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Larvicidal activities of some Euro-Asiatic plants against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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ABSTRACT

Methanol extracts of the aerial parts from 31 Euro-Asiatic plant fourth instar larvae species were tested for larvicidal activity against the mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae) fourth instar larvae. under laboratory conditions. The mortality from six concentrations (5, 10, 25, 50, 100 and 200 ppm) was determined and LD_{50} was calculated. All plant extracts showed larvicidal activity in 24 h exposure tests. The methanolic extracts of plants *Otanthus maritimus* displayad the highest larvicidal activities with LD_{50} 7 ppm, followed by *Ammi visnaga* , *Acer pseudoplatanus*, *Acer platanoides* and *Satureja hortensis* with LO_{50} 9, 23, 28 and 28 ppm, respectively.

Keywords: *Culex quinquefasciatus, Otanthus maritimus, Ammi visnaga* , *Acer pseudoplatanus*, *Acer platanoides* and *Satureja hortensis*.

INTRODUCTION

Mosquitoes not only cause nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries (Jang *et al.,* 2002). *Culex quinquefasciatus* Say (Diptera: Culicidae) is a pantropical pest and urban vector of *Wuchereria bancrofti, Plasmodium* (avian malaria), myxomatosis, and other diseases in some parts of the world (Holder, 1999). It has been shown to be able to carry Murray Valley encephalitis (MVE) virus in laboratory studies and MVE virus has been isolated from it in northern Western Australia. *Culex quinquefasciatus* has yielded an isolate of Ross River (RR) virus during an outbreak in New Caledonia, but from a number of laboratory studies in Australia it appears to be a poor and unlikely vector of MVE, Kunjin, RR and other arboviruses. It is a poor vector of dog heartworm, and of human filariasis in more northern tropical regions (Russell, 1993).

Synthetic insecticides are today in the fore of the mosquito controlling agents. Compared to other controlling measures in the past few decades, synthetic insecticides have been used and have produced a feed back of environmental ill effect, non-target organisms being affected and most of mosquitoes species have becoming physiologically resistant to these synthetic insecticides (VCRC, 1989; Severini *et al.,* 1993). On the other hand, some mosquito species have developed high levels of resistance to microbial control agents (Rao *et al.,* 1995). These factors have created a search for ecofriendly, biodegradable and target – specific insecticides against the mosquitoes.

Plant products have been used traditionally by human communities in many parts of the world against the vector and pest species of insects (Jacobson, 1958; Pavela, 2007). Over the past 35 years, the search for plants with novel insecticidal constituents has been intensive. Among the plant families studied Asteraceae, Lamiaceae, Meliaceae, Piperaceae, Rutaceae crude extracts or their compounds have showed toxicity (Pavela, 2006), antifeedants (Sadek, 2003; Pavela, 2004a), insect growth regulators (Pavela, 2004b; Pavela, 2005), oviposition deterrence (Dimock and Renwick, 1991; Zhao *et al.,* 1998), suppression of calling behaviour (Khan and Saxana, 1986) and reduction of fecundity and fertility (Pavela *et al.,* 2005). Such a wide variety of effects provides potential alternatives for the use of synthetic chemical insecticides.In the present investigation an attempt was made to evaluate the larvicidal efficacy of some plant extracts against *Culex quinquefasciatus*.

MATERIAL AND METHODS

Plant extracts

Fresh plant material of each of the selected species (see Table 1) was collected in 2006. Voucher specimens of all the plant species studied were deposited in herbaria of our institute. The plant material was shade-dried and powdered. The dry powder was extracted eith excess of methylalcohol (500 ml of MeOH for 100g of plant powder) for 24 h. The crude extracts were filtrated and evaporated under reduced pressure in a rotary evaporator.

Test organism

The test organism *Culex quinquefasciatus* Say was reared in the laboratory conditions. (28 ± 2°C, 70± 5% RH, and a photo regime of 16:8 (L:D) h.)on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week – old chick for blood feeding.

Larvicidal activity

Mosquito larvicidal assays were carried out according to WHO standard procedures (1996), with slight modifications. The extracts were diluted in dimethyl sulphoxide (DMSO) to prepare a serial dilution of test dosage. Early fourth instar larvae of *C. quinquefasciata* were selected and transferred in 25 ml of distilled water.

Table1. Plants selected for the screening of larvicidal activity against *Culex quinquefasciata*

Species	Family assayed	Plant part	Yield (%) references	Voucher	Origin
Acer campestre L.	Aceraceae	Leaves	8.7	0760	Prague, Czech Republic
Acer cissifolium (Siebold & Zuccarini) Koch	Aceraceae	Leaves	8.3	0762	Prague, Czech Republic
Acer negundo L.	Aceraceae	Leaves	9.5	0763	Prague, Czech Republic
Acer platanoides L.	Aceraceae	Leaves	9.2	0761	Prague, Czech Republic
Acer pseudoplatanus L.	Aceraceae	Leaves	7.6	0759	Prague, Czech Republic
Achilea millefolium L.	Asteraceae	Stem	8.9	0740	Prague, Czech Republic
Ajuga reptans L.	Lamiaceae	Stem	10.2	0722	Prague, Czech Republic
Ammi visnaga (L.) LAM.	Apiaceae	Seeds	19.3	0732	Prague, Czech Republic
Circium arvense (Savi) Ten.	Asteraceae	Stem	5.8	0750	Prague, Czech Republic
Fraxinus exelsior L.	Oleaceae	Leaves	5.3	0765	Prague, Czech Republic
Glebionis coronarium (L.) Tzvelev. Russia	Asteraceae	Flower	9.2	0758	Krasnodarskiy region,
Humulus japonicus Sieb. & Zucc.	Cannabidaceae	Leaves	7.9	0725	Prague, Czech Republic
Hysopus officinalis L.	Lamiaceae	Stem	8.7	0748	Prague, Czech Republic
Laburnum anagyroides Medik.	Legiminosae	Leaves	15.0	0734	Prague, Czech Republic
Lavandula officinalis L.	Lamiaceae	Flower	3.1	0227	Prague, Czech Republic
Limonium. Bonduelii. O. Kuntze	Plumbaginaceae	Stem	2.8	0746	Prague, Czech Republic
Matricaria maritima L.	Asteraceae	Flower	6.8	0766	Prague, Czech Republic
Matthiola tricuspidata (L.) R. Brown Russia	Brassicaceae	Stem	7.2	0754	Krasnodarskiy region,
Melisa officinalis L.	Lamiaceae	Stem	5.7	0742	Prague, Czech Republic
Ocimum basilicum L.	Lamiaceae	Stem	7.6	0753	Prague, Czech Republic
Origanum vulgare L.	Lamiaceae	Stem	7.4	0729	Prague, Czech Republica
Otanthus maritimus (L.) Hoffmanns & Link Russia	Asteraceae	Stem	5.6	0755	Krasnodarskiy region,
Salvia farinacea Benth.	Lamiaceae	Stem	5.4	0713	Prague, Czech Republic
Salvia viridis L.	Lamiaceae	Flower	4.7	0731	Prague, Czech Republic
Salvia officinalis L.	Lamiaceae	Stem	11.3	0721	Prague, Czech Republic
Satureja hortensis L.	Lamiaceae	Stem	7.7	0717	Prague, Czech Republic
Satureja nervosa Desf .	Lamiaceae	Stem	4.2	0756	Crete, Greece
Sonchus arvensis L	Asteraceae	Stem	5.8	0744	Prague, Czech Republic
Tanacetum vulgare L.	Asteraceae	Flower	6.1	0736	Krasnodarskiy region, Russia
Teuricum capitatum L.	Lamiaceae	Stem	5.8	0757	Crete, Greece
Thymus vulgaris L.	Lamiaceae	Stem	5.0	0718	Prague, Czech Republic

For experimental treatment, 1 ml of serial dilutions was added to 224 ml of distilled water in a 500-ml glass bowl and shaken lightly to ensure a homogenous test solution. The selected larvae were transferred in distilled water into a bowl of prepared test solution with final surface area 125 cm2 (25 larvae/beaker). Four replicates were of maintained for all the five dosages (5, 10, 25, 50, 100 and 200 ppm) separetly. The assays were placed in a growth chamber (L16:D9, 26 °C). The mortality was determined after 24 h of exposure, during which time no food was offered to the larvae. The control mortality was corrected by Abbott's formula (Abbott, 1925) and LD_{50}, LD_{90}, regression equation and the 95% confidence limit was calculated by using probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

The results of larvicidal activity of plant extracts are presented in Table 2. All plant extracts showed larvicidal activity in 24 h exposure tests. The methanolic extracts of plants *Otanthus maritimus* displayad the highest larvicidal activities with LD_{50} 7 ppm, followed by *Ammi visnaga* , *Acer pseudoplatanus*, *Acer platanoides* and *Satureja hortensis* with LD_{50} 9, 23, 28 and 28 ppm, respectively.

Table 2. Insecticidal activity of plant extracts against fourth stage larvae of *Culex quinquefasciata*

Species	Average mortality[a](%) ± SE	LD_{50}(CI95)[b]	LD_{90}(CI95)
Acer campestre L.	100.0 ± 0.0	52 (48-75)	86(82-98)
Acer cissifolium (Siebold & Zuccarini) Koch	100.0 ± 0.0	70 (62-84)	123(110-132)
Acer negundo L.	100.0 ± 0.0	51(45-63)	89(80-93)
Acer platanoides L.	100.0 ± 0.0	28(25-39)	62(54-73)
Acer pseudoplatanus L.	100.0 ± 0.0	23(18-33)	76(60-89)
Achilea millefolium L.	100.0 ± 0.0	120(115-126)	159(142-169)
Ajuga reptans L.	46.0 ± 3.8	>200	>200
Ammi visnaga (L.) LAM.	100.0 ± 0.0	9(8-18)	45(38-52)
Circium arvense (Savi) Ten.	44.6 ± 3.8	>200	>200
Fraxinus exelsior L.	21.0 ± 3.2	>200	>200
Glebionis coronarium (L.) Tzvelev.	100.0 ± 0.0	53(46-58)	110(102-123)
Humulus japonicus Sieb. & Zucc.	100.0 ± 0.0	25 (21-32)	87 (76-110)
Hysopus officinalis L.	92.7 ± 5.9	150 (140-163)	>200
Laburnum anagyroides Medik.	98.0 ± 8.1	111 (103- 126)	198 (156-220)
Lavandula officinalis L.	100.0 ± 0.0	59(57-72)	123(118-128)
Limonium. Bonduelii. O. Kuntze	52.2 ± 12.3	>200	>200
Matricaria maritima L.	100.0 ± 0.0	72(60-90)	139(126-142)
Matthiola tricuspidata (L.) R. Brown	9.0 ± 3.3	>200	>200
Melisa officinalis L.	44.5 ± 6.5	>200	>200
Ocimum basilicum L.	100.0 ± 0.0	32(29-45)	69(53-82)
Origanum vulgare L.	88.6 ± 7.2	156 (148-172)	>200
Otanthus maritimus (L.) Hoffmanns & Link	100.0 ± 0.0	7.3(6.5-9.3)	15(12-18)
Salvia farinacea Benth.	68.3 ± 3.3	195(183-199)	>200
Salvia viridis L.	100.0 ± 0.0	110(105-132)	183(153-191)
Salvia officinalis L.	84.6 ± 10.2	159(133-168)	>200
Satureja hortensis L.	100.0 ± 0.0	28(22-37)	56(48-72)
Satureja nervosa Desf .	32.5 ± 8.5	>200	>200
Sonchus arvensis L	100.0 ± 0.0	68(55-78)	118(110-132)
Tanacetum vulgare L.	92.1 ± 4.4	178(158-188)	198(186-201)
Teuricum capitatum L.	48.8 ± 12.9	>200	>200
Thymus vulgaris L.	100.0 ± 0.0	48.2(39-82)	98(79-102)
control	0.0 ± 0.0		

[a] The average mortality (± standard error) established in concentration 200 ppm as a maximal tested concentration.
[b] The lethal concentrations with the corresponding 95% confidence intervals are shown in are shown in parenthesis.

Today, the environmental safety of an insecticide is considered to be of paramount importance. Phyto chemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world (Pavela, 2007). The screening of locally available plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.

The crude extracts of the five plant has been found to possess larvicidal activity against the mosquito *Culex quinquefasciatus*. The biological activity of this plant extracts might be due to the various compound, including phenolics, terpenoids, and alkaloids exist in plants these compounds may jointly or independently contribute to produce larvicidal and adult emergence inhibition activity against *Culex quinquefasciatus*.

The larvicidal efficacy of *O. maritimus, A. visnaga* , *A. pseudoplatanus*, *A. platanoides* and *S. hortensis* are comparable to well established insecticidal plant species. Omena *et al*. (2007) studied larvicidal activities of 51 Brazilian medicinal plants against Aedes aegypti. Eleven of 84 extracts (obtained from different plant parts) showed significant LD_{50} < 100 ppm and seven species showed significant LD_{50} < 10 ppm (from genus *Annona* sp., *Derris* sp., *Erythrina* sp. and *Pterodon* sp.). Pizzarro *et al*. (1999) studied the activity of the saponine fraction of *Agave sisalana* and estimated the LD_{50} and LD_{90} against 3^{rd} instar larvae of *Culex quinquefasciatus,* that were 183 and 408 ppm, respectively. These concentrations were much higher than those reported in this study, but these authors suggested its use for control of this mosquito. The leaf extracts of *O. maritimus, A. visnaga* , *A. pseudoplatanus*, *A. platanoides* and *S. hortensis* is superior to various neem extracts, which are reported to be effective with LD_{50} values ranging from 55-65 ppm against mosquito larvae (Ascher and Meisner, 1989). The median lethal concentrations (LD_{50}) of various parts of *Melia azederach* ranging from 30-40 ppm against larva of *Culex pipiens* (Al-Sharook *et al*., 1991). The effect of the various neem extracts and various parts of *Melia azederach* was slightly lower than that reported for the *C. asiatica* leaf extract. The adult emergence inhibition activity of *C. asiatica* is also comparable to different species of plant extract in different families (Muthukrishnan *et al*., 1999; Pushpalatha and Muthukrishnan, 1999).

In our study, five extracts possess remarkable larvicidal activity against mosquito *Culex quinquefasciatus*. Further investigations are needed to elucidate this activity against a wide range of all stages mosquito species and also the active ingredients of the extract responsible for larvicidal and adult emergence inhibition activity in *Culex quinquefasciatus* should be identified and utilized, if possible, in preparing a commercial product / formulation to be used as a mosquitocidal.

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