb. 20, 2005. Watering, weeding etc were carried out as necessary. No chemicals (fertilizers or others) were applied. Observations were made on growth and flowering of the plants weekly, the accessions were given numbers, as previously reported by Abduelrahman *et al*. (2009). Essential oil of 4 basil accessions obtained by steam distillation, fresh leaves of plant material (150 g) was subjected to stem distillation. The extraction was carried out at a rate not exceeding 3 mL min^{-1}, distillation was continued for 2-4 h. This procedure was repeated until at least 10 mL of oil had recovered. The volume of the obtained essential oil was dried over anhydrous Na_2SO_4, stored in a dark bottle and kept at 4°C until it analysis or were evaluated for mosquito repellency as described below.

Rearing of mosquitoes: The mosquitoes, *Anopheles* mosquito's larvae, were collected at various sites in Gezira state; the larvae were transferred separately in metal dishes (40 cm diameter) and their rearing was continued until pupation. The larvae were reared continuously for several generations and kept under 25-30°C, 70-80% relative humidity and photoperiod of 13:11 h (light/dark) on 4, August, 2006 to 15, September, 2006, in the insectary of Blue Nile Research and Training Institute, Wad Medani, Sudan. Larvae were fed on ground commercial biscuit. The adults were reared in humidified cages and supplied with 10% sugar solution and 10% multivitamin syrup supplied in plates. Female mosquitoes were periodically blood-fed on restrained rabbits to obtain protein used principally for egg production. Under these conditions, the full development from egg to adult lasted about 3-5 weeks. Batches of 3-5 day-old healthy female mosquitoes were used in the repellency bioassay.

Repellency tests: The repellency of the essential oils of 4 basil accessions was evaluated using the human-bait technique (Schreck and McGovern, 1989; WHO, 1996). The tests were carried out at night as the test organism, anopheles, is a night biter. Evaluations were carried out by placing the arms of volunteers inside 40×40×40 cm cages. The temperature was maintained at 25-30°C and the relative humidity at 60-80%. Three human volunteers were employed for one repellency tests. Both arms of volunteers, which would later be placed one in each cage, were carefully covered with thick paper, except for a cut area of 3×10 cm exposing the arm to mosquito bites. This area (of 3×10 cm) was not treated with anything for control (one arm) or pretreated by 0.1 mL of basil essential oil (the other arm). Each arm of a volunteer was placed inside the cage containing 50 female mosquitoes for 3 min. Exposure was repeated every 30 min without renewing the essential oil treatments. During the 3 min exposure the number of mosquitoes landing on each 3×10 cm test area was observed. Mosquitoes that land and bite are clearly distinguished by the visible blood in their bodies. The number of these mosquitoes taken throughout the test period 6 h (20:00 to 02:00 h) was used as the number of bites calculated per hour. Each mosquito that has bitten once was removed from cage by the visible blood in their bodies and replaced by a new one. This test repeated for all 4 accessions with same conditions.

Data analysis: The median protection time was used as a standard measure of the repellency of the volatile oil against mosquito in the laboratory. Percent protection from mosquito landing/biting or repellency was computed as compared to control by the following equation (Tawatsin *et al*., 2001):

$$\text{Repellency (\%)} = \left[(C - T) \times 100\right] / C$$

where, C is the number of mosquitoes collected from control area and T is the number of mosquitoes collected from the treated areas of volunteer's arms. Statistical analysis was conducted using a statistical analysis program, the Origin version 6.1.

RESULTS AND DISCUSSION

Insect repellency of the crude essential oils of four basil accessions: Certain basil crude essential oils were claimed to have a repellent activity towards mosquitoes (Tawatsin *et al*., 2001). To test this claim the essential oils of four accessions of basil were selected and tested for mosquito repellency on human volunteers using the method described above.

Table 1 shows the numbers of mosquitoes (*Anopheles*) biting on the control and treated areas of the arm and Fig. 1 shows insect repellency of four volatile oils against *Anopheles* mosquito. The same volume

Table 1: Number of mosquito bites on arms of volunteers

Time	Mean number of mosquito biting arms of volunteers											
(h) A.	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
10	0.0	0.0	0.0	0.80	2.5	5.9	13.0	15.0	20.4	27.4	31.6	32.4
16	0.0	0.0	0.0	0.65	0.8	2.7	4.3	8.2	11.7	15.4	19.5	25.5
17	0.0	0.0	0.0	0.00	0.0	0.8	2.5	4.5	08.8	13.3	21.8	25.4
20	0.0	0.0	0.0	0.00	0.6	3.1	5.1	9.6	11.1	15.8	19.5	21.3
Control	4.8	8.5	17.1	18.00	21.1	25.7	30.0	31.0	34.0	38.1	40.0	40.8

*A. Accession; No. of bites are means of three replicates, Control: Means of three replicates of untreated arms. The same volume (0.1 mL) of the essential oil from each accession tested was uniformly applied (just ones) to a volunteer's arm, which was exposed to the mosquitoes and repellency evaluated

Table 2: GC-MS analysis (%) compositions of essential oils of 4 basil accessions cultivated in Nishishiba

	Basil accession			
Component	10	16	17	20
Cineol	11.31	09.48	06.01	04.28
Cymene	-	-	-	02.21
Menthanol	-	01.01	-	-
Linalool	50.92	29.43	03.86	0.99
Caravone	-	-	-	t
Camphor	01.14	00.91	03.11	08.43
Caranol	-	-	-	-
Geranial	00.94	00.29	18.10	11.82
Geraniol	26.76	00.55	60.10	58.16
Estragol	01.02	06.37	00.04	00.16
Thujo	-	09.01	-	08.03
Cy. citral	-	00.41	-	00.2
Eugenol	-	01.24	00.56	00.05
M. cinnamate	-	40.42	10.3	-
M. eugenol	-	-	-	t
Bergamotene	01.83	00.09	-	0.35
Bisabolene	-	-	-	t
Isoledene	-	00.42	-	t

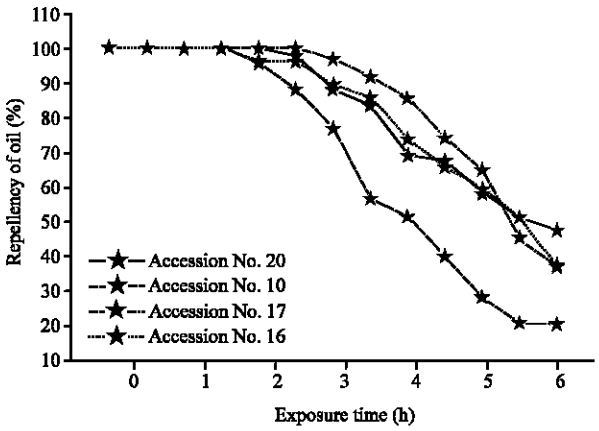

Fig. 1: Insect repellency of four volatile oils against *Anopheles* mosquito. The essential oil was applied only once onto hands of volunteers and repellency followed for up to 6 h

(0.1 mL) of the essential oil from each accession tested was uniformly applied (just once) to a volunteer's arm, which was exposed to the mosquitoes and repellency evaluated. All basil accessions showed complete (100%) repellency toward mosquitoes till 1½ h of exposure of the volunteer's arm to the insects. Accessions No. 17 and 20 provided complete protection against bites for 2 ½ and 2 h, respectively (Fig. 1). With long exposure times observed repellency decreased markedly especially in accession No. 10. This could be due to increased volatilization of the applied essential oil with time, reducing efficacy.

Table 2 shows the GC-MS analysis (%) compositions of essential oil of 4 basil accessions cultivated in Nishishiba. It is noteworthy that GC-MS separations of the essential oil in the previously study (Abduelrahman *et al*., 2009) for these accessions. We classify the basil accessions analyzed according to the major essential oil constituents present. The 4 accessions were classified into 3 classes where either one essential oil constituent dominated in proportions exceeding 50% of the oil constituents or/otherwise, the first two major constituents designed the group, the more dominant compound named first. The major 3 classes (chemotypes) were: linalool chemotype (accession No. 10), methyl cinnamate-linalool (accession No. 16) and geraniol-geranial class (accession No. 17 and 20). An attempt was made to correlate between repellency with essential oil chemical constituents. No clear-cut correlations were obtained. One generalization that could be made, the two accession No. 17 and 20 which provided complete protection against bites for 2- 2 ½ h, both have geraniol as dominant component, other two accession No. 10 and 16 the dominant component were linalool and *M. cinnamate*, respectively. So, may be the reason of high protection due to present of geraniol, however, this claim may warrant further research.

Chokechaijaroenporn *et al.* (1994) showed repellency of the volatile oil of several basil species against Ae. Aegypti lasting between 15 and 105 min, depending on the basil type. On the other hand, in a study in Guinea Bissau, West Africa, fresh *Ocimum canum* Sims (syn. *Ocimum americanum*) could reduce biting by anopheline mosquitoes, about 63.6%. Kweka *et al.* (2008) reported the repellency of plant species including *O. kilimandscharium* and *O. suave* with high biting protection reached (83 to 91%) and feeding inhibition (71.2 to 92.5%), respectively. It was observed against three species of mosquitoes. Other essential oil e.g., oil of turmeric, kaffir lime, citronella grass and citronella cream were shown to possess repellency against mosquitoes (Assabgui *et al*., 1997; Palsson and Jaenson, 1999; Rajkumar and Jebanesan, 2007, 2008; Oyewole *et al*., 2008;

Tawatsin *et al*., 2001). In this study, we tested the repellent activity of four accessions of *O. basilicum* essential oil against *Anopheles* mosquito. The results obtained from this study indicate that all four essential oil of basil should promising repellent activity against *Anopheles* mosquito. These results, somewhat, same with some earlier studies in repellent activity of basil essential oil against mosquito; but these also differ with some reported studies, the differences can be attributed to many factors; perhaps the most obvious is the difference in concentration or amount of essential oil, also some researchers obtained the oil by solvent extraction, while we obtained by steam distillation. However, comparisons, such as the preceding, are confounded by differences in the mosquito species tested, environmental factors.

From knowledge of the essential oil components of the 4 accessions mosquito repellent activity could not be assigned to a particular compound. So, further investigations are needed to elucidate the four essential oil against a wide range of mosquito species and also to identify the active ingredient.

CONCLUSION

In conclusion, this study clearly demonstrated the potential of volatile oil derived from some basil species for use as topical repellents against nocturnal mosquitoes. Repellent activity is subject to improvement. These basil oil can, therefore, serve as a substitute for some synthetic insecticides which are expensive and have toxic residual effects to human.

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REFERENCES

Abduelrahman, A.H.N., S.A. Elhussein, N.A. Osman and A.H. Nour, 2009. Morphological variability and chemical composition of essential oils from nineteen varieties of basil (*Ocimum basilicum* L.) growing in Sudan. Int. J. Chem. Technol., 1: 1-10.

Assabgui, R., F. Lorenzetti, L. Terradot, P. Hedin, W. Holling, E. Masler, J. Miyamoto and D. Thompson, 1997. Efficacy of the botanicals from the Meliaceae and Piperaceae. J. Phytochemicals Pest Contr., 1: 38-48.

Burfield, T. and S. Reekie, 2005. Mosquitoes, malaria and essential oils. Int. J. Aromather., 15: 30-41.

Cavalcanti, E., S. Morais, M. Alima and E. Santana, 2004. Larvicidal activity of essential oils from Brazilian plants against Aedes Aegyptil. J. Oswaldo Cruz, Rio De Janeiro, 99: 541-544.

Chokechaijaroenporn, O., N. Bunyapraphatsara and S. Kongchuenisn, 1994. Mosquito repellent activities of Ocimum volatile oils. Phytomedicine, 1: 135-139.

Kweka, E., F. Mosha, A. Lowassa, A. Mahande and J. Kitau *et al*., 2008. Ethnobotanical study of some mosquito repellent plants in North Eastern Tanzania. Malaria J., 7: 152-152.

Lee, E., 2000. Mosquito larvicidal activity of piper nonaline, piperidine alkaloid derived from long pepper, piper longum. J. Am. Mosq. Contr. Assoc., 16: 245-247.

Odalo, J., M. Omolo, H. Malebo, J. Angira, P. Njeru, I. Ndiege and A. Hassanali, 2005. Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*. Acta Trop., 95: 210-218.

Oyewole, I., C. Ibidapo, D. Moronkola, A. Doula, G. Adeoye, G. Anyasor and J. Obansa, 2008. Anti-malarial and repellent activities of *Tithonia diversifolia* (HemsL.) leaf extracts. J. Medicinal Plants Res., 2: 171-175.

Palsson, K. and T. Jaenson, 1999. Plant products used as mosquito repellents in Guinea Bissau, West Africa. Acta Trop., 72: 39-52.

Rajkumar, S. and A. Jebanesan, 2007. Repellent activity of selected plant essential oils against the malarial fever mosquito *Anopheles stephens*. J. Trop. Biomed., 24: 71-75.

Rajkumar, S. and A. Jebanesan, 2008. Bioactivity of flavonoid compounds from *Poncirus trifoliate* L. (Family: Rutaceae) against the dengue vector, *Aedes aegypti* L. (Dipteria: Culicidae). J. Parasitol. Res., 104: 19-25.

Schreck, C.E. and T.P. McGovern, 1989. Repellents and other personal protection strategies *Aedes albopicstus*. J. Am. Mosq. Control Assoc., 5: 247-250.

Tawatsin, A., S. Wratten, R. Scott, U. Thavara and Y. Techadamrongsin, 2001. Repellency of volatile oils from plants against three mosquetoe vectors. J. Vector Ecol., 26: 76-82.

WHO., 1996. Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES/IC/96.1, Control of Tropical Diseases Division, World Health Organization, Geneva. http://whqlibdoc.who.int/hq/1996/CTD_WHOPES_IC_96.1.pdf.