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Full Length Research Paper

A study of the larvicidal properties of the essential oils of some aromatic plants against larvae of *A. arabiensis* and *A. aegypti* in the laboratory and anophelines in simulated field condition

***Gelila Haile Marcus[1] Zenawi Marley[1] and Abune Adere**

[1]Department of Pathobiology, Institute of Health Sciences, [2]Essential Oil Research Institute, Mekelle University, Mek'ele, Ethiopia.

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The concern for environmental safety and increased development of resistance to chemical insecticides by major arthropod vectors is rekindling interest in the search for botanical products that may be used against major vectors. Essential oils of 11 local plants were evaluated for larvicidal activities against laboratory colonies of *Anopheles arabiensis* and *Aedes aegypti* early fourth instar larvae. Those oils which induced higher larvicidal activities in the laboratory were also evaluated in the field. In the laboratory, the LC_{50} values of the oils ranged from 17.5 to 85.9 ppm against *A. arabiensis* and from 9.1 to 67.8 ppm against *A. aegypti.* Similarly, the LC_{90} values of the oils ranged from 33.2 to 128.4 ppm and from 14.3 to 96.4 ppm against the respective mosquito species. However, *Chenopodium ambrosioides* Linnaeus oil with LC_{50} of 17.5 and 9.1 ppm against *A. arabiensis* and *A. aegypti,* respectively, and *Ocimum lamiifolium* Hochst oil with LC_{50} of 20.9 and 8.6 ppm against *A. arabiensis* and *A. aegypti,* respectively, were the most effective oils. *A. aegypti,* were more sensitive to most oils than *A. arabiensis* larvae. Of the five essential oils which exhibited relatively strong larvicidal effects in the laboratory and further tested in the field against wild-collected anopheline larvae, the LC_{50} and LC_{90} values ranged from 35 to 110 ppm, and from 63.7 to 162.9 ppm, respectively. *O. lamiifolium* and *C. ambrosioides* still induced the highest larvicidal effects with LC_{50} = 34 ppm; LC_{90} = 97. 9 ppm and LC_{50} = 47.3 ppm; LC_{90} = 97.9 ppm, respectively. However, it was revealed that laboratory bred mosquito larvae were more sensitive to the essential oils than wild-collected larvae.

Key words: *Anopheles arabiensis, Aedes aegypti,* essential oils, botanical larvicides.

INTRODUCTION

Mosquitoes pose the greatest threat to public health because of their ability to act as vectors of pathogens causing malaria, dengue, yellow fever, encephalitis and filariasis (Service, 2004). Mosquito-borne diseases contribute significantly to disease burden, death, poverty, and social debility all over the world, particularly in tropical countries. Among these diseases, malaria remains the most serious vector-borne disease affecting some 300 - 500 million people and 1.4 to 2.6 million deaths annually throughout the world. More than 40% of the world's populations live in malarious areas (Ghai and Gupta, 2000). In Ethiopia, about 75% of the landmass is considered to be malarious, and about two-thirds of the populations (over 40%) are at risk of the disease (Ghebreyesus et al., 2005).

Current mosquito control strategies depend primarily on synthetic insecticides. The discovery, development and use of synthetic insecticides have reduced the interest in plant origin products. However, widespread use of these insecticides in public health and agriculture for the control of vector and pest species has created different problems, such as the development of physiological resistance in major vector species, environmental pollution and toxic

*Corresponding author. E-mail: gh_marcus@gmail.com

hazards to human and other non-target organisms due to their broad spectrum of activity (Minjas and Sarda, 1986; WHO, 1992; Hemingway and Craig, 2004). As a result, there has been an increased interest in developing potential alternative or additional control methods/materials that are effective against the target vector species, environmentally safe, biodegradable, with low cost, and can be used by individual and communities in specific situations (Redwane et al., 2002). One of these potential alternatives or additional control methods/tools is the use of selected botanical derivatives against the target mosquito species (Perich et al., 1995). Insecticidal activities of different plant essential oils have been reported against different mosquito species. For example, Tare et al. (2004) reported the larvicidal activity of essential oils of 11 plants grown in the Himalayan region against *A. aegypti* larvae. Likewise, Pitasawat et al. (1998) screened the larvicidal effects of ten plant species and found three plant essential oils (*Kaempferia galangal* L., *Illicium verum* Hook. f. and *Spilanthes acmella* Murray) to have larvicidal properties against *Culex quinquefasciatus* Say. Similarly, Jantan et al. (2005) evaluated the leaf essential oils of eight *Cinnamomum* species for larvicidal activity against *A. aegypti* and *A. albopictus* Skuse and found 5 species (*Cinnamomum impressico-statum* Kostern, *Cinnamomum microphyllum* Meisen, *Cinnamomum pubescens* Kochummen, *Cinnamomum mollissimum* Hook and *Cinnamomum rhyncophyllum* Miq.) to have significant larvicidal effects. Recently, Morais et al. (2006) evaluated the larvicidal activity of essential oil of four

Croton species and found *Cinnamomum zehntneri* Pax and *Cladonia macilenta* Hoffm. to be highly toxic against larvae of *A. aegypti.* Furthermore, Amer and Mehlhorn (2006a) evaluated the larvicidal effects of essential oils of 41 plants against *Aedes*, *Anopheles* and *Culex* mosquitoes and reported 13 plant oils [*Cinnamomum camphora* (L.) J. Presel, *Thymus serpyllum* L., *Amyris balsamifera* L., *Citrus limon* (L.) Burm. f ., *Juniperus virginiana* L., Juniperus *communis* L., *Boswellia carteri* Birdw, *Anethum graveolens* L., *Myrtus communis* L., *Piper nigrum* L., *Lippia citriodora* Kunth, *Helichrysum italicum* (Roth) and
Santalum album L.] to have significant effects.

The identification and eventual use of local plants in the control of mosquito larvae may be very valuable for developing countries. Besides being more readily available, they are more economical to use and the methods employed are usually simpler (Monzon et al., 1994). This study reports on the larvicidal properties of the essential oils of some aromatic plants found in Ethiopia against larvae of *A. arabiensis* and *A. aegypti* in the laboratory and anophelines in simulated field condition.

MATERIALS AND METHODS

Collection of plant materials

The samples of test plants were collected from different localities of the country including Addis Ababa. The accessible parts of eleven aromatic plants (mostly their leaves) were collected for extraction and testing. These included *Chenopodium ambrosioides (*aerial parts), *Ocimum lamiifolium* (leaves), *Ocimum suave* Wild (leaves), *Schinus molle* L. (leaves and seeds), *Piper nigrum* L. (seeds), *Corymbia citriodora* (Hook) Hill and Johnson (leaves), *Eucalyptus globules* Labill. (Leaves), *Nigella sativa* L. (leaves), *Lippia adoensis* Hochst (leaves), *Mentha spicata* L. (leaves) and *Thymus vulgaris L.* (leaves). Taxonomic confirmation of these plants was preformed by botanists in the National Herbarium (Department of Biology), Addis Ababa University.

Distillation of essential oils

Essential oils were extracted from leaves or seeds of the test plants by hydro-steam distillation in a Clevenger-type apparatus for 3 h. Distillation were repeated to obtain sufficient oils for the experiment. The oils thus obtained were separated from water in the condenser and stored in airtight containers under refrigeration (4^{O}C) till their later use for larval bioassays.

Test mosquitoes

Laboratory tests of the oils were conducted on larvae from colonies of *A. arabiensis* and *A. aegypti* maintained at the Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University at 27 ± 2^{O}C and 70 ± 5% relative humidity.

Larvicidal bioassays in the laboratory

The larval bioassay tests were carried out following the standard World Health Organization larval bioassay test method (WHO, 2005). White enamel cups with capacities of 300 ml each were used for the larvicidal bioassays. Appropriate amount of each essential oil was dissolved in acetone to prepare 5 ml of stock solution of each concentration (0.09 to 2.5%, v/v). Fresh stock solutions of each of the above concentration were prepared to produce the required test concentrations ranging from 6 to 333.3 ppm. Four replicates were carried out for each test concentration and species of mosquito larvae. Twenty-five active early fourth instar larvae of *A. arabiensis* and *A. aegypti* in 19 ml distilled water were transferred into each white enamel cup which contained 130 ml distilled water. One ml of the stock solution was added to each cup which contained 149 ml distilled water to give a final solution of 150 ml with the desired test concentrations. Two replicates of control were carried out simultaneously with 149 ml of distilled water and 1 ml of acetone.

Larvicidal bioassay under field conditions.

Essential oils of seven plants which had shown relatively strong larvicidal efficacies in the laboratory were also evaluated for their efficacies in field situations and for comparison with laboratory results. Tests were conducted according to the methods of WHO (2005) and Mwaiko and Savaeli (1994) . Artificial containers (plastic bowls) of 18 cm wide (diameter) by 7.5 cm depth of 1.5 litre capacity were used for larvicidal bioassays in the field. The containers were half-buried in the ground, and 299 ml of water from the natural breeding habitats were added into each bowl. Each container was then treated with 1 ml of the stock solution of each plant oil so that final volume was 300 ml each. Concentrations ranging from 16 to 200 ppm were used for the tests in the field. Batch of 40 wild-collected early fourth instar anopheline larvae were released into each container and for each test concentration. The containers were then covered with nylon mosquito netting to prevent debris and other mosquitoes from egg laying. Four replicates were conducted for the treatments and two for the controls as described above.

Table 1. LC $_{50}$ and LC $_{90}$ (ppm) values of the essential oils of different plants against *A. arabiensis* and *A. aegypti* larvae after 24 h exposure.

Plant oil	LC	*A. arabiensis*	*A. aegypti*
C. ambrosioides	50	17.5 (13.3-22.2)	9.1 (7.8-10.7)
	90	33.2 (27.3-45.4)	14.3 (12.2 –18.6)
O. lamiifolium	50	20.9 (16.2-26.7)	8.6 (7.3-10.0)
	90	39.9 (32.6-55.5)	13.4 (11.5-17.5)
S. molle (leaves)	50	21.0 (16.8-26.3)	9.6 (8.2-11.4)
	90	37.3 (30.9-50.3)	15.0 (12.8-19.9)
N. sativa	50	23.4 (18.2-28.5)	32.1 (27.1-36.7)
	90	45.4 (38.8-55.9)	48.4 (42.9-57.6)
S. molle (seeds)	50	26.5 (18.2-32.6)	14.5 (11.4-18.4)
	90	45.4 (38.8-55.9)	28.5 (23.3-38.6)
P. nigrum	50	33.5 (28.5-37.9)	9.1 (7.9-10.5)
	90	48.2 (43.0-57.0)	13.5 (11.7-16.9)
T. vulgaris	50	33.7 (27.4-39.4)	17.3 (12.2-22.0)
	90	57.5 (50.2-70.1)	36.6 (30.3-48.2)
C. citriodora	50	40.3 (33.2-47.6)	38.7 (31.3-46.5)
	90	65.4 (56.3-81.7)	65.5 (55.9-82.6)
O. suave	50	53.5 (47.9-59.6)	29.8 (23.5-35.0)
	90	75.3 (67.4-91.1)	50.9 (44.6-61.8)
L. adoensis	50	56.4 (47.7-65.6)	47.1 (40.5-54.6)
	90	90.3 (79.5-109.7)	68.7 (60.2-83.1)
E. globulus	50	68.3 (57.1-78.3)	52. 9 (41.8-63.6)
	90	109.7 (97.3-130)	102.0 (87.7-125.9)
M. spicata	50	85.9 (76.6-96.1)	67.8 (59.4-76.3)
	90	128.4 (115.7-148.7)	96.4 (86.3-113.8)

Numbers in parenthesis are the 95% confidence intervals.

In both laboratory and field tests, mortality was recorded after 24 h exposure period. Dead and moribund larvae in four replicates were combined and expressed as a percentage of larval mortality in each concentration. Dead larvae were those that failed to move when probed with a needle at the terminal segments, siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed.

Data analysis

The LC50, LC90 and the 95% confidence intervals were calculated by probit analysis using SPSS computer soft ware programs version 11.0 in order to compare the larvicidal potency of the plants and susceptibility of the test mosquito larvae. LC$_{50}$ and LC $_{90}$ values were judged as significantly different between the essential oils ($p < 0.05$) if the confidence intervals did not overlap (Bassole et al., 2003; Petersen et al., 2004). In all the tests, no control mortality was detected after the 24 h exposure; hence, no correction was required based on Abbot's formula.

RESULTS

Larvicidal activities of essential oils under laboratory conditions

Table 1 shows the LC_{50} and LC_{90} values of the essential oils of different plants tested against early fourth instar *A. arabiensis* and *A. aegypti* larvae in the laboratory. Against *A. arabiensis* larvae, the LC_{50} and LC_{90} values ranged from 17.5 to 85.9 ppm and from 33.2 to 128 ppm, respectively. On both values, *C.ambrosioides* exhibited the highest larvicidal activity (LC_{50} = 17.5 ppm; LC_{90} = 33.2 ppm) and *M. spicata*, the weakest larvicidal activity (LC_{50} = 85.9 ppm; LC $_{90}$ = 128.4 ppm) against *A. arabiensis*. Furthermore, oils of *O. lamiifolium*, *S. molle* (leaves), *N. sativa*, *S. molle* (seeds), *P. nigrum*, and *T. vulgaris* still showed strong larvicidal activity after *C.ambrosioides* with LC_{50} values 35 ppm against *A. arabiensis.*

Table 2. LC $_{50}$ and LC $_{90}$ (ppm) values of the essential oils of different plants against anopheline larvae after 24 h exposure in simulated field conditions.

Plant oil	LC $_{50}$ (95% CI)	LC$_{90}$ (95% CI)
O. lamiifolium	34 (27.6-40.2)	63.7 (54.9-79.4)
C. ambrosioides	47.3 (42.0-56.9)	97.9 (89.6-114.4)
S. molle	63.5 (57.0-71.4)	100.7 (89.8-119.0)
O. suave	86.4 (76.4-94.6)	127.6 (117.2-144.0)
P. nigrum	110.6 (99.7-121.4)	162.9 (148.9-183.0)

Numbers in parenthesis are the 95% confidence intervals.

Based on the overlapping of the confidence intervals of the LC_{50} and LC_{90} values, there were many significant and insignificant differences between the oils. For example, *C. ambrosioides*, *O. lamiiflolium*, *S. molle* (leaves), *N. sativa* and *S. molle* (seeds) differed significantly from others.

Against *A. aegypti,* the LC_{50} and LC_{90} values of the different oils were generally much lower than that against *A. arabiensis*, and ranged from 8.6 to 67. 8 ppm and from 13.4 to 96.4 ppm, respectively. However, unlike in *A. arabiensis,* highest larvicidal activity was recorded for *O. lamiiflolium* oil (LC_{50} = 8.6 ppm; LC_{90} = 13.4 ppm) although the next highest potent essential oil was that of *C. ambrosioides.* Oils of *S. molle* (leaves), *N. sativa*, *S. molle* (seeds), *P. nigrum*, *T. vulgaris* and *O. suave* still showed strong larvicidal activity against *A. aegypti* following *O. lamiiflolium* and *C. ambrosioides* and with LC_{50} values 35 ppm. Based on the overlapping of the confidence intervals of the LC_{50} and LC_{90} values, there were many significant and insignificant differences between the oils as in *A. arabiensis*. The same plants, *C. ambrosioides*, *O. lamiiflolium*, *S. molle* (leaves), *N. sativa* and *S. molle* (seeds) differed significantly from others. Moderately toxic plants against both species of mosquito larvae included *C. citriodora*, and *L. adoensis* with LC_{50} values between 38.7 and 56.4 ppm and LC_{90} values between 65.4 and 90.3 ppm. The least toxic plants against both species of mosquito larvae were *E. globulus* and *M. spicata* with LC_{50} values between 52 .9 and 85.9 ppm and LC_{90} values between 96.4 and 128.4 ppm.

Based on the LC_{50} values and LC_{90} values, it can be seen (Table 1) that *A. aegypti* was more susceptible than *A. arabiensis* to all the essential oils tested except *N. sativa* to which it was slightly more tolerant than *A. arabiensis*.

Larvicidal effects of essential oils in simulated field conditions

The toxicity of five essential oils against third and fourth stage wild-collected anopheline larvae in simulated field conditions is shown in Table 2. Treatments of two other plant oils (*N. sativa* and *T. vulgaris*) were halted because of theft of the containers. Essential oil of *O. lamiifolium* showed highest larvicidal activity (LC_{50} = 34 ppm and LC_{90} = 63.5 ppm) followed by *C. ambrosioides* (LC_{50} = 47.3ppm and LC_{90} = 97.9 ppm); the least activity was exhibited by *P. nigrum* (LC_{50} = 110.6 ppm; LC_{90} = 162.9 ppm). In all cases however, wild-collected anopheline larvae had higher LC_{50} and LC_{90} values of the essential oils than laboratory reared *A. arabiensis* larvae.

DISCUSSION

From the LC_{50} and LC_{90} values, the essential oils from *C. ambrosioides*, *O. lamiifolium*, *S. molle* (leaves), *N. sativa, S. molle* (seeds), *P. nigrum*, and *T. vulgaris* exhibited higher larvicidal activity against fourth instar laboratory reared larvae of *A. arabiensis* after 24 h of exposure(LC_{50} 33.7 ppm), the most toxic of all being that of *C. ambrosioides*. Similarly, the same plant oils plus that of *O. suave* produced higher larvicidal activity against laboratory *A. aegypti* fourth instar larvae; the most toxic of all was *O. lamiifolium* oil.

Elsewhere, larvicidal activity had been reported for some of the oils used in the present work or for similar oils. Earlier studies involving the petroleum ether extract of thyme plant, *Thymus capitatus* (L.) Hoff. and Link was found to be toxic (LC $_{50}$ = 49.0 ppm) against larvae of *Culex pipiens* (Mansur et al*.,* 2000). Similarly, Amer and Mehlhorn (2006a) reported larvicidal activity of *Thymus serpyllum* against *Anopheles stephensi* Liston*, A. aegypti,* and *C. quinquefasciatus* with LC_{50} 10 ppm after 24 h of exposure. However, the effects of the essential oil of our local thyme tested (*T. vulgaris*) in the present study were much lower with LC_{50} values of 33.7 and 17.3 ppm against *A arabiensis* and *A. aegypti* larvae, respectively. Amer and Mehlhorn (2006a) also reported larvicidal activity of black pepper (*Piper nigrum*) with LC_{50} values of between 10 and 105 ppm against the above three mosquito species after 24 h exposure, the highest value being for *Anopheles* larvae. In the present work however, the same oil resulted in LC_{50} of 9.1 ppm against *Aedes* larvae and 33.5 ppm against *Anopheles* larvae, the latter being more tolerant. Similarly, Amer and Mehlhorn (2006a) reported LC_{50} values ranging between 10 and

100 ppm for verbena (*Lippia citriodora*): *A. aegypti* was the most resistant and *A. stephensi* was the most susceptible. In contrast, our local verbena (*Lippia adoensis)* in the present work had LC_{50} values of 47.1 and 56.4 ppm for *A. aegypti* and *A. arabiensis*, respectively. Further-more, our oil from *L. adoensis* is also about 1.3 more toxic to *A. aegypti* larvae compared to that of *Lippia sidoides* Cham. oil with LC_{50} = 63 ppm) (Carvalho et al., 2003; Cavalcanti et al., 2004).

The activity of *M. spicata* was 2.59 times less effective than *M. piperita* L. (LC_{50} = 26.192 ppm) (Pathak et al., 2000) against *A. aegypti* larvae. Though the essential oil of *E. globulus* in present study showed poor larvicidal activity against *A. arabiensis* and *A. aegypti* larvae, it had more potency than *E. globulus* from Philippines (LC_{50} 92.0123% and LC_{90} 810.6377%; w/w) against *A. aegypti* larvae (Monzon et al., 1994).

The laboratory reared *A. arabiensis* larvae were found to be more susceptible to essential oils than field population of anopheline larvae. No essential oil exhibited similar activity against laboratory reared *A. arabiensis* and field population of anopheline mosquitoes. Essential oils from *O. lamiifolium*, *C. ambrosioides*, *S. molle*, *O. suave* and *P. nigrum* showed the highest toxicity against laboratory reared *A. arabiensis* larvae than against field population of anopheline larvae. In the area, several anopheline species (*A. arabiensis, A. paharoensis* Theobald, *A. funestus* Giles, *A. nili* Theobald, *A. coustani* Laveran, *A. marshallii* Theobald, and *A. demeilloni* Evans) had earlier been reported, the former being the predominant (Adugna et al., 1998; Taye et al., 2006). Thus, the presence of several species in the test solution may have resulted in higher tolerance to the oils since variations in susceptibilities to toxic products exist between species. However, even with the same mosquito species, variations in susceptibilities between laboratory and field strains are expected. Recently, George and Vincent (2005) evaluated the larvicidal activity of petroleum ether seed extract of *Annona squamosa* L. and *Pongamia glabra* L. against field collected and laboratory reared *C. quinquefasciatus* larvae and noted that the field collected larvae were apparently better adapted to adjust to stress variations in the environment and hence required a higher concentration of extract to bring about the required mortality. More recently, Sun et al. (2006) evaluated the larvicidal effects of ethanol extract of *Ginkgo biloba* L. against laboratory and field strain of *C. pipiens* and reported that the field strain were more resistant than laboratory reared strain. The possible reasons are that the field strains were genetically more heterogeneous (Kabir et al., 2003) and are routinely exposed to diverse insecticides. Therefore, they probably have a higher general tolerance to toxic compounds.

Though several plants from different families have been reported to have mosquitocidal activities, only very few botanicals have moved from the laboratory to field use (Sukumar et al., 1991; Mulla and Su, 1999; Awad and Shimaila, 2003). This might be due to light and heat instability of phytochemicals compared to synthetic insecticides (Green et al., 1991). However, Mwaiko and Savaesi (1994) reported that light did not affect the larvicidal activity of essential oil from lemon peel. Recently, Amer and Mehlhorn (2006b) tested essential oil solutions stored in light and dark against different mosquito species and found identical toxicity of the essential oils against the tested mosquito species. In the present study, it was clear from the data obtained that the essential oils have also shown some promising results in the simulated field conditions regardless of light effects. However, further investigation on the persistency of essential oils may be needed.

As a whole, essential oils individually evaluated in this study had higher LC_{50} values as seen in some of the synthetic larvicides such as permethrin and chlorfenapyr (Paul et al., 2006) . Higher LC_{50} values of plant products are expected and acceptable, considering that they are generally more biodegradable, have lower non-target toxicity and environmentally friendly. Furthermore, unlike conventional insecticides, which are based on a single active ingredient, plant derived insecticides comprise variety of components with different mechanisms of action. Thus, the chances of insects developing resistance to plant products seem likely to be low (Saxena, 1987; Dhar et al., 1996). Chemical control of vectors is increasingly becoming difficult because of the development of insecticide resistance in many groups that serve as vectors of diseases. Our results thus provide further promises that they could be useful in the search of newer, more selective, and biodegradable larvicidal natural products to be used in local mosquito management programmes.

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