Parassitologia 45: 23-26, 2003

Ovicidal and larvicidal activity against *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes of essential oils extracted from three spontaneous plants of Burkina Faso

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Abstract. Essential oils extracted from dried leaves of three spontaneous plants naturally growing in Burkina Faso, i.e. *Cymbopogon proximus*, *Lippia multiflora* and *Ocimum canum*, exhibited larvicidal activity by the WHO standard protocol against 3rd and 4th instar F_1-larvae of field-collected mosquitoes vectors of human disease, namely *Aedes aegypti* and members of the *Anopheles gambiae* complex, *An. arabiensis* and *An. gambiae*. The median lethal concentration (LC_{50}) for *Ae. aegypti* and *An. gambiae* s.l. larvae ranged between 53.5-258.5 ppm and 61.9-301.6 ppm, respectively. The LC_{90} estimates ranged 74.8-334.8 ppm for *Ae. aegypti*, and 121.6-582.9 ppm for *An. gambiae* s.l. Ovicidal activity against eggs of *An. gambiae* s.l. was also demonstrated. The LC_{50} values for *An. gambiae* s.l. eggs ranged between 17.1-188.7 ppm, while LC_{90} values ranged between 33.5-488 ppm. *Lippia multiflora* showed the highest activity against *An. gambiae* s.l. eggs and *Ae. aegypti* larvae, whereas no difference was found among *C. proximus* and *L. multiflora* in their activity against *An. gambiae* s.l. larvae. Of the three plants, essential oils from *O. canum* had the lowest activity against both eggs and larvae. Eggs were more susceptible than larvae. *Ae. aegypti* larvae were more susceptible than larvae of *An. gambiae* s.l.

Key words: *Aedes aegypti*, *Anopheles gambiae* complex, *Cymbopogon proximus*, *Lippia multiflora*, *Ocimum canum*, essential oils, ovicidal effects, larvicides.

Mosquito-borne diseases still constitute a major public health problem, particularly in sub-Saharan Africa where there is 90% of the world malaria burden. This is due to the efficacy and diversity here of the vectorial system providing high inoculation rates, and a wide range of geographical, ecological, and socio-economic conditions leading to the failure of the early eradication programmes, and hindering the present efforts to control this disease (Coluzzi, 1984, 1992, 1993; Nájera, 2001; Sachs, 2002).

Development by the *Plasmodium* parasite and by mosquitoes of resistance to drugs and insecticides compounds to the problem (Hemingway *et al.*, 2002; Wellems, 2002), thereby encouraging the search for innovative vector control methods such as transmission-blocking vaccines (Ballou *et al.*, 1999), genetically-modified refractory mosquitoes (Boëte and Koella, 2002; Scott *et al.*, 2002), or the use of alternative tools such as biological agents (Davidson and Becker, 1996), or phytochemicals (Sukumar *et al.*, 1991). Vaccines and genetically-modified mosquitoes, however, will not be available as control tools before several years, whereas biological agents such as *Bacillus thuringensis israeliensis* are often not affordable by developing countries.

Phytochemicals are effective mosquito control agents offering a biorational alternative to organic synthetic pesticides. They are affordable, environmentally safe, generally available in developing countries, and are known to produce several biological effects in mosquitoes (Sukumar *et al.*, 1991). For example, Saxena and Sumithra (1989) reported on the larvicidal activity of *Ipomea carneafistolosa* against *Anopheles* spp. and *Aedes* spp. Here, we tested the ovicidal and larvicidal activity of essential oils extracted from three plants that grow naturally in Burkina Faso, *Cymbopogon proximus*, *Lippia multiflora* and *Ocimum canum*, with a view to use them against mosquitoes vectors of disease such as *Aedes aegypti* and *Anopheles gambiae*.

Materials and methods

Mosquitoes

Aedes aegypti larvae were collected in various urban settings in Ouagadougou, Burkina Faso, reared to adults in the insectary, and the F_1 proge-

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ny was used in the bioassays. Similarly, *An. gambiae* s.l. adults were manually collected indoor-resting from huts of the rural village of Goden, near Ouagadougou. Members of the *An. gambiae* complex were not identified, but from previous collections in Goden and in nearby villages it is known that three taxa are present in the area, namely *An. arabiensis* and *An. gambiae* s.s. molecular forms M and S. Gravid females were allowed to lay their eggs in the insectary, and the ensuing F_1 progeny (eggs or larvae) was subsequently tested. Rearing and testing conditions were 26-28°C and 70-80% relative humidity.

Essential oils

Essential oils were extracted by hydrodistillation from dried leaves of wild-collected *C. proximus*, *L. multiflora* and *O. canum* using a Clevenger-type apparatus. The essentials oils consisted mainly of hydrocarbon and phenolic monoterpenes. The *L. multiflora* oil contained three major components: thymol (29.9%), *p*-cymen (26.2%) and thymyl acetate (11.8%) (Bassolé *et al.*, 2003). The *C. proximus* and *O. canum* oils were mainly characterised by piperitone (70.2%) and 1,8-cineole (61.2%), respectively (Bassolé *et al.*, 2003b).

Bioassays

Serial dilutions (v/v) of each essential oil were made in absolute alcohol. A series of five dilutions and an alcoholic control were selected for testing. Test concentrations varied from 40 to 90 ppm for *C. proximus* and *L. multiflora*, and from 100 to 600 ppm for *O. canum*.

For ovicidal effect bioassays, 1 ml of test solution was mixed with 29 ml of distilled water in six plastic cups (of 115 mm diameter and 80 mm depth). Then, 20 recently-laid eggs were introduced into each cup. These cups were held in the insectary and egg mortality was scored 48 hrs post-treatment. Eggs that did not hatch after this period were considered as dead. A total of three replicates were carried out.

Larvicidal effects of plant extracts were tested according to the standard WHO protocol (WHO, 1970), with slight modifications. For the experimental treatment, 1 ml of test solution was mixed with 224 ml of distilled water in a plastic cup. Then, twenty 3rd and 4th instar larvae gathered in 25 ml of distilled water were transferred to the cup. Each replicate set included one (*Ae. aegypti*) or two (*An. gambiae* s.l.) batches for each dose tested and one control, which consisted of 1 ml of absolute ethanol in 249 ml of distilled water. After a period of 24 hrs, mortality counts were performed. Dead larvae were identified when they did not arouse after probing with a needle on the siphon or the cervical region. Moribund larvae were those unable to rise to the surface (within a reasonable period of time), or unable to show the characteristic diving reaction when the water was disturbed. They also showed discoloration, unnatural positions, tremors, uncoordination, or rigor. After each replicate, moribund and dead larvae were combined and expressed as percent mortality at each concentration. Each test spanned 3 or 4 replicates. Replicates with ≥15% mortality in the control were discarded from the analysis.

Data analysis

Acute toxicity data were analysed by logistic binomial regression with the software GLIM v.3.77 (Payne, 1987) to determine median lethal concentrations (LC_{50}) and LC_{90}. During ovicidal tests non-hatching rates in control batches usually exceeded 15%, therefore treatment data were corrected using Abbott's formula (Collett, 1991). Overdispersion in binomial data was corrected using Williams' algorithm (Collett, 1991). Confidence limits for median LCs were calculated applying the Fieller theorem (Collett, 1991). Confidence limits for LC_{90} were estimated calculating the profile deviance for binomial data (Aitkin *et al.*, 1993, p. 191). Statistical inference for comparisons of the response between different plant extracts and mosquitoes was performed by fitting a generalised linear logistic regression binomial model with all factors (mosquito species, plant extracts, log-dose) included, and then performing an analysis of deviance by F-tests following stepwise removal of factor interactions and main effects from the maximal model (Crawley, 1993).

Results

The results of the acute toxicity tests on *An. gambiae* s.l. eggs for the three plant extracts are presented in Table 1. The essential oils of *L. multiflora* had the lowest LC_{50} and LC_{90} values, followed by *C. proximus* and *O. canum* in that order. Confidence limits (95%) in Table 1 do not overlap, thus indicating that the response was significantly different between the different extracts. Larval susceptibility

Table 1. Ovicidal effect of essential oils extracted from spontaneous plants of Burkina Faso: lethal concentration values (expressed in ppm) against *Anopheles gambiae* s.l. eggs. 95% confidence intervals are shown in brackets.

Plant extracts	LC_{50}	LC_{90}
Cymbopogon proximus	52.8 (49.5-55.9)	91.1 (80.4-107.7)
Lippia multiflora	17.1 (7.9-21.1)	33.5 (28.2-49.2)
Ocimum canum	188.7 (120.4-249.1)	488.0 (470.0-669.5)

Table 2. Larvicidal effect of essential oils extracted from spontaneous plants of Burkina Faso: LC_{50} and LC_{90} values (expressed in ppm) against *Aedes aegypti* and *Anopheles gambiae* s.l. 3rd and 4th instar larvae.

Plant extracts	*Aedes aegypti*		*Anopheles gambiae* s.l.	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Cymbopogon proximus	71.8 (48.2-79.1)	106.7 (98.6-119.9)	69.7 (63.7-76.9)	121.6 (111.5-138.1)
Lippia multiflora	53.5 (47.4-59.3)	74.8 (70.7-82.0)	61.9 (55.4-68.0)	136.7 (120.1-167.0)
Ocimum canum	258.5 (222.3-290.3)	334.8 (325.1-478.2)	301.6 (264.0-342.7)	582.9 (509.3-688.8)

Table 3. Logistic regression parameters of the minimal adequate statistical model relating the log-dose mortality response of larvae of *Aedes aegypti* and *Anopheles gambiae* s.l. exposed to different essential oils extracts. Parameters followed by different letters are significantly different at $P<0.01$ by Student's *t* tests.

Plant extracts	*Aedes aegypti*		*Anopheles gambiae* s.l.	
	Slope	Intercept	Slope	Intercept
Cymbopogon proximus	6.35 [a]	−26.85 [b]	3.31 [e]	−14.08 [f]
Lippia multiflora	6.35 [a]	−25.20 [c]	3.31 [e]	−13.69 [f]
Ocimum canum	6.35 [a]	−34.83 [d]	3.31 [e]	−18.91 [g]

tests showed that *C. proximus* and *L. multiflora* had marked larvicidal activity against *Ae. aegypti* and *An. gambiae* s.l. 3rd and 4th instars (Table 2). *L. multiflora* had consistently the lowest LC_{50} and LC_{90} values whereas *O. canum* had consistently the highest. The minimal adequate model fitted to the data indicates that the functional response to the three essential oils did not change within species, but it did differ across species, with *Ae. aegypti* showing a steeper dose-response curve than *An. gambiae* s.l. (Table 3). All the intercepts of the logistic regression lines were significantly different, except for extracts from *C. proximus* and *L. multiflora* for *An. gambiae* s.l. larvae, indicating that in this case the functional response to the two essential oils was the same.

Discussion

Of the three essential oils screened, those extracted from *L. multiflora* showed the highest activity against *Ae. aegypti* larvae and *An. gambiae* eggs. *C. proximus* and *L. multiflora* exhibited the most potent activity against *An. gambiae* larvae. Essential oils from *O. canum* were the least active against both species and life stages. The low activity of *O. canum* against *An. gambiae* larvae has been previously reported (Lukwa, 1994). Comparison of results presented here with earlier investigations (Novak, 1985) shows that *Ae. aegypti* larvae are generally more susceptible to essential oils than *An. gambiae*.

The results obtained also showed that *An. gambiae* eggs are more sensitive than their larvae. Saraç and Tunç (1995) have reported similar results for an insect pest of stored products, *Ephestia kuehniella*. The ovicidal properties of our essential oils, however, must be treated with some caution as they were evaluated by scoring hatching rates 48 hrs post-treatment, although hatching could have occurred after this time if the effect of the treatment was simply a delay in embryo development and hatching. Further tests with longer scoring delays are needed to confirm our results.

Apart from ovicidal and larvicidal activity, it is known that *C. proximus*, *L. mutiflora* and *O. canum* essential oils possess antimicrobial activitiy (Jansen *et al.*, 1989; Bassolé *et al.*, 2003a; Bassolé *et al.*, 2003b), exhibit adulticidal properties against the insect pest *Callosobruchus maculatus* Fab. (Koumaglo *et al.*, 1996, 1998; Keïta *et al.*, 2000), and provide repellency against mosquitoes (Lukwa, 1994). These biological properties vary with the chemical composition of the oil. Thus, the relative effect of the *L. multiflora* essential oil could result from its three major components thymol, *p*-cymen, and thymyl acetate. Conversely, the lower ovicidal and larvicidal activity of *C. proximus* and *O. canum* oils could be explained by the weaker effect of piperitone and 1,8-cineole against *Ae. aegypti* larvae, and of piperitone against *An. gambiae* s.l. eggs. Synergetic and antagonistic activities of essential oil compounds against some bacteria reported by Cosentino *et al.* (1999) could also exist for *Ae. aegypti* and *An. gambiae* s.l. eggs and larvae. This could explain the differences observed in the biological activity among the essential oils.

Even though the *L. multiflora* essential oil had a relatively strong activity, its potency is still much lower than current synthetic larvicides such as malathion, fenthion, fenitrothion and carbosulfan (WHO, 1970, 1992), which exhibit larvicidal activity in the range of 0.00054-0.64 ppm. Yet, essential oils can offer a promising alternative for mosquito control because they are environmentally safe, locally available at community level in developing coun-

tries, and easy to extract. Moreover, they are readily biodegradable (Baysal, 1997). In addition to their general toxicant properties against various mosquito life stages, plant essential oils also show potential as growth and reproduction inhibitors, repellents, and oviposition deterrents (Klocke *et al.*, 1987; Lukwa, 1994).

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