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Comparative Study of Different Species of Tulsi for Lanvicidal Activity

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Abstract*: Basil is a fragrant, small tree or shrub native to warm and temperate regions of the world. The larvicidal activity of essential and different oils of B. santo, B. basilicum, and B. fragrans was compared on laboratory-collected and field-collected Culex quinquefasciatus larvae. Thin layer chromatography analysis showed that all three strains were similar; the results indicated the presence of steroids and triterpenoids. Larvicidal activity is determined by the 24-hour LD50 against third in star or early fourth instar larvae. Comparison of LD50 values showed that O. basilicum was the more effective of the two species. His LD50 value. Basilicum and O. sainttum oil were determined to be 39.31 and 40.02, respectively, in larvae reared in the laboratory, and 129.53 and 139.49, respectively, in larvae collected in the field. Laboratory-reared larvae are more sensitive than field-collected larvae.*

Keywords: Basil quinquefasciatus Larvised LD50 value

I. INTRODUCTION

The acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of Ocimum sanctum were studied against fourth instar larvae of Aedes aegypti and Culex quinquefasciatus. The highest larval mortality was found in leaf extract of O. sanctum against the larvae of A. aegypti and C. quinquefasciatus. The LC50 values of O. sanctum against the larvae of A. aegypti were 425.94, 150.40, 350.78, 575.26, and 175.67 and against the larvae of C. quinquefasciatus were 592.60, 93.92, 212.36, 76.61, and 82.12 ppm, respectively.Ocimum is a genus of aromatic herbs, undershurbs or shrubs distributed in the tropical and warm temperate regions of the world. Larvicidal activity of essential oils and different extracts of O. sanctum, O. basilicum and O. gratissimum were compared on laboratory reared and field collected larvae of Culex quinquefasciatus. Thin layer chromatographic analysis revealed that all the three species have similar components and results showed the presence of steroids and triterpenoids. The larvicidal activity was determined in terms of LD_{50} value on late third or early fourth instar larvae for a period of 24 h. A comparison of LD_{50} value has shown that O. basilicum is more active than the other two species. The LD_{50} value of O. basilicum and O. sanctum oil were 39.31 and 40.02 on laboratory reared larvae and 129.53 and 139.49 on field collected larvae. Laboratory reared larvae were more sensitive than field collected larvae.

Extracts from 56 species of plants in the Euro-Asiatic region were tested for larvicidal activity against the fourth larval instar of the mosquito Culex quinquefasciatus Say (Diptera: Culicidae). All plant extracts showed larvicidal activity after 24 h of exposure to the plant extracts in a maximal dose of 500 ppm. The extracts of the plants Otanthus maritimus and Ammi visnaga displayed the highest larvicidal effect (LD(50) 7 and 9 ppm, respectively) followed by Acer pseudoplatanus, Humulus japonicus, Acer platanoides, Satureja hortensis, Ocimum basilicum and Thymus vulgaris (LD(50) 23, 25, 28, 28, 32 and 48 ppm respectively). For eight species, the appraisal value of LD(50) was between 51 and 100 ppm, another eight species from 101 to 200 ppm, 15 species from 201 to 500 ppm and for 17 species, low mortality showed no lethal dose (LD(50)>500 ppm).

In recent years, use of environment friendly and biodegradable natural insecticides of plant origin have received renewed attention as agents for vector control because they are rich in bioactive chemicals, active against a limited number of species including specific target insects, and biodegradable. The present study was carried out to evaluate the adulticidal, repellent, and larvicidal activity of crude hexane, ethyl acetate, and methanol extracts of eight plants, viz. Aristolochia indica L., Cassia angustifolia Vahl, Diospyros melanoxylon Roxb., Dolichos biflorus L., Gymnema sylvestre (Retz) Schult, Justicia procumbens L., Mimosa pudica L., and Zingiber zerumbet L., were tested against adult and early fourth instar larvae of Culex gelidus Theobald and Culex quinquefasciatus Say (Diptera: Culicidae). The effective adult mortality was observed in methanol extract of A. indica, ethyl acetate extract of D. biflorus, and ethyl acetate and hexane extract of Z. zerumbet against C. gelidus and C. quinquefasciatus (LD(50) =37.75, 78.56, 129.44, 86.13, 80.06, 112.42, 53.83, and 46.61; LD(90) =166.83, 379.14, 521.50, 289.83, 328.18, 455.72, 181.15, and 354.50 ppm, respectively). Complete protections for 150 min were found in hexane and methanol extract of A. indica and Z. zerumbet at 1,000 ppm against mosquito bites. The highest larval mortality was found in the hexane extract of Z. zerumbet, ethyl acetate extract of D. biflorus, and methanol extracts of A. indica against C. gelidus (LC(50) =26.48, 33.02, and 12.47 ppm; LC(90) =127.73, 128.79, and 62.33 ppm) and against C. quinquefasciatus (LC(50) =69.18, 34.76, and 25.60 ppm; LC(90) =324.40, 172.78, and 105.52 ppm), respectively, after 24 h. The plant extracts are potential to be used as an ideal eco-friendly approach for the control of the Japanese encephalitis vector, C. gelidus, and lymphatic filariasis vector, C. quinquefasciatus.

A screening for larvicidal activity of plant extracts with some known medicinal attributes could lead to the discovery of new agents for pest and vector control. In the backdrop of recent revival of interest in developing plant-based insecticides, the present study was carried out to evaluate the larvicidal properties in three medicinal plants growing abundantly in the region of Chitheri Hills, Dharmapuri District, India. Antifeedant and larvicidal activity of the acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of Ocimum canum, Ocimum sanctum and Rhinacanthus nasutus were studied against fourth instar larvae of Spodoptera litura (Lepidoptera: Noctuidae), Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in methanol extract of O. canum, R. nasutus and acetone extract of O. sanctum against the larvae of S. litura (LC(50) = 36.46, 68.08 and 68.84 ppm), against A. aegypti (LC(50) = 99.42, 94.43 and 81.56 ppm) and against C. quinquefasciatus (LC(50) = 44.54, 73.40 and 38.30 ppm), respectively. This is an ideal eco-friendly approach for the control of the agricultural pest, S. litura, and medically important vectors, A.

II. MATERIALS AND METHODS

Preparation of Plant Extracts

For methanolic extract, 250 g of finely powered leaves were extracted with methanol in a Soxhlet apparatus (boiling point range 50–80 ºC) for 8 hours. For aqueous extracts, 250 g of leaf powder was extracted with water in a boiling water bath (60 ºC) for 4 hours and filtered through muslin cloth. Both the extracts were concentrated under reduced pressure (22–26 mm Hg) at 45 ºC. The obtained residue was stored at room temperature.

Phytochemical Screening

The phytochemical screening test was performed as described by Trease and Evans [11] and Sofowora [12].

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

FTIR identifies the presence of organic and inorganic compounds in the sample. FTIR spectrum of aqueous and methanolic extracts of O. sanctum, O. basilicum, L. aspera, C. amboinicus was recorded in the range of 4000–400 cm^{-1} using FTIR spectrophotometer (Spectrum RX I, PERKIN ELMER).

Chemicals and reactions The following chemicals were purchased from their respective companies: Dimethyl sulfoxide (DMSO) from Fisher Scientific Ltd., Loughborough, United Kingdom; absolute ethanol, n-hexane (chromatography grade) and Silica 60 F254 from Tatemer, Darmsch, Germany; acetone, hexane and methanol (analytical grade) from Prochem Chemicals Inc., High Point, NC, USA; and temephos from the Vector Control Research Laboratory, Universiti Sains Malaysia, Penang, Malaysia.

Extraction of Plant Material

Fresh leaves of O. basilicum L. were collected from Universiti Tunku Abdul Rahman Kampar Agricultural Park, Malaysia, in January 2015. The plant is named after ethnobotanist Dr. Hean Chooi Ong is a former professor at the Faculty of Science at the University of Malaya, Malaysia. Wash the leaves under running water to remove dirt and grime and air dry in a cool place for 24 hours. The leaves (1.3 kg) are then chopped into small pieces using a stainless steel blender before extraction. Mixed leaves were soaked in hexane for two days at room temperature and mixed at 120

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rpm using an orbital shaker (Orbit LS, Labnet International Inc., Woodbridge, NJ, USA). The immersion was repeated for five cycles. The filtrate was concentrated to dryness at 40 °C using a rotary evaporator (Rotavapor R-200, Buchi Labortechnik AG, Flawil, Switzerland), and the dry extracts were stored at −20 °C until bioassays.

Mosquito sampling and larval culture

From February 2015 to June 2015, 60 oviposition devices (height: 9.0 cm x depth: 7.8 cm) were placed outdoors around the West Lake Garden (Kampar, Malaysia) for mosquitoes to lay eggs. Garden is a suburb full of middle-class families and a former mining lake. Spawners are hung on trees or bamboo at a distance of approximately 20-30 m and 1 m above the ground. A chipboard paddle (height: 10.0 cm × length: 2.5 cm × width: 0.3 cm) was placed in each spawning device containing dechlorinated tap water. Change the water in the rowing and spawning unit every two to three days. The spoons containing the eggs are taken back to the laboratory and air dried for two days before being placed in distilled water for incubation. The resulting larvae are fed ground cat food (Cuties Catz, Perfect Companion (M) Sdn. Bhd., Kuala Lumpur, Malaysia) until they develop into pupae and adults. Temperature and humidity ranged from 22.0 °C to 27.7 °C and 67% to 78%, respectively [33]. Emerging adults were used for species identification using a stereomicroscope (Zoom 2000, Leica Microsystems Inc., Buffalo, NY, USA) [34]. Only third instar

Larvicidal Activity against Different Species of Mosquito Larvae

The cultures of *Armigeres subalbatus*, *Aedes aegypti*, and *Culex tritaeniorhynchus* were maintained for 10 generations. The third instar larvae (50 Nos) was collected from the colony and placed in a 50 mL beaker and various concentrations of UAE Ocimum essential oils were added by dissolving in DMSO (0.5 mL) up to 50 mL using deionized distilled water/milliQ water.

The mortality at the end of 24 h in each concentration of essential oil was determined by counting the dead larvae (using magnifying lens on a colony counter) and percentage of death and LC50 was estimated.

III. RESULTS

In this study, the hexane extract of the leaves of *O. basilicum* was evaluated against third instar larvae of *Ae. albopictus* using six concentrations ranging from 6.25 to 200 µg/mL for 24 and 48 h of exposure. As shown in **Figure 1**, the larval mortality rates increased from 3.3% at 6.25 µg/mL to 100% at 100 and 200 µg/mL after 24 h of exposure. Further exposure for another 24 h resulted in higher larval mortality rates for 12.5 µg/mL (51.7% to 66.7%), 25.0 µg/mL (68.3% to 78.3%), and 50 µg/mL (91.7% to 100%). Statistical comparisons of the larval mortality performed using one-way ANOVA revealed that there were significant differences between groups for both 24 h ($F(6, 14) = [360.205]$, $p = 0.00$) and 48 h of exposures ($F(6, 14) = [277.130]$, $p = 0.00$).

IV. CONCLUSION

The hexane extract and fractionations obtained from the leaves of *O. basilicum* have demonstrated significant larvicidal effects against wild strain *Ae. albopictus* that are both concentration and time dependent. GC-MS analysis revealed the presence of methyl chavicol, methyl eugenol, cedrelanol, and 2,4-di-tert-butylphenol as the major components in the active subfractions with reported larvicidal activities against disease-carrying mosquitoes, including *Ae. albopictus*. In addition, the larvicidal effects were also contributed by germacrene D, α-humulene, or β-elemene, which were minor components with known larvicidal property. These results are indicative of the use of *O. basilicum* leaves as a potential source of natural mosquitocidal agents in integrated vector management programs. Application of these natural larvicides alongside the reduction of usage of synthetic larvicides may serve to decrease the population of *Aedes* mosquitoes, and eventually the transmission of arboviruses to humans without the decremental effects to the environment. Further investigations using laboratory strains of both dengue vectors are necessary to substantiate the herb's efficacy.

REFERENCES

[1]. Wakasugi, M.; Kawamura, K.; Yamamoto, S.; Kazama, J.J.; Narita, I. High mortality rate of infectious diseases in dialysis pa-tients: A comparison with the general population in Japan. *Ther. Apher. Dial.* 2012, *16*, 226–231. [Google Scholar] [CrossRef] [PubMed]

[2]. Casadevall, A. Climate change brings the specter of new infectious diseases. *J. Clin. Investig.* 2019, *130*, 553–555. [Google Scholar] [CrossRef] [PubMed][Green Version]

[3]. Thaden, J.T.; Li, Y.; Ruffin, F.; Maskarinec, S.A.; Hill-Rorie, J.M.; Wanda, L.C.; Reed, S.D.; Fowler, V.G., Jr. Increased Costs Associated with Bloodstream Infections Caused by Multidrug-Resistant Gram-Negative Bacteria Are Due Primarily to Patients with Hospital-Acquired Infections. *Antimicrob. Agents Chemother.* 2017, *61*, 01709–01716. [Google Scholar] [CrossRef] [PubMed][Green Version]

[4]. Peterson, E.; Kaur, P. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Front. Microbiol.* 2018, *9*, 2928. [Google Scholar] [CrossRef]

[5]. Govindarajan, M.; Sivakumar, R.; Rajeswary, M.; Yogalakshmi, K. Chemical composition and larvicidal activity of essential oil from *Ocimum basilicum* (L.) against Culex tritaeniorhynchus, Aedes albopictus and Anopheles subpictus (Diptera: Culicidae). *Exp. Parasitol.* 2013, *134*, 7–11. [Google Scholar] [CrossRef]

[6]. Sumitha, K.V.; Thoppil, J.E. Larvicidal efficacy and chemical constituents of *O. gratissimum* L. (Lamiaceae) essential oil against Aedes albopictus Skuse (Diptera: Culicidae). *Parasitol. Res.* 2015, *115*, 673–680. [Google Scholar] [CrossRef]

[7]. Scalvenzi, L.; Radice, M.; Toma, L.; Severini, F.; Boccolini, D.; Bella, A.; Guerrini, A.; Tacchini, M.; Sacchetti, G.; Chiurato, M.; et al. Larvicidal activity of Ocimum campechianum, Ocotea quixos and Piper aduncum essential oils against Aedes aegypti. *Parasite* 2019, *26*, 23. [Google Scholar] [CrossRef][Green Version]

[8]. Gbolade, A.A.; Lockwood, G.B. Toxicity ofOcimum sanctumL. Essential oil toAedes aegyptiLarvae and its Chemical Composition. *J. Essent. Oil Bear. Plants* 2008, *11*, 148–153. [Google Scholar] [CrossRef]

[9]. Cavalcanti, E.S.B.; De Morais, S.M.; Lima, M.A.A.; Santana, E.W.P. Larvicidal Activity of essential oils from Brazilian plants against *Aedes aegypti* L. *Memórias Do Inst. Oswaldo Cruz* 2004, *99*, 541–544. [Google Scholar] [CrossRef]

[10]. Yamani, H.A.; Pang, E.C.; Mantri, N.; Deighton, M.A. Antimicrobial Activity of Tulsi (Ocimum tenuiflorum) Essential Oil and Their Major Constituents against Three Species of Bacteria. *Front. Microbiol.* 2016, *7*, 681. [Google Scholar] [CrossRef][Green Version]

[11]. Matasyoh, L.G.; Matasyoh, J.C.; Wachira, F.; Kinyua, M.G.; Muigai, A.W.T.; Mukiama, T.K. Antimicrobial activity of essential oils of Ocimum gratissimum l. from different populations of Kenya. *Afr. J. Tradit. Complement. Altern. Med.* 2008, *5*, 187–193. [Google Scholar] [CrossRef] [PubMed][Green Version]

[12]. Benitez, N.P.; León, E.M.M.; Stashenko, E.E. Eugenol and methyl eugenol chemotypes of essential oil of species Ocimum gratissimum L. and Ocimum campechianum Mill. from Colombia. *J. Chromatogr. Sci.* 2009, *47*, 800–803. [Google Scholar] [CrossRef] [PubMed][Green Version]

[13]. Ilić, Z.S.; Milenković, L.; Šunić, L.; Tmušić, N.; Mastilović, J.; Kevrešan, Ž.; Stanojević, L.; Danilović, B.; Stanojević, J. Efficiency of Basil Essential Oil Antimicrobial Agents under Different Shading Treatments and Harvest Times. *Agronomy* 2021, *11*, 1574. [Google Scholar] [CrossRef]

[14]. Karamaouna, F.; Kimbaris, A.; Michaelakis, A.; Papachristos, D.; Polissiou, M.; Papatsakona, P.; Tsora, E. Insecticidal Activity of Plant Essential Oils Against the Vine Mealybug, Planococcus ficus. *J. Insect Sci.* 2013, *13*, 1–13. [Google Scholar] [CrossRef] [PubMed][Green Version]

[15]. Rodríguez-González, Á.; Álvarez-García, S.; González-López, Ó.; Da Silva, F.; Casquero, P.A. Insecticidal Properties of Ocimum basilicum and Cymbopogon winterianus against Acanthoscelides obtectus, Insect Pest of the Common Bean (*Phaseolus vulgaris* L.). *Insects* 2019, *10*, 151. [Google Scholar] [CrossRef]