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Bioactive Compounds from Basil (*Ocimumbasilicum*) Essential Oils with Larvicidal Activity against *Aedesaegypti* Larvae

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Abstract. Dengue, yellow fever and dengue haemorrhagic fever are health problems in many countries. The *Aedesaegypti* mosquito is the major vector for these diseases. The development of insect resistance and side effects associated with synthetic pesticides make essential oils the focus of intense research efforts because of their biological activities and long, safe use as natural products. In this study, the essential oils from two basil (*O. basilicum*) accessions were tested in a laboratory bioassay for larvicidal activity against third instar *A. aegypti* larvae. The essential oils were extracted by steam distillation, and their chemical compositions were determined by GC-MS. Approximately 13 compounds (>1%) were detected; most compounds were oxygenated monoterpenes. Methyl chavicol and geranial were the predominant compounds in the two observed accessions. For the bioassay, six concentrations (50, 100, 200, 300, 400 and 500 µg/ml) of essential oil solutions were prepared from the two basil accessions. For each concentration of oil solution, 25 third instar larvae were inserted. Controls, 1% DMSO or untreated larvae in tap water were also performed for comparison. The active ingredients in the essential oils were separated and identified by TLC and GC-MS. The larval assays provided LC_{50} and LC_{90} values for the methyl chavicol accession (MCV) were 160 and 262µg/ml, respectively; the values for the geranial-geraniol accession (GGV) were 174 and 356µg/ml, respectively. Linalool, geraniol, geranial, methyl chavicol and eugenol were active components against *A. aegypti* larvae.

Keywords: Basil, *O. basilicum*, Essential oil, Larvicidal, *A. aegypti,* Larvae

1. Introduction

Mosquitoes are generalvectors that carry disease-causing viruses and parasites from person to person. Some of these diseases can be life threatening, such as malaria, yellow fever, dengue fever, filariasis, dengue haemorrhagic fever and Japanese encephalitis. For instance, yellow fever, haemorrhagic fever and dengue fever are transmitted by the *Aedesaegypti*mosquito[1,2]. These diseases exist worldwide in tropical and subtropical stagnant water. To minimise and eradicate the occurrence of mosquito-borne diseases, many steps have been taken to prevent their spread to different extents;for example, mosquito eradication atearly or late stages, disease prevention via prophylactic drugs and vaccines and the prevention of mosquito bite using repellents. In all cases, larval mosquito control should be considered as the first option for abatement.By controlling larval mosquitoes, 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 are thuseasy to deal with.

Aromatic plants and their essential oils are very important sources of many compounds that are used for different applications[3]. The use of conventional pesticides in the water supply, however, introduces risks to people andto the environment. Essential oil and plant extracts are still important natural resources,and they are more promisingthan pesticides or insecticides for control of mosquito populations[4].

The goalsof this study were to evaluate the toxicity of essential oils from two basil accession (*Ocimumbasilicum* grown in Malaysia) againstthird instar *Aedesaegypti*mosquito larvae and todetermine the bioactive ingredients of these essential oils.

2. Material and Methods

2.1. Plant Material (Seed Sources)

Two commercial basil seed accessions were obtained from Sudan. Seeds were sown on 16 April 2009directly on the ridges at University Malaysia Pahang-UMP, Kuantan, Malaysia. Taxonomic identification of the plants was performed by botanists in the School of Envi. Sci.s and Nat. Res, National University Malaysia, Selangor, Malaysia. Thebasil accessions were given theacronyms MCV (methyl chavicolaccession) and GGV (geranial-geraniolaccession). Essential oilsfrom both basil accessions were obtained by steam distillation of fresh plant leaves. The essential oils were then evaluated for larvicidal activity as described below.

2.2. GC-MS

The composition of the essential oils was determined using an Agilent 7890A Gas Chromatography – Mass Spectrometry instrument. Oxygen-free nitrogen was used as a carrier gas, and hydrogen was used for the flame. The GC conditions used were as follows: capillary column; fused silica (polydimethylsiloxane, 0.25 μm film thickness); temperature program: 70 °C (2 min^{-1}), 70 – 230 °C (3 min^{-1}), 230 – 240 °C (5 min^{-1}), 270 °C (5 min^{-1}); carrier gas, He at 5 bar, linear velocity of 20 cm min^{-1}; injection port splitless at 250 °C; injection volume, 0.1 μL. The MS conditions were as follows: ionisation EI at 70 eV; m/z range, 30-300 °C; scan rate 1 sec^{-1}; ionisation chamber at 180 °C; and transfer line at 280°C. The identification of the essential oil constituents was based on a comparison of their retention times, and these constituents were further identified and authenticated using their MS data compared to the NIST mass spectral library.

2.3. Larvicidal Bioassay

For the larvicidal bioassay,essential oil solutions (derived from the two basil accessions) were prepared. For each essential oil concentration, twenty-five larvae (third instars) were placed into a 50 ml beaker containing 20 ml of oil solution. For each experiment, controls were run for comparison. Larvae mortality was recorded after incubation for 1, 3, 6, 9, 12 and 24 hours without any nutritional supplements. The experiments were performed at 28 ± 1 °C. Each test comprised three replicates with six concentrations (50, 100, 200, 300, 400 and 500μg/ml). Data were evaluated by a regression analysis. The $LC_{50}$and LC_{90} values of *Aedesaegypti* mosquito larvae (representing the lethal concentration at which 50% and 90% of the larvae showed mortality, respectively) were derived from the regression line.

3. Results and discussion

3.1. Larvicidal activity of the crude essential oils from two basil accessions

Larval mortality increased as the essential oil concentration increased, andthe essential oils from the different accessions had different activities.For example, the highest dose (500μg/ml) caused 100% mortality after 3-hour incubation with oils of the MCV accession, whereas the same dose caused 100% mortality after 6 hours with oils of the GGV accession. The MCV accession was more potent than the GGV accession for all treatment periods and concentrations.

The LC_{50} and LC_{90} values were 160 and 262μg/ml, respectively, for the methyl chavicol (MCV) accession and 174 and 356μg/ml, respectively, for the geranial-geraniol (GGV) accession. Essential oils from both accessions had larvicidal activity until 9 hours of incubation. Thereafter, the activity remained constant between 9 and 12 hours until 24 hours of exposure of the larvae to the essential oils. This phenomenon may be explained by the volatility of essential oils. Figs. 1, 2 and 3 show the larvicidal activity of different essential oil concentrations (for the two basil accessions) at different time points (3, 6 and 9hours); the results are expressed as the percentageof mortality of the mosquito larvae.

Previous studies showed that essential oil from different plants hadlarvicidal activities against *Aedesaegypti*larvae and that the activities were different for different instar stages[5]. In another studies also

demonstrated that the essential oil extracted from*Ocimum sanctum* leaves had different larvicidal activities against *Aedesaegypti*larvae depending on the extraction solvent[6].Whole-plant extracts of some basil species showed larvicidal properties against *Anopheles* mosquito larvae[1,7,8].

3.2. Identification of the Active Ingredients

The essential oils of basil accessions were subjected to a thin layer chromatography (TLC) analysis. Eight different TLC bands from two plates (0.25 mm silica-gel precoated plate) were scraped off, eluted and appropriately tested for larvicidal activity. Five bands corresponded to the methyl chavicolaccession, and three bands corresponded to the geranial-geraniolaccession. Five of the eight bands were highly active in the larvicidal activity assay; 100% larvae mortality was reached within half an hour of exposure to the essential oil. The five active TLC elutions that contained larvicidal compounds were subjected to GC-MS (in dichloromethane). The GC-MS data showed that the three active compounds in the methyl chavicolaccession were eugenol, methyl chavicol and linalool; and that the two active compounds in the geranial-geraniolaccession were geranial and geraniol.

Notably, the main essential oil components of the methyl chavicol accession (determined by GC-MS) were methyl chavicol (43%), linalool (17.55%), geranial (13.73%), eucalyptol (5.00%), methyl cinnamate (5.00%), α-bergamotene (3.22%) and eugenol (2.79%) as well as β-pinene, β-myrcene, caryophyllene, germacrene and β-ocimene (1.00-1.89%). The second basil accession was composed of geranial (68.42%), geraniol (9.61%) and linalool (8.12%) as well as β-ocimene and caryophyllene (1.05-2.90%).

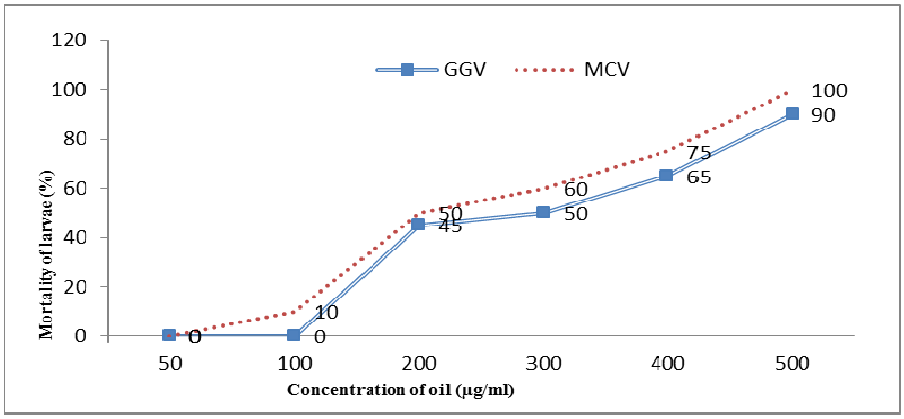

Fig. 1: Mortality of larvae (%) after incubation for 3 hours with different essential oil concentrations.

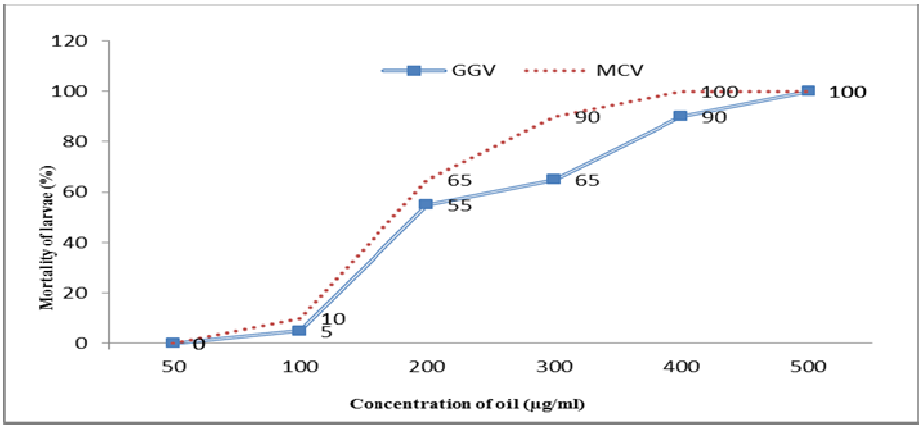

Fig. 2: Mortality of larvae (%) after incubation for 6 hours with different essential oil concentrations.

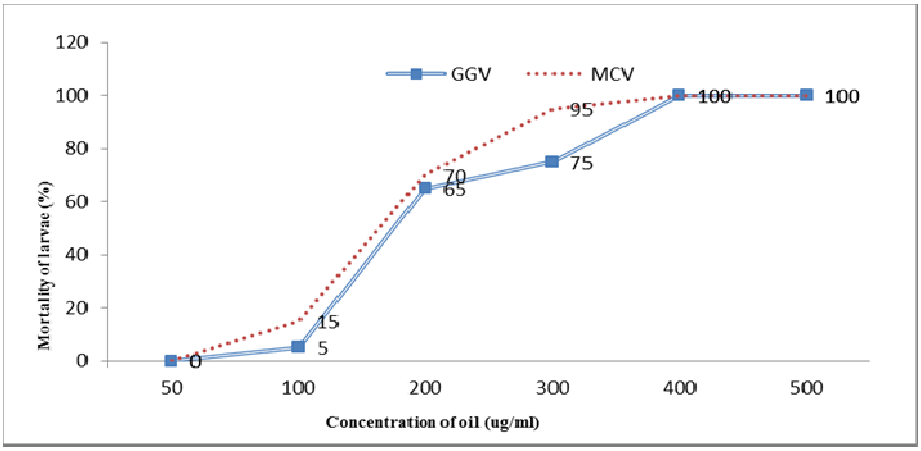

Fig. 3: Mortality of larvae (%) after incubation for 9 hours with different essential oil concentrations.

4. Acknowledgements

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