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A Study of the Essential Oils of Four Sudanese Accessions of Basil (*Ocimum basilicum* L.) Against *Anopheles* Mosquito Larvae

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Abstract: Problem statement: Certain basil essential oils were claimed to have a larvicidal activity towards mosquito's larvae. To test this claim the essential oils of four accessions of basil grown in Sudan were selected and tested for *Anopheles* larvae. Malaria is the major health problem in the Sudan and the whole country is now considered endemic, with varying degrees, about 35,000 deaths every year due to malaria. *Anopheles* mosquito is the major vector of malaria disease in Sudan. Search for larvicidal active compound(s) is one of several attempts to fine effective and affordable ways to control this mosquito. To determine the toxic effects of basil essential oils extracted by steam distillation against *Anopheles* larvae. **Approach:** For the larvicidal bioassay, three concentrations (100, 300, 500 ppm) of essential oil solutions of four basil accessions were prepared; 1 mL of ethanol was used to solubilize the oil in water (999 mL). In each concentration of oil solution were inserted 20 larvae (third instars). A set of controls using 0.1% ethanol and untreated sets of larvae in (tap) water, were also run for comparison. Data were evaluated through regression analysis, from the regression line; the LC_{50} values were read. The active ingredients were separated and/ or identified by TLC, IR and GC-MS. **Results:** Larvicidal activity of the essential oils is varied, lasted for about 9 h and thereafter decreased, LC_{50} values ranging from 190-300 ppm. Linalool, geraniol and eugenol are active components of basil essential oil against *Anopheles* larvae. Two accessions were caused 100% mortality at a concentration range 300-500 ppm for 3 h. **Conclusion:** These results indicated that basil essential oils have larvicidal activity towards *Anopheles* larvae. Therefore, could be affordable way to control this mosquito.

Key words: Basil, *Ocimum basilicum*, essential oil, larvicidal activity, *Anopheles* mosquito

INTRODUCTION

Mosquitoes are insects belonging to the order Diptera and as other true flies; develop through four distinct life stages-egg, larva, pupa and adult. It is hard to comprehend the amount of disease and the resulting sickness, death and economic loss caused by the mosquito[1]. 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 and dengue. Malaria is transmitted by different *Anopheles* species, depending on the region and the environment[2].

To control mosquito, any type of control should involve careful consideration of the biology of the mosquitoes and be based on scientific surveillance. A response to control nuisance mosquitoes may look very different from a response to control disease-vectoring mosquitoes[3]. 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[4]. By controlling larval mosquitoes, the adults may never become a problem. Larviciding has the greatest control impact on mosquito populations because the larvae are concentrated, immobile and accessible. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat.

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The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment. Aromatic plants and their essential oils are very important sources of many compounds that are used in different respects. Essential oils and plant extracts are still an important natural resource and more promising for pesticides/ insecticides[5].

Therefore, the purpose of this study is to determine the activities of essential oils from four basil accessions (*Ocimum basilicum* L.) grown in Sudan were tested against third instars *Anopheles* mosquito larvae.

MATERIALS AND METHODS

Plant material (seed sources): Basil seeds used in these studies were obtained from different parts of Sudan. Commercial basil accession seeds were obtained from Germany and the United Arab Emirates (UAE). 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 was carried out as necessary. No chemicals (fertilizers or others) were applied. Observations were made on growth and flowering of the plants weekly. Taxonomic identification of plants was performed by botanists of the National Centre for Research, department of biology, Khartoum, Sudan. The accessions were given numbers, as previously reported[6]. Essential oils of 4 basil accessions obtained from fresh leaves of the plants by steam distillation were evaluated for larvicidal activity as described.

Rearing of mosquitoes larvae: 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. Third instars of *Anopheles* mosquito larvae were used in the larvicidal bioassay.

Larvicidal activity: For the larvicidal bioassay, the essential oil solutions of the four basil accessions were prepared; one ml of ethanol was used to solubilize the oil in water (999 mL). In 50 m L beakers containing 20 mL of each concentration of oil solution were inserted 20 larvae (third instars). With each experiment, a set of controls using 0.1% ethanol and untreated sets of larvae in (tap and distilled) water, were also run for comparison. Mortality was recorded after 1, 3, 6, 9, 12 and 24 h, of test during which no nutritional supplement was added. The experiments were carried out at 30±1°C. Each test comprised of three replicates with three concentrations (100, 300, 500 µL L^{-1}). Data were evaluated through regression analysis. From the regression line; the LC_{50} values were read representing the lethal concentration for 50% larval mortality of *Anopheles* mosquito.

RESULTS

Larvicidal activity of the crude essential oils of four basil accessions: Table 1 shows larvicidal activity of essential oils of 4 basil accessions against *Anopheles* mosquito larvae. These accessions included basil grown in Sudan as ornamental plants (one accession, no. 10), one accession introduced from UA Emirates (no. 16), one accession introduced from Germany (no. 17) and accession no. 20 is grown in Sudan as wild basil.

Table 1: Larvicidal activity of essential oils of 4 basil accessions against mosquitoes (*Anopheles*) larvae

Percentage of mortality of larvae																														
	Time of incubation																													
	3						6						9						12						24					
	Tr.																													
Oi.	10	16	17	20	Ci	Cii	10	16	17	20	Ci	Cii	10	16	17	20	Ci	cii	10	16	17	20	Ci	Cii	10	16	17	20	Ci	Cii
100	10	15	20	0	0	0	40	30	37	0	0	0	40	35	37	10	0	0	40	35	37	10	0	0	40	35	40	10	0	0
300	40	75	60	55	0	0	50	85	95	55	0	0	60	95	95	75	0	0	60	95	95	75	0	0	60	95	95	75	0	0
500	60	100	100	90	0	0	70	100	100	95	0	0	75	100	100	95	0	0	75	100	100	95	0	0	75	100	100	95	0	0

*Tr. = Treatment, Oi = Oil does, ci = Control 1(0.1% ethanol set), cii = Control 2 (untreated set)

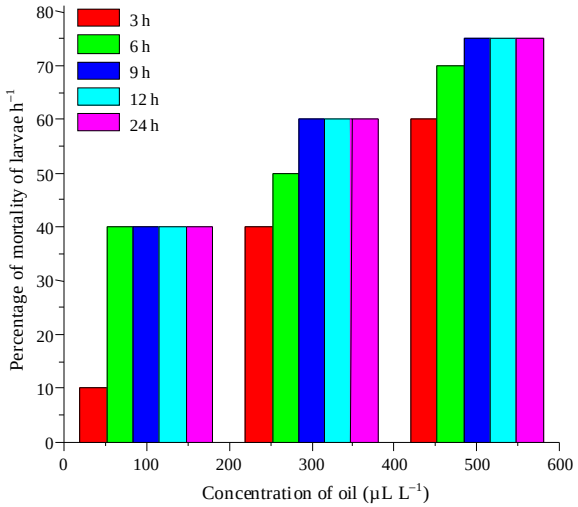

Fig. 1: Larvicidal activity of different concentrations of the essential oil of accession no. 10. (Measured at different time intervals (3-24 h) the results are expressed as % mortality of mosquito larvae)

The oils were used in three concentrations and the % mortality of the larvae was observed after 3, 6, 9, 12 and 24 h of incubation. All these essential oils of 4 accessions showed considerable larvicidal activity when tested against larvae of *Anopheles* mosquito.

The LC_{50} value (the lethal concentration for 50% of larval mortality of *Anopheles* larvae) was 190 µL L^{-1} for accession no. 16, 200 µL L^{-1} for accession no. 17, 280 µL L^{-1} for accession no. 20 and 300 µL L^{-1} for accession no. 10. All essential oils of the 4 accessions were active larvicidally till 9 h of incubation. Thereafter, the activity remained constant between 9 and 12 h till 24 h of exposure of the larvae to the essential oils. Fig. 1 shows the Larvicidal activity of different concentrations of the essential oil of accession no. 10 measured at different time intervals (3-24 h) the results are expressed as % mortality of mosquito larvae and Fig. 2a-c shows the mortality of larvae (%) with different concentrations of oils (A = 100 µL L^{-1}, B = 300 µL L^{-1} and C = 500 µL L^{-1}).

The essential oil of accession no. 16 was subjected to Thin Layer Chromatography (TLC) analysis. Five TLC bands (corresponding zones detected using Anisaldehyde-sulfuric acid) were scraped off the plates, eluted and appropriately tested for larvicidal activity.

Three bands were found larvicidally very active, 100%, mortality reached within just half-an-hour of exposure (the concentration was not accounted for, roughly equivalent to 50 mg of crude essential oil). The remaining two bands were inactive.

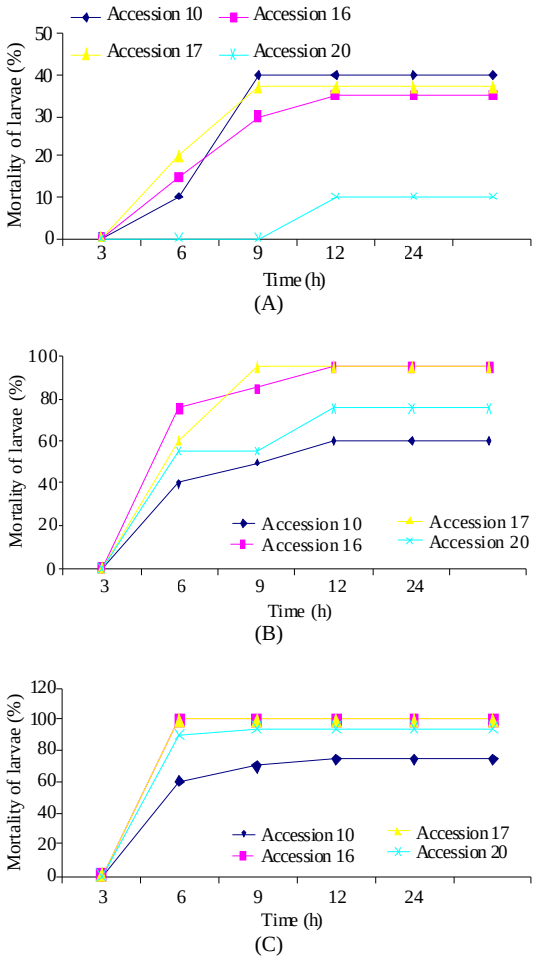

Fig. 2A-C: Mortality of larvae (%) with difference concentrations of oils (A = 100 µL L^{-1}, B = 300 µL L^{-1} and C = 500 µL L^{-1})

TLC elutes of the three larvicidally active compounds were subjected to i.r. spectrum analysis (in chloroform solution). We studied the i.r. spectra, absorption frequencies observed and the possible functional groups present in the three bands (compounds) as interpreted from the literature[7]. These data suggest that the active bands (compounds) are eugenol, geraniol and linalool.

DISCUSSION

Larval mortality increased as the essential oil concentration increased. The essential oils of the different accessions had differential activity. After 3 h of treatment, for example, the highest dose (500 µL L^{-1}) caused 100% mortality in the case of accessions (no. 16) and (no. 17), 90% and 60% mortality with

accessions (no. 20) and (no. 10) respectively. This order of larvicidal potency (16 and 17 > 20 > 10) remained the same for all treatment periods and concentrations except for the anomaly of accession no. 20 having exceptionally little activity at the lower concentration.

In previous study, authors reported larvicidal activity of essential oil against *Aedes aegypti* at concentration of (2, 4, 6, 8 and 10%). Activity was different for the different instars stages[8]. However, these authors used a concentration much higher than the one used in this study (the concentration used here was 0.01%). In another study author recorded different mortalities for larvicidal activity of *Ocimum sanctum* leaf extraction with acetone, chloroform, ethyl acetate, hexane and methanol against *Aedes aegypti* larvae[9]. Previous studies reported that some basil species showed solvent extracts from the whole plant have larvicidal properties against *Anopheles* mosquitoes' larvae[10-12].

The i.r. suggestion of three bands of accession no. 16 shown eugenol, geraniol and linalool are active compounds. It is noteworthy that the major components of the essential oil of accession no. 16 as determined by GLC were methyl cinnamate (40.42%), linalool (29.43%), cineole (9.48%), thujone (9.01%), methyl chavicol (6.37%) and eugenol (1.24%), as previously reported[6]. Thus Linalool, a major component, is likely to be one of the active compounds. This is supported by reported literature[13]. The two other larvicidally active compounds are eugenol, geraniol, the structure of which was suggested by i.r. spectra and are present as minor components in accession 16.

In this study, we tested the larvicidal activity of four accessions of basil essential oils grown in Sudan against *Anopheles* mosquito larvae. The results obtained from this study indicate that all four essential oils of basil should promising larvicidal activity against *Anopheles* mosquito larvae. These results, somewhat are same with some previous studies in larvicidal activity of basil essential oil against mosquito larvae; but there also some 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 and oil obtaining method (some authors obtained the oil by solvent extraction, while for this study the essential oils obtained by steam distillation). However, comparisons, such as the preceding, are confounded by differences in the mosquito species larvae tested, basil species and environmental factors. So, further investigations are needed to elucidate the four essential oils against a wide range of mosquito species larvae and so far the formulation of active ingredients.

CONCLUSSION

In conclusion, the essential oils of *Ocimum* spp., namely accessions no. 16 and no. 17 were shown to be quite potent as larvicidal agents against *Anopheles* mosquito larvae and caused 100% mortality at a concentration range of 300-500 μL L^{-1} for 3 h and caused 95% mortality at a concentration of 300 μL L^{-1} for 6 h. These results are very promising in creating new effective and affordable approaches to the control of *Anopheles* mosquito and larvae.

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