International Journal of Mosquito Research 2017; 4(2): 52-56

International Journal of Mosquito Research

ISSN: 2348-5906
CODEN: IJMRK2
IJMR 2017; 4(2): 52-56

Received: 09-01-2017
Accepted: 10-02-2017

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Effect of basil (*Ocimum basilicum*) Leaves Powder and Ethanolic-Extract on the 3rd Larval Instar of *Anopheles arabiensis* (Patton, 1905) (Culicidae: Diptera)

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Abstract
Malaria is transmitted by *Anopheles arabiensis* (Diptera: Culicidae), and the disease is a significant health problem in the Sudan, affecting 52% of outpatients and accounting for 9% of all hospital deaths. A cross-sectional study was conducted by collecting first to third instar larvae from Barakat area of Wad Medani town, Gezira State, Central Sudan. The third instar (L3) was used for the bioassay. Susceptibility to Basil (*Ocimum basilicum*), which is an abundant weed during the rainy-season, as a leaf powder, leaves ethanolic –extracts and the standard organophosphate larvicide temephos were investigated. Five concentrations (2, 4, 6, 8, 10 g/L) of the powder, and 1,2,…,5 mg/L of the extract were tested in a 1L beakers. Ten L3 larvae/beaker were exposed for 24 hr to each of these concentrations. Each concentration was replicated 3X, and the experiment was repeated twice for verification. The results showed that the extract LC_{50} was 58mg/L and LC_{90} was 143 mg/L; the slope of log-dose-probability line was 3.04. For the powder, the respective values were 9.19 g/L, 19.88 g/L and 3.82. The temephos resulted in 0.033 mg/L, 0.16 mg/L and 1.85, respectively. It can be concluded that using this natural, botanical extract and its leaf powder are effective, safe, economic and environmentally-sound in controlling the aquatic stages. It can be easily prepared and applied by villagers and others.

Keywords: *Anopheles arabiensis*, Sudan, Wad Medani. Basil, *Ocimum basilicum*, ethanolic extract, leaf powder, temephos, mosquitoes, Sudan

1. Introduction

Malaria is a major health problem in the tropical countries, especially sub-Saharan Africa, where about 90% of the clinical cases occur. There are nearly 500 million clinical cases of malaria worldwide each year and 1.1 to 2.7 million people die annually [1]. Malaria is the leading cause of morbidity and mortality in the Sudan, with 7.5 million cases and 35, 000 deaths recorded annually [2]. The Federal Ministry of Health in the year 2006 stated that malaria is a significant health problem in the Sudan, affecting 52% of outpatients and accounting for 9% of all hospital deaths.

The use of insecticides is the main strategy for controlling malaria vectors in the Sudan through indoor residual spraying (IRS), and more recently, the use of insecticide-treated bed nets (ITNs) [3]. Larviciding has the greatest control impact on mosquito populations, because the larvae are concentrated, immobile and accessible. The use of conventional pesticides in the water sources, however, introduces many risks to people, their animals, wildlife and/or the environment. In recent times, the use of environment -friendly and biodegradable natural insecticides of plant origin (botanicals) to control insect vectors of diseases and others pests is very helpful and proved to be promising; therefore, aromatic plants and their essential oils, for example, are considered to be very important sources of many compounds that are used in different respects. Essential oils and some plant-extracts are still an important natural resource and more promising for pesticides/insecticides to control mosquitoes and several other pests [4, 5, 6, 7, 8, 9].

Identification and recognition of the biological properties of natural products I (viz botanicals) became one of the fastest growing scientific fields in Pharmacognosy and pesticides [10, 11, 12].

Unlike compounds synthesized in the laboratory, primary and secondary compounds (metabolites) from plants are produced by the plants to protect them from the pests and disease or for attracting the pollinators. Thus, knowledge of pest to which the producing plant is resistant may provide useful guidance in predicting what pest may be controlled by phytochemicals from a particular plant species. This approach has led to several commercial pesticides, such as pyrethroid insecticides [13].

Natural product can be used either by itself as powders or with suitable carrier or additives after being extracted by a proper solvent(s) and then formulated as required [14]. *Basil, Ocimum basilicum* L. (Lamiaceae), vernacularly called Rehan, native to tropical Africa, Asia and the Pacific Islands, and may be either annual or perennial. Basil chemically contains low percentage of essential oils, as well as volatile oils, linalool, lineol, geraniol and polyphenolic acids. It is used in the traditional medicine to sooth pain, treat vomiting and stress and commonly as insect repellent [9, 15]. Oils from some *Ocimum* spp. have been shown to repel insects and have larvicidal activity against houseflies, blue bottle flies, and mosquitoes. The effective concentration of the oil to kill 90% (LD90) of the larvae ranged from 113-283 ppm. Camphor, d-limonene, myrcene, and thymol are some of the compounds in the oil that may provide the repellent properties. Eugenol and methylchavicol could be responsible for the larvicidal activity. Certain basil essential oils were claimed to have a larvicidal activity against mosquito larvae [8]. These compounds are known as apolar and cannot dissolve in water, where the larvae live.

The present work aims at studying the potential of the leaf powder (LP) of this abundant wild plant and the ethanolic - extract (LPE) of that powder to investigate the effects of the polar ingredients of basil on *A. arabiensis* 3rd instar larvae (L3), and comparing their results (viz. LC50, LC90, LC90/LC50 ratio and the slopes of the log-dose probability lines (Ld-P lines)) with those of the commonly used organophosphate larvicide temephos.

2. Materials and Methods

2.1. Study design and study area

This study was conducted as a cross-sectional in which larvae of *A. arabiensis* mosquitoes were collected randomly from water ponds in Barakat area, 5 km south of Wad Medani Town (population half a million), the capital of the Gezira State (population 4 million), central Sudan. Gezira state is the most agriculturally productive state in the Sudan; >2.5 million acres irrigated and >7 million acres rainfed). It lies between the Blue Nile and the White Nile rivers. It is bounded by Khartoum state in the North, Gadarif state in the East, White Nile state in the West and Sennar state in the South. Gezira lies in the rich Savanna region between latitude 13-15.20 N and longitude 32.5 – 340 E. The area has a hot dry summer from April to June, with daily temperature between 32-42 °C and relative humidity (R.H.) of 20%. The rainy-season starts in late June and ends in mid- October. Winter is cold and dry and is from December to February with daily average temperature between 15-21 °C, and R.H. of 30% [16].

2.2. Sampling and Rearing of insects

The *Anopheles* mosquito's larvae were collected randomly; transferred in metal dishes (40 cm. dia.) and reared for several generations under 25-30 °C, 70-80% R.H. and 13:11 hr light/dark photoperiods in the insectary of the Blue Nile National Institute for Communicable Disease (BNNICD), U of Gezira, 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 for egg production. Under these conditions, the full development from egg to adult lasted about 3-5 wk. Third instars (L3) larvae were used for the bioassay.

2.3. Identification of *Anopheles* mosquitoes:

The collected larvae were identified as Anopheles species using the key (Anopheles larvae of the Sudan) based on the following characters (no siphon, they lie parallel on the water surface, the tergal plates and paired palmate hairs it dorsally appear on the abdominal segments [18].

2.4. Basil Collection

Basil leaves were obtained from the Demonstration Farm, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan. Taxonomic identification of plants was performed by botanists of the Agricultural Research Corporation, Centre of Crop protection, Department of weeds, Wad Medani, Sudan.

2.5. Preparation of Powder

The collected leaves of the basil were put in the shade for 7 days for drying, finely powdered by electric blender, stored in glass jars, covered by aluminum foil and left in the laboratory at 25 °C until used.

2.6. Preparation of the Ethanolic- Extract

LP (20 g) was extracted by using a Soxhlet apparatus for 6 hr, using 100 ml absolute ethanol (LPE). The extract was put in a flask (50 ml) and kept under laboratory conditions until used.

2.7. Bioassay

2.7.1. Ethanolic -extract (LPE)

Five concentrations (2, 3, 4, 5and 6 mg/L) were taken from LPE and the volume was completed to 1000 ml by adding distilled water (DW). Ten larvae from L3 were transferred separately to beakers. The exposure period was 24 hr. The control was exposed to DW only. Three replicates were used in each treatment, and the experiment was repeated twice. The knock-down (Kd) was taken from each concentration after 10, 20, 30, 40, 50 and 60 min of exposure, in addition to the acute toxicity after 24 hr.

2.7.2. Leaf Powder (LP)

Five rates of LP (2, 4, 6, 8 and 10 g) were taken, and the volume was completed to 1000 ml by adding DW. Ten larvae from L3 instars were transferred separately to beakers. The exposure period and the rest of the procedure was as above.

2.7.3. Temephos

Five concentrations (2, 4, 6, 8, 10 mg/L) of temephos were used as above for comparison with a standard larvicide.

After 24 hr of exposure both the dead and moribund larvae were counted and the percent mortality (%M) was calculated in both the treatments and control.

2.7.4 Statistical Analysis
Probit analysis program was used to determine, LC50 and LC90 values with 95% confidence intervals [18].

3. Results
3.1. Basil leaf powder extract (LPE)
The results of the lethal time (LT50 and LT90) of all tested concentrations (2, 4, 6, 8 and 10 mg/L) are shown in table (1). The LT50 and LT90 values of concentrations 2mg/L and 10 mg/L were 79 and 156 min, and 44 and 108 min, respectively. Table 1 and Fig. 1 showed the percent mortalities of *A. arabiensis* larvae exposed to LPE tested concentrations after 24 resulted in an LC50 of 58mg/L, an LC90 of 143 mg/L, with an Ld-P line slope 3.04. The lowest dose 2 mg/L results in 20% mortality, whereas the highest dose 10 mg/L results in 90% mortality. At 2% and 4%, morality was not recorded during the first 20 min, whereas in the rest of the concentrations, the mortality was observed starting the first 10 min of exposure.

Table 1: Dose–response of basil leaf powder ethanolic-extract (LPE) on the percent mortality following exposure for 10 to 60 min and 24 hr (*A. arabiensis* larvae)

LPE (mg/L)	Exposure time (min)						%Mortality after 24 hr
	10 (%)	20 (%)	30 (%)	40 (%)	50 (%)	60 (%)	
2	0	0	10	10	20	20	20
4	0	0	10	20	30	30	40
6	10	20	20	30	40	40	60
8	10	30	30	30	50	50	80
10	20	30	30	40	60	60	90
Control	0	0	0	0	0	0	0

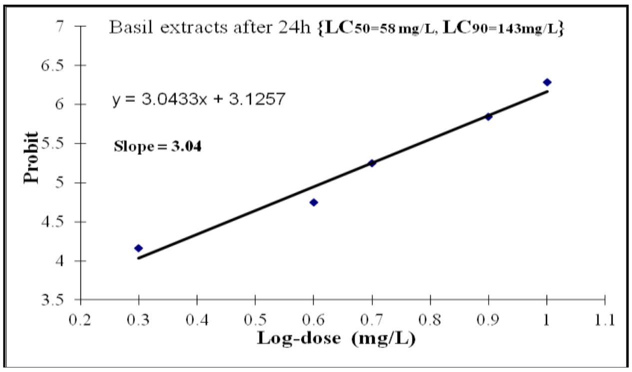

Fig 1: Dose-response relationship of basil leaves ethanolic- extract (LPE) after 24 hr on the third instar larvae of *A. arabiensis* from the Gezira state, Sudan.

3.2. Basil leaf powder (LP)
The LT50 and LT90 of all concentrations (2, 4, 6, 8, and 10 g/L) were shown in table 2; fig. (2). The LT50 and LT90 values of concentrations 6g/L and 10 g/L were 71.5 and 174 min, and 49 and 82 min, respectively. The treatments with 2 and 4 g LP/L were unable to kill the larvae within the first 40 min of exposure. When the concentration was increased to 6g/L, mortality occurred after 30 min of exposure. However, at 8 and 10g/L mortality was reported at 20 min. After 24 hr, there was no difference in % mortality between 2 and 4 g/l (10%). Mortality % was doubled when 6g/L was used (20%). Another doubling occurred when the 8g/L was used (40%).The LC50 for LP was 9.19 g/L, whereas the LC90 was 19.88g/L, with an Ld-P line slope 3.82.

Table 2: Dose - response of the basil leaf powder (LP) on the percent mortality following exposure for 10 to 60 min and 24 hr on *A. arabiensis* third instar larvae

LP (g/L)	Exposure time (min)						%Mortality after 24 hr
	10 (%)	20 (%)	30 (%)	40 (%)	50 (%)	60 (%)	
2	0	0	0	0	10	10	10
4	0	0	0	0	10	10	10
6	0	0	10	20	20	20	20
8	0	10	10	20	30	50	40
10	0	10	10	30	60	70	60
Control	0	0	0	0	0	0	0

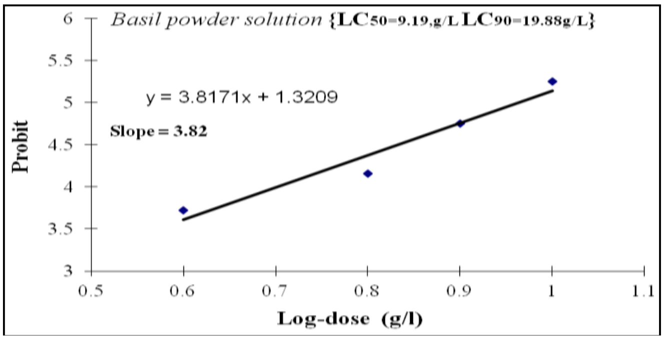

Fig 2: Effect of basil powder (LP) after 24hr on the mean number of the *Anopheles arabiensis* larvae.

3.3. Temephos
The LT50 and LT90 values for the standard temephos were 58 and 143 and 52 and 92 min, respectively (Table 3; Fig. 3). However, the LC50 and LC90 were 0.033 and 0.16 mg/L, following the same order. The Ld-P line slope was 1.85. The 2mg/L treatment killed 40% of the population after 24 hr, but mortality did not occur during the first 30 min. Regarding the 4mg/l treatment, the mortality started at 30 min, and after 24 hr the % mortality was 53%. In all other treatments (6-10 mg/L), mortality was reported at 10 min of exposure.

Table 3: The effect of the different tested concentrations of temephos in terms of percent mortality after 10 -60 min of exposure and the overall mortality after 24 hr.

Temephos (mg/L)	Exposure time (min)						
	10 (%)	20 (%)	30 (%)	40 (%)	50 (%)	60 (%)	%Mortality after 24hr
2	0	0	0	10	10	10	40
4	0	0	10	10	10	30	53
6	10	10	10	20	30	50	57
8	10	10	10	20	30	50	77
10	10	10	10	30	40	70	87
control	0	0	0	0	0	0	0

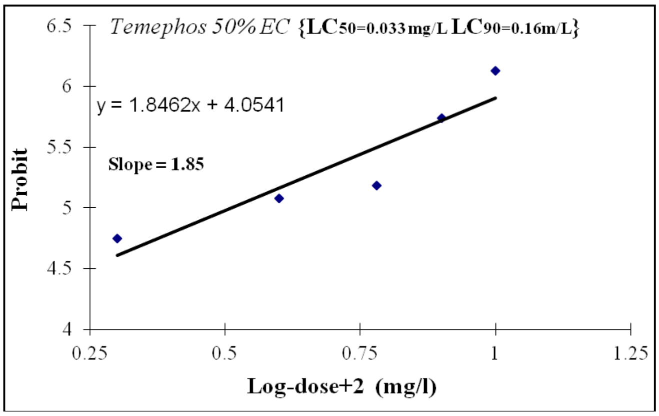

Fig 3: Dose-response of temephos on the third instar larvae of *A. arabiensis* from Gezira state, Sudan.

4. Discussion

Over the last 5 decades the indiscriminate use of synthetic insecticides in agriculture and public health programs for the control of pest species has created multifarious problems, *viz*. resistance, environmental pollution, toxic hazards to humans and other non-target organism. Thus, great emphasis has been recently placed on the research and development of alternatives using natural plant products. Studies in our laboratories and others on botanicals as larvicide have indicated that they could provide alternatives solution to synthetic chemical insecticides [3, 4].

As mentioned earlier, Chemical analysis showed that basil oil is rich in monoterpenes, sesquiterpenes and phenyl-propane derivatives [19]. Desphande [20] reported the larvicidal activity of *O. basilicum* against mosquito. *Ocimum*, proved to have an insect-repellency effect, and possesses juvenile hormones [21]. The plant contains volatiles well-smelled oil [22].

In the present study, the LPE was by far more toxic to the larvae than the LP. The LC50= 58mg/L vs. 9.19 g/L, respectively. Regarding the LC90, it was of the extracts 143mg/L for LPE vs. 19.88g/L for the LP. The Ld-p line of the extract was 3.04 and the powder was 3.82, which indicated that the larval population was more homogeneous to the LP than to the LPE.

Kehail [3] concluded that 3g of basil LP, and after 7 days of exposure resulted in LD50 of 976.05 mg/L using a population collected the same location of the present work. The present work results agreed and confirmed Kehail findings.

Temephos, the standard OP larvicide, was used for the control of *A. arabiensis* larvae since 1976 [23]. Elsayed [6] found that *A. arabiensis* larvae in Khartoum state showed an LD50 of 0.06 ppm when treated with temephos, whereas in study by Kehail [4] found that the same species larvae from Wad Medani area showed an LD50 of 0.029 ppm, and an LD95 of 0.1688 ppm. The present study showed that temephos gave higher LC50 (0.033mg/L) and almost similar LC90 (0.16mg/L) to LD95 obtained by Kahail [4]. This indicates that temephos is less effective to the larval population in Wad Medani, Gezira state than in Khartoum state. This can be attributed to the continuous use of temephos since 1976 up to now. Ld-P line slope for temephos was the lowest when compared with LP and LPE.

In this study the Ld-P lines slopes of temephos, basil extract and basil powder showed that *A. arabiensis* larvae population proved to be more heterogeneous towards temephos (1.85). This indicates that the population of *A. arabiensis* in the Gezira state, *viz*. in Wad Medani Greater Locality is still heterogeneous to temephos despite its application for >40yr. It can be concluded that *O. basilicum* LP can be used by the villagers and the community of the towns where breeding sites for mosquitoes are available, especially during the rainy-season broken water pipes, evaporative colors, etc. But, for the malaria control programs of the Ministry of health the LPE can be prepared in the universities or research centers laboratories to be applied by the Environmental Health personnel; ethanol is locally produced by the sugar factories and its price is affordable.

5. References

1. WHO. WHO expert committee on malaria. World Health Organ Technical Report Ser. 2000; 892:1-71.
2. Abdalla HB. Insecticide resistance in malaria vectors in Central Sudan. M.Sc. Thesis. U. of Witwatersrand, Johannesburg, RSA. 2007.
3. Kehail MAA. Larvicidal potentialities of 20 plant species from wad Medani – Gezira state on *Anopheles arabiensis* Patton and *Culex quinquefasciatus* Say (Culicidae, Diptera).Ph.D. thesis, U. of Gezira, Sudan. 2004.
4. Kehail MAA. Susceptibility of *Anopheles arabiensis* Patton and *Culex quinquefasciatus* Say (Culicidae, Diptera) larvae to selected insecticides and some Natural products in Wad Medani. M.Sc. thesis. U. of Gezira, Sudan. 1995.
5. Harborne JB. Plant phenolics. In Bell E. A.; Charlwood BV. Encyclopedia of Plant Physiology, Secondary Plant Products. Berlin Heidelberg New York: Springer-Verlag. 1980; 8:329-395.
6. El Sayed BB. Susceptibility of *Anopheles arabiensis* and *Culex quinquefasciatus* larvae to some control agents in Khartoum state. M.Sc. thesis. U. of Khartoum, Sudan. 1992.
7. Chavasse Dc, Yap HH. Chemical methods for the control of vectors and pests of public health importance. WHO/CTD/WHOPES/97.2.WHO,Geneva. 1997.
8. Abo-Elseoud MA, Sorhan MM, Omar AE, Helal MM. Biocides formulation of essential oils having antimicrobial activity. Archives of physiopathology and plant protection. 2005; 38:175-184.
9. El Gazali G, Khalid H, El Tohami M, Abdalla W, Yagi S. Medicinal plants commonly used in Khartoum State. National Centre for Research, Khartoum. 1998; 35:65.
10. El-Shemy HA, Aboul-Enein AM, Aboul-Enein KM, Fujita KW. Leaves extract contain Anti-tumor agent effective against three cell type. PLOS one. 2007; 2:e178.
11. Dan B, Steven C, Erich S, Andrew G. Chinese herbal medicine. Materia Medica, 3rd edn. 2004.
12. Hernan G, Antonia S, Hibetta B, Jeff C. Wind in the blood. Mayan healing and Chinese Medicine. 1999.
13. Duke SO. Natural pesticide from plants. J. Janick and J.E. Siman (eds), Advances in new crop. Timber press. Portland, OR. 1990, 511-517.
14. Satriana MJ. Insecticide Manufacturing Recent Processes and applications. Nayes Data Corporation. Park Ridge, New Jersey U.S.A. 1983.
15. Chatterjee A, Pakrashi SC. The treatise on Indian medicinal plants. National Institute of Science Communication, New Delhi. 1997, 5.

16. Sulieman AEM. Identification, monitoring and control of the fruit fly *Bacterocera invadens* (Drew, Tsuruta and White) (Diptera: Tephritidae) in Guava orchards in Greater Wad Medani Locality, Gezira State, Sudan. MSc Thesis, University of Gezira, Sudan. 2013.
17. Nugud AD**.** Key for Identification of Common Adult Anophelines.
18. Alonso PL. Malaria: deploying a candidate vaccine (RTS, S/ASO2A) for and old scourge of human kind. International Microbiology. 2006; 9:83-93.
19. Kenaga EE. Pesticide reference standards of the Entomological society of America. Bull. Entomol. Soc. Amer. 1966; 12(2):117-127.
20. Tajima PC. Sesquiterpenes from basil oil.US Patent. 1992; 5:173-479.
21. Desphande EL. Mosquito larvicidal activity of *Ocimum basilicum* Linn. Pesticides. 1977; 11(5):11-12.
22. White GB. Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Dipt., Culicidae). The distribution, ecology, behavior and vectorial importance of D in Bwamba Country, Uganda, with an analysis of biological, ecological, morphological and cytogenetical relationships of Uganda species D. Bulletin of the WHO. 1973; 63:65-97.
23. AOAD. Medicinal, aromatic and poisonous plants in the Arab World. Khartoum, National Center for Research Proceeding. 1988.
24. WHO. Manual on environmental management for mosquito control with special emphasis on malaria vectors. WHO Offset Publication Geneva. 1982, 66.