Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 7897–7901
BIORESOURCE TECHNOLOGY

Short Communication

Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* L. (Lepidoptera: *Limantriidae*)

Miroslav Kostić [a], Zorica Popović [b,*], Dejan Brkić [c], Slobodan Milanović [d], Ivan Sivčev [e], Sladjan Stanković [f]

[a] *Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška, 11000 Belgrade, Serbia*
[b] *Department of Ecology, Institute for Biological Research, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia*
[c] *Johnson and Johnson S.E. Incorporation, Bulevar Mihajla Pupina 10B/I, 11070 Belgrade, Serbia*
[d] *Institute of Forestry, Kneza Višeslava 3, 11030 Beograd, Serbia*
[e] *Institute for Plant Protection and Environment, Banatska 33, 11080 Zemun, Serbia*
[f] *Institute for the Application of Science in Agriculture, Bulevar Despota Stefana 68b, 11060 Belgrade, Serbia*

Received 27 March 2007; received in revised form 6 February 2008; accepted 10 February 2008
Available online 24 March 2008

Abstract

Ethanol solutions of essential oil of *Ocimum basilicum* and its main component, linalool (both isomer forms), all in three concentrations, as well as botanical standard Bioneem (0.5%), were tested for their toxicity and antifeedant activity against the second instar gypsy moth larvae in the laboratory bioassay. The essential oil of *O. basilicum* was subjected to gas chromatography analysis, and totally 37 compounds were detected, of which linalool was predominantly present.

All tested solutions showed low to moderate larvicidal effect in both residual toxicity test and in chronic larval mortality bioassay. Chronic mortality tests showed that obtained mortality was a consequence of starving rather than ingestion of treated leaves. However, antifeedant index achieved by application of tested solutions in feeding choice assay was remarkable. Foliar application of all tested compounds deterred feeding by L2 in the same percent as Bioneem. Antifeedant index was relatively high at all tested treatments (85–94%); moreover, the larval desensitization to repelling volatiles has not occurred after five days of observation. Low toxic and high antifeedant properties make these plant-derived compounds suitable for incorporation in integrated pest management programs, especially in urban environments.

Keywords: *Lymantria dispar*; *Ocimum basilicum*; Larvicide; Antifeedant; Botanical pest control agents

1. Introduction

The caterpillars of gypsy moth, *Lymantria dispar* (L.), are major defoliators of deciduous forests, being also very harmful in urban environment, i.e., orchards, parks and tree rows. Population of this pest oscillate with periodic eruptions, and despite the enormous research effort, there remain many unanswered questions about the causes for yearly fluctuations in gypsy moth abundance (Liebhold et al., 2000). Among a number of biotic and abiotic factors, the quality and quantity of food is still the most important factor for regulation the population density of this pest (Lance, 1983; Montgomery and Wallner, 1988). In order to reduce a major damage caused by *L. dispar*, besides Bt insecticides, conventional pesticides were applied frequently and sometimes in an inadequate way in forest management and crop protection, which consequently have lead to serious environmental damages.

Due to increased environmental demands, the promotion of pest control agents of botanical origin became actual in recent years. Plant protective function of low-toxic semiochemicals derived by commonly used,

* Corresponding author. Tel.: +381 11 2078 360; fax: +381 11 2761 433.
E-mail address: zoricaj@ibiss.bg.ac.yu (Z. Popović).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.02.010

non-noxious plant species which would not cause ecosystem disturbance due to high mortality of target (and/or non-target) insect population, are the objective of number of studies. Azadirachtin (accessible as the commercial formulation 'Bioneem') derived from the seeds of the neem tree, *Azadiracta indica* A. Juss (Meliaceae), has already been incorporated in integrative pest management as the efficient toxic and antifeedant agent against insects.

One of the most known aromatic herbs, the common sweet basil, *Ocimum basilicum* L. (Lamiaceae), besides it is widely usage for the various human purposes, is also known by it's biological activities against various groups of pests. In search for alternatives to conventional pesticides, the essential oil of *O. basilicum* has been widely investigated, and showed toxic, anti-reproductive and repellent effects against stored product pests, bean and rice weevils (Popović et al., 2006) and moderate toxicity against the greenhouse pests, spider mites and white fly (Aslan et al., 2004). Extract of whole leaf of basil was highly insecticidal to mosquito (Umerie et al., 1998), and showed lethal and repellent effect against the larvae of cotton worm (Pavela, 2004) and yellow fever mosquito (Murugan et al., 2007). The main component of basil's essential oil, linalool has already been recognized as a strong volatile repellent building up a non-host status of the green ash to gypsy moth larvae (Marković et al., 1996a,b).

This study was undertaken to evaluate toxic and antifeedant activities of essential oil obtained from *O. basilicum* and linalool (both isomer forms) against the second instar larvae of *L. dispar*.

2. Methods

2.1. Plant material and isolation of essential oil

Fresh leaves of *O. basilicum* collected during the blooming period from the location Pančevo (Serbia) were air-dried at room temperature (22–25 °C) for 7 days. The essential oil from dried leaves was obtained using a Clevenger-type apparatus (European Directorate for the quality of Medicines, 2002), and transferred into a dark glass flask filled to the top and kept at temperature of 4 °C until used.

2.2. Chemical characterization of essential oil

The composition of the examined essential oil was determined by gas chromatography (GC) and mass spectra (MS) analyses, as described in Block et al. (2005). GC analyses were performed using HP-5890 Series II gas chromatograph, with split/splitless injector, fused silica capillary column (25 m × 0.32 mm) coated with non-polar stationary phase HP-1 (cross-linked methylsilicone, 0.5 μm film thickness) and flame ionization detector (FID). GC/MS analyses were done on a Hewlett–Packard 5890 gas chromatograph directly coupled to a Hewlett–Packard HP 5971 A (70 eV) mass selective detector. Component identification was carried out by comparing the obtained MS data with those reported in Library Wiley on MS-ChemStation HP v. B.00.01.

2.3. Preparation of test solutions

The essential oil of *O. basilicum* and its dominant component linalool in both isomer forms, D-linalool and (+/−) linalool, dissolved in technical ethanol (96%) were used. Each crude solution was serially diluted with 96% ethanol to prepare test solutions of 0.05%, 0.10% and 0.50%. D-linalool used in analysis was extracted from the essential oil of coriander by the vacuum rectification, whereas (+/−) linalool was commercial preparation (Fluka, Milan, Italy).

2.4. Botanical insecticide standard

'Bioneem' (0.09% azadirachtin, Safer) was used as botanical standard control (BS) in all experiments. The commercial preparation was diluted with 96% ethanol to prepare test solution of 0.50%.

2.5. Gypsy moth culture

Eggs of gypsy moth were collected on a locality at National Park 'Djerdap' (Eastern Serbia) in the oak forest during the autumn period. Egg masses were maintained at 4 °C till the next year spring. Prior to starting the bioassays, eggs were mechanically cleaned from hairs and disinfected (dipped in 0.1% Na-hypochloride for 5 min); than washed with distilled water for 10 min and air dried. Selected eggs from 25 egg masses were intermixed and put into flasks for hatching (at 25 °C). Newly hatched larvae were selected and maintained together in Petri dishes ($R = 10$ cm) until they reached the second larval stage. Caterpillars were daily nourished with fresh leaves of cherry plum. They were maintained, and all experiments were carried out, in a microclimate chamber, at 25 ± 1 °C, 65 ± 5% RH and neon diffuse light with 30159.29 candelas with 16:8 h L/D.

2.6. Residual toxicity test

All tested solutions were deposited onto the bottom of Petri dishes ($R = 9$ cm) in the quantity of 0.3 ml, dried about 20 min at 21 °C and than 10 larvae per repetition were introduced. Experiments with BS (0.50%) and ethanol (96%) were performed under the same conditions. Dead larvae were removed after 24 and 48 h. The treatments were replicated six times (total, $n = 60$). Percentage insect mortalities were calculated using the corrected Abbott's formula (Abbott, 1925): $\text{Corrected}\% = \left(1 - \frac{T}{C}\right) \times 100$, where T and C were the number of living larvae at treatment and control, respectively, after the observation time. Efficiency of treatments were obtained using the formula: $E = 100 - \left(\frac{\text{St}-T}{\text{St}} \times 100\right)2$, where St and T were larval mortalities at BS and treatment, respectively.