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Mosquitocidal activity of some volatile oils against *Aedes caspius* mosquitoes

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Vector borne diseases are a major source of illness and death worldwide. Mosquitoes are one of the most important vectors that alone transmit diseases to more than 700 million people per annum[1]. For instances, malaria alone kills 3 million each year, including 1 child every 30 sec[2], is transmitted by *Anopheles gambiae* and *Aedes caspius* is the vector of Tahyna (TAH) virus in the Mediterranean region, a potential reservoir of "Rift Valley Fever Virus" (RVF) during interepizootic periods, harbor some microsporidia and the West Nile virus (WNV), and the bacterium *Francisella tularensis*, the causative agent of tularemia could be detected in natural populations[3]. Control of such diseases is becoming increasingly difficult because of increasing resistance in mosquitoes to synthetic insecticides[4]. An alternative and recent approach for mosquito control is the use of natural products of plant origin, botanical derivatives. Beside their toxicity to pests, these botanical insecticides are readily biodegradable and usually lack toxicity to higher animals[5] that means they are eco-friendly. Pyrethrum and neem are well-established commercial pesticides based on plant essential oils and have recently entered the market place. A huge number of botanical derivatives exhibited mosquitocidal activity[6,7]. They act as ovicides, larvicides, pupicides, adulticides, repellents, oviposition deterrents beside their insect growth regulating activity.

Cinnamon (*Cinnamomum cassia*), Sabina (*Sabina vulgaris*), White camphor (*Cinnamomum glanduliferum*), and Wintergreen (*Liex chinensis*) oils exhibited larvicidal activity against the dengue vector *Aedes aegypti* larvae[8]. Furthermore, Cinnamon oils exhibited antimicrobial activity[9], antimycotic activity[10], insecticidal activity against the oak nut weevil *Mechoris ursulus*[11] and mosquito repellent activity against the dengue vector *Ae. aegypti*[12]. Since there is no available information about utilizing these oils in *Ae. caspius* control, this study mainly compares between the mosquitocidal capacities of these oils and the mosquitocidal capacities of a couple of their mixtures against *Ae. caspius*.

Field collected IV instar larvae of *Ae. caspius* were used in the present work. Volatile oils used in the present work are extracted from Cinnamon, Sabina, White camphor and Wintergreen and were supplied by Biopesticide Research Centre, Yangling, China. Larvae were exposed to one sublethal concentration 5 ppm, and selection of this dose was based on previous study against *Ae. aegypti*[8] whereas LC_{50} of white camphor, cinnamon and wintergreen oils were 43.0, 58.4 and 81.3 mg/L respectively but Sabina was inactive (Table 1). Hence, the present study investigated the influence of a very low dose, 5 ppm (10th time lower than the most active oil), on the larval development until mosquito emergence compared to mixtures from different oils at the same dose. WHO methodology[13] was adopted for evaluating the activity of the volatile oils against mosquitoes. The corresponding dose of 1 ml was added to 99 ml of distilled water. Lots of 25 newly moulted IV instar *Ae. caspius* larvae were used per cup. Control received 1 ml of ethanol only. Four replicates for each dose were conducted. For accurate determination of the mosquitocidal activity, the larval and pupal mortality as well as adult emergence were daily recorded up to emergence of the adults or death of the last larva or pupa. Due

Table 1. LC_{50} and LC_{90} of some volatile oils against *Ae. aegypti* IV instar larvae

Oils	LC_{50}		LC_{90}		Slope $\pm$ SE
	mg/L	95% FL	mg/L	95% FL	
Cinnamon (*Cinnamomum cassia*)	58.41	53.8–62.8	82.36	76.5–90.4	0.054 $\pm$ 0.004
White camphor (*Cinnamomum glanduliferum*)	42.98	40.1–45.4	55.35	52.2–60.05	0.104 $\pm$ 0.009
Wintergreen (*Liex chinensis*)	81.32	75.9–88.3	107.689	98.1–125.7	0.049 $\pm$ 0.005

Table 2. Effect of some oils and their mixtures on the development of the IV instar *Ae. caspius* larvae

Scientific name	Common name	Mean % of mosquitocidal activity ± SE				
		Dead larvae	Dead pupae	Dead adults	Malformations	Emerged adults
Cinnamomum cassia	Cinnamon	24 ± 4.6[a]	10 ± 2.5[a]	8 ± 1.6	2 ±1.1	56 ± 8.6[a]
Cinnamomum glanduliferum	White camphor	60 ± 5.8[ab]	26 ± 4.7	3 ± 1.9	8 ±2.8	3 ± 1.9[ab]
Liex chinensis	Wintergreen	46 ± 8.7[c]	41 ± 6.1[ab]	5 ± 3	6 ±3.6	2 ± 1.1[ac]
Sabina vulgaris	Sabina	47 ± 2.6[d]	37 ± 7.0[ac]	16 ± 8	0 ± 0	0 ± 0[ad]
Cinnamomum cassia & *Liex chinensis*	Cinnamon & Wintergreen	84 ± 2.8[acde]	16 ± 2.8	0 ± 0	0 ± 0	0 ± 0[ae]
Cinnamomum glanduliferum & *Liex chinensis*	White camphor & Wintergreen	50 ± 12[e]	33 ± 6[ad]	5 ± 5	3 ± 1	9 ± 4.1[af]
Control		7 ± 4.1[bcde]	12 ± 1.6[bcd]	3 ± 1.0	0 ± 0	78 ± 3.4[abcdef]

*Means within columns followed by the same letter are significantly different at $p = 0.05$.

to the long duration of the test, larvae provided with food at intervals of 2 days during the test period. From the overall results of the test, percentage of dead larvae, both emerged and dead pupae and percentage of dead and emerged adults were determined. For bioassay of mixtures, the oils mixture were combined from 5 ppm of each oil in a 1:1 volume and the same previously mentioned bioassays were conducted. SPSS version 16 software was used for statistical analysis.

Results in Table 2 showed that all oils and their mixtures exhibited mosquitocidal activity. The mixture results were better and more effective than the individual oil and mixture of cinnamon and wintergreen was more effective than the mixture of wintergreen and white camphor. The former mixture completely inhibited mosquito emergence compared to the latter mixture. Both oils and their mixtures exhibited significant larval (df=6, F=13.7, $p < 0.05$) and pupal (df=6, F=7.3, $p < 0.05$) mortalities compared to control. They also significantly inhibited adult emergence (df=6, F=62.05, $p < 0.05$). Cinnamon, white camphor, wintergreen and mixture of white camphor and Wintergreen significantly produced more malformations (df=6, F=2.9, $p < 0.05$) than control.

These results indicated that mosquitocidal activity of such oils depends upon plant species. In coincidence with these results Prajapati *et al*[14] found that essential oils of various parts of 10 medicinal plants showed different mosquitocidal activity against *An. stephensi*, *Ae. aegypti* and *Culex quinquefasciatus*. The larvicidal activity of the tested oils considered promising if compared with the larvicidal activity of the 11 Indian medicinal plants that produced 22–100% larval mortality against *An. stephensi* and *Cx. quinquefasciatus* at 1000 ppm[15]. Whilst white camphor, cinnamon and wintergreen oils produced relatively high LC_{50} against *Ae. aegypti* larvae (43.0, 58.4 and 81.3 mg/L respectively)[8], the present results against *Ae. caspius* larvae are promising since they produced higher mosquitocidal activity at very low dose and significantly inhibited mosquito emergence in particular Sabina oil that was inactive against *Ae. aegypti*.

Additionally, malformations, dead intermediate stages such as larval-pupal individuals and half-ecdysed adults, pupicidal activity associated with adult emergence inhibition suggesting growth regulation for these oils. Sublethal concentrations of botanical extracts greatly affect mosquito development and this effect is dose dependant[16] and factors that affecting the bioactivity of essential oils include plant species (variety), cultivating conditions, maturation of harvested plants, plant storage, plant preparation and methods of extraction[11, 17].

In contrast to previous study against *Ae. aegypti*[8], these mixtures were highly active against *Ae. caspius* whereas white camphor and wintergreen mixture largely reduced emergence of *Ae. caspius* mosquitoes whilst cinnamon and wintergreen mixture completely stopped mosquito emergence. Similarly, a mixture of the peel oils extract of three citrus species (lemon, orange, and bitter orange) was much more effective than for the peel oils extract for the individual species[18]. Generally speaking, mixtures are always stronger and better than their single oils[6,19] and this strong activity is due to synergistic action of these mixtures. The mechanism of synergism is not known, but it might be due to phytochemicals inhibiting the ability of mosquito larvae to employ detoxifying enzymes against synthetic chemicals[20].

In conclusion, the present result showed that tested oils affected the development of *Ae. caspius* at very low concentration (5 ppm) and this effect depended on the plant species. Fractionation and separation of main compounds of these oils may lead to discovery of more potent compound that is comparable to synthetic insecticides. Results of mixtures are surprising and doughtily better than the sublethal concentration of the oils if they applied individually and reduce the amount of used toxins.

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