Industrial Crops and Products 30 (2009) 311–315

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Larvicidal property of essential oils against *Culex quinquefasciatus* Say (Diptera: Culicidae)

Roman Pavela

Crop Research Institute, Drnovska 507, 161 06 Prague 6 – Ruzyne, Czech Republic

ARTICLE INFO

Article history:
Received 27 April 2009
Received in revised form 9 June 2009
Accepted 12 June 2009

Keywords:
Thymus
Satureja
Essential oils
Larvicidal
Mosquito
Botanical insecticides

ABSTRACT

Essential oils from 22 aromatic plant species were tested for mortality of the mosquito larvae *Culex quinquefasciatus*. Lethal concentrations were determined for individual essential oils. Essential oils obtained from *Thymus vulgaris*, *Satureja hortensis* and *Thymus satureioides* plants showed the highest effect, with LC_{50} found lower than 50 μg/ml (33, 36 and 44 μg/ml, respectively). Analyses showed that majority substances for *T. vulgaris* were thymol and p-cymene (60.3 and 10.1%, respectively); carvacrol and γ-terpinene for *S. hortensis* (48.1 and 36.7%, respectively), and borneol and thymol for *T. satureioides* (30.3 and 32.5%, respectively).

The selected essential oils also showed very good effectiveness with respect to mortality and percentage of adult emergence upon short-term exposure in water contaminated with lethal doses of individual oils. While there was 77% adult emergence from the larvae in the control, in *T. vulgaris*, *T. satureoides* and *S. hortensis* there was only 12.3, 15.3 and 16.0% adult emergence, respectively. High antioviposition effectiveness was found in all selected oils. Almost 100% deterrence of female oviposition was determined for all oils in concentrations of 0.02%. Significant differences were seen with tested concentrations of 0.01 and 0.005%, where the oil of *T. vulgaris* proved most effective (repellency about 99.8 and 62.3%, respectively).

1. Introduction

Insect-transmitted disease remains a major cause of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually (Taubes, 2000). Therefore, the control of mosquitoes is an important public health concern around the world. For example, *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 (Goddard et al., 2002). The only efficacious approach to minimizing the incidence of these diseases is to eradicate and control mosquito vectors, mainly by applying insecticides to larval habitants, and educating the public (Corbel et al., 2004).

Chemical control is an effective strategy used extensively in daily life. Synthetic insecticides are today at the forefront of mosquito controlling agents. Nevertheless, controlling the mosquitoes has become complicated because of their resistance to synthetic insecticides, as well as the toxicity of insecticides to fish and other non-target organisms (Wattanachai and Tintanon, 1999; Rohani et al., 2001). There is an urgent need to develop new materials for controlling mosquitoes in an environmentally safe way, using biodegradable and target-specific insecticides against them.

Plant essential oils have been suggested as alternative sources for insect control, because some are selective, biodegrade to non-toxic products, and have few effects on non-target organisms and the environment (Singh and Upadhyay, 1993; Isman, 2006; Pavela, 2007a).

Essential oils and their volatile constituents are widely used in the prevention and treatment of human illnesses. Various essential oils have also been documented to exhibit acute toxic effects against the insect. Several experiments have been conducted on the insecticidal properties of essential oils against various mosquitoes (Shalaby et al., 1998; Zaridah et al., 2006; Knio et al., 2008). All the screening papers mentioned are very important on the path to the development of new botanical insecticides. The number of plant species that can provide essential oils is high. Nevertheless, only a part of them can be successfully cultivated to provide sufficient quantities of biologically active compounds, moreover for relatively favourable production prices. Such plants show a high potential for the production of botanical insecticides or larvicides, respectively. A comparison of the biological activity of commercially produced essential oils is therefore highly valuable in the narrow selection of suitable plant species, development of a suitable cultivation technology, extraction and subsequent formulation of plant insecticides.

This study was aimed at assessing the potential of plant essential oils for use as commercial insecticides – larvicides. The toxicity

E-mail address: pavela@vurv.cz.

0926-6690/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2009.06.005

Table 1
Plant species, origin and parts evaluated in their larvicidal activity of essential oils.

Scientific name	Family	Plant part used	Origin
Amyris balsamifera L.	Rutaceae	Wood	Haiti
Anthemis nobilis L.	Asteraceae	Flower	USA
Cannabis sativa L.	Cannabinaceae	Herb	Canada
Citrus aurantium L.	Rutaceae	Flower	Tunisia
Erigeron canadensis L.	Asteraceae	Herb	USA
Juniperus communis L.	Cupressaceae	Berry/twig	Yugoslavia
Laurus nobilis L.	Lauraceae	Leaf	Crete
Lavandula angustifolia Mill.	Lamiaceae	Flower	USA
Melaleuca alternifolia L.	Myrtaceae	Leaf	Australia
Nepeta cataria L.	Lamiaceae	Flowering tops	Canada
Ocimum basilicum L.	Lamiaceae	Leaf	Egypt
Pelargonium roseum Willd.	Geraniaceae	Leaf	Madagascar
Pimenta dioica (L.) Merr.	Myrtaceae	Fruit/berry	Jamaica
Ravensara aromatica Sonn.	Lauraceae	Leaf	Madagascar
Rosmarinus officinalis L.	Lamiaceae	Flowering herb	Corsica
Salvia sclarea L.	Lamiaceae	Flower	USA
Santalum album L.	Santalaceae	Heartwood	India
Satureja hortensis L.	Lamiaceae	Flowering tops	Czech Republic
Tanacetum vulgare L.	Asteraceae	Flowering tops	USA
Thymus satureoides Boiss.	Lamiaceae	Herb	Morocco
Thymus vulgare L.	Lamiaceae	Flowering tops	Czech Republic
Zingiber cassumunar Roxb.	Zingiberaceae	Root	Thailand

of 22 essential oils against *C. quinquefasciatus* larvae was assessed, and the effectiveness of the three most effective oils on larval development, as well as oviposition deterrent effect on mosquito adults, was compared.

2. Materials and methods

2.1. Essential oils

The essential oils (excluding oils from *Thymus vulgaris* and *Satureja hortensis*) used in this study (Table 1) were purchased from Essential Oil University, 16224 Charlestown-Bethlehem Rd., Charlestown, IN 47111, USA. The essential oils were obtained by steam or hydrodistillation of botanicals.

The plants of *T. vulgaris* and *S. hortensis* were collected from plants growing in areas of CRI, Prague, Czech Republic. The essential oils of the dry herb were extracted by hydrodistillation using a modified Clevenger-apparatus. In each case, 20 g of the plant material was distilled in 300 ml of distilled water in a 500 ml flask for 60 min. All oil samples were stored at 4 °C until bioassays.

2.2. Test organisms

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

2.3. Chemical analyses

GC–MS analyses were performed on a Finnigan GCQ instrument with a Zebron ZB-5 column (Phenomenex, USA), 30 m × 0.25 mm × 0.25 μm, using the following temperature program: initial temperature 60 °C, hold for 1 min, then gradient 4 °C min^{-1} to 180 °C, then gradient 10 °C min^{-1} to 275 °C and hold 5 min at this temperature. Temperature of the transfer line was 275 °C, ion source was 200 °C. Linear velocity of the carrier gas (helium) was 40 cm s^{-1}. Full scan spectra in the range of relative mass m/z 50–450 Da were obtained (Adams, 2007).

2.3.1. Larvicidal activity

Mosquito larvicidal assays were carried out according to WHO Standard Procedures (1996), with slight modifications (Pavela, 2008b). Early third instar larvae of *C. quinquefasciata* were used. The oils were diluted in dimethyl sulphoxide (DMSO) to prepare a serial dilution of test dosage. 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 cm^2 (25 larvae/beaker). Four replications were run simultaneously with at least six dosages (from 5 to 500 μg/ml). The assays were placed in a growth chamber (L16:D9, 26 °C). Mortality was determined after 24 h of exposure, during which time no food was offered to the larvae.

Supplemental tests were determined for the three most effective essential oils (*T. vulgaris*, *S. hortensis* and *Thymus satureoides*), described in Sections 2.3.2 and 2.3.3.

2.3.2. The effect of lethal doses on larval development for selected essential oils

At the beginning of the fourth instar, the larvae were put into a plastic container (20 cm × 20 cm × 20 cm) with 3 l of drinking water and let to stand still. Upon acclimatization (after approximately 1 h), a dose of essential oil was mixed into the water, corresponding to the calculated dose LD_{50} (for *T. vulgaris*, *S. hortensis* and *Thymus satureoides*; 33, 36 and 44 μg/ml, respectively). The essential oils were emulsified using DMSO; water with an adequate DMSO content was used for the control larvae. Each time, 100 larvae were introduced into a vessel treated with an appropriate dose of essential oil, corresponding to the lethal dose. The larvae were left in the treated water for 5 h. Afterwards, the larvae were removed using a sieve into new vessels with only clean water. Regular food was given to the larvae. The larvae were left in clean water for the whole development period until the emergence of adults. Larval mortality was observed during the experiment, 24 and 48 h following termination of exposure. The percent mortality of larvae that did not finish development was also determined, as well as the percentage of emerged adults. Three repetitions of the experiment were performed. The assays were placed in a growth chamber (L16:D9, 26 °C).

2.3.3. Oviposition deterrent effect for selected essential oils

The oviposition deterrent test was performed using the method of Xue et al. (2001). Fifteen gravid females (8–10 days old, 4 days after blood feeding) were transferred to each mosquito cage (45 cm × 30 cm × 30 cm), which was covered with a plastic screen, with a glass top and muslin sleeve for access. Serial dilutions of essential oils were made in DMSO. A plastic bowl containing 100 ml of rainwater was treated with oil to obtain test solutions of 0.005, 0.01 and 0.02%. Two plastic bowls holding 100 ml of rainwater were placed in opposite corners of each cage. One was treated with the test material, and the other with a solvent control that contained DMSO. The positions of the bowls were alternated between different replications so as to nullify any effect of position on oviposition. Five replications for each concentration were run, with cages placed side by side for each bioassay. A sucrose solution was available at all times. The assays were placed in a growth chamber (L16:D9, 26 °C). After 24 h, the number of eggs laid in the bowls was recorded.

The percent effectiveness of repellency for the essential oils was calculated by the formula:

$$ER\,(\%) = \frac{NC - NT}{NC + NT} \times 100$$

where ER = percent effectiveness of repellency; NC = number of eggs in control; NT = number of eggs in treatment.