Industrial Crops and Products 34 (2011) 888–892

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Insecticidal and repellent activity of selected essential oils against of the pollen beetle, *Meligethes aeneus* (Fabricius) adults

Roman Pavela

Crop Research Institute, Drnovská 507, Praha 6 – Ruzyne 161 06, Czech Republic

ARTICLE INFO

Article history:
Received 26 July 2010
Received in revised form 1 February 2011
Accepted 17 February 2011
Available online 17 March 2011

Keywords:
Meligethes aeneus
Essential oils
Botanical insecticides
Lethal doses
Thymus vulgaris
Carum carvi

ABSTRACT

The essential oils from 9 aromatic plants were tested on repellency and mortality of *Meligethes aeneus* adults. All the tested essential oils caused high mortality of *M. aeneus* adults in the tarsal tests. The lethal doses after 6 h exposure were ranged between 197 and 1508 μg cm^{-2}. Essential oils obtained from *Carum carvi* and *Thymus vulgaris* were most efficient where LD_{50} was estimated as 197 and 250 μg cm^{-2}, respectively.

Repellency declined in all the essential oils as a function of time. The longest persistence time was determined for essences obtained from *C. carvi* and *T. vulgaris* where significantly the highest repellent index of 65.6% and 63.8%, respectively, was determined. Repellent index lower than 15% was determined for the remaining essential oils.

1. Introduction

The pollen beetle, *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae), is the most important pest of oilseed rape, *Brassica napus* L., crops throughout Europe (Ekbom, 1995).

Adult beetles of *M. aeneus* may damage any of the flowering structures during the green to yellow bud stages. Feeding by the pollen beetles reduces the number of buds that are able to develop into pods, and the damage to the ovary results in podless stalks (Williams and Free, 1978; Evans and Scarisbrick, 1994).

The adults can cause serious yield losses in both winter and spring oilseed rape crops, and for spring oilseed rape, more than 80% yield reduction can occur. Consequently, in most years, spring oilseed rape crops are treated with insecticides (Bichel, 1999; Hansen, 1996).

Annual application of synthetic insecticides based on several active substances whose structures exhibit only few differences causes development of resistant pest populations (Beeman and Schmidt, 1982; Evans and Scarisbrick, 1994). Pest populations resistant against one or more active substances in insecticides pose one of the present key problems to conventional agriculture (Helander and Delin, 2004). This is also one of the main reasons why new, environmentally friendly chemicals applicable in the production of new insecticides have been sought.

Moreover, oilseed rape, one of the most important present commodities in the EU (which occupies 1/3–1/2 of crop fields in conventional systems), could find its application also in the systems of organic farming. Winter oilseed rape is particularly useful for in crop rotations and animal fodder, but organic farmers hesitate to grow it because it is attacked by numerous insects, which are difficult to control without chemical treatments (Alford et al., 2003; Valantin-Morisona et al., 2007). No extensive study has investigated the effects of crop management on winter oilseed rape in organic systems, accounting for the current lack of pesticide free crop protection strategies for this crop (Valantin-Morisona et al., 2007).

Botanical insecticides use a mixture of biologically active substances or secondary plant metabolites of defensive nature that provide insecticidal effects. Botanical insecticides have been finding increasing popularity both in integrated and in ecological pest management. This is due to their special nature. Firstly, these products are considered as safe for the health and for the environment; secondly, they usually contain a mixture of several dozens of active substances, and thus do not cause pest resistance (Isman, 2000; Pavela, 2007). Moreover, for organic farmers they represent the only possible way of protecting plants. Nevertheless, considering the limited sales volume and high interest in these products, ever new substances must be sought, which could be used in plant protection.

Essential oils are one of the highly perspective substances available, besides others, also in plant protection. Aromatic hydrocarbon mixtures are obtained from aromatic plants, most often by means

E-mail address: pavela@vurv.cz

0926-6690/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2011.02.014

of water distillation or supercritical fluid extraction (Isman, 2000; Pavela et al., 2009a).

Essential oils exhibit significant insecticidal, bactericidal and fungicidal effects (Pavela, 2005, 2006, 2009; Pavela et al., 2009a,b; Zabka et al., 2009; Nerio et al., 2010). They find their application as repellents very often, although this effect is relatively short-lived (Isman, 2000; Nerio et al., 2010).

This paper presents our test of the hypothesis of use of essential oils in oilseed rape protection against *M. aeneus* adults. Considering that adults cause damage predominantly by eating out buds, there are two possible ways how to effectively prevent their damage: (1) reduce the number of adults per area using an insecticidal effect and/or (2) use the antifeedant and repellent effect of essential oils to prevent bud damage due to feeding. As the adults prefer flowers in bloom where they cause no more damage, it would be sufficient to delay the feeding time of the adults by 5–10 days so that a sufficient number of flowers can burst into blossom, thus the buds would be protected against damage (Sedivy and Kocourek, 1994).

Essential oils are known as highly efficient substances that cause acute toxicity (Hummelbrunner and Isman, 2001; Pavela, 2005, 2006, 2009; Pavela et al., 2009a), reduce fertility and modify behaviour (Pavela, 2008; Pavela et al., 2009b), and that also cause repellency and antifeedancy (Koul, 2005, 2008; Nerio et al., 2010) in many insect species. Nevertheless, information on their effects on *M. aeneus* adults is insufficient.

This paper presents the results of the first screening of the insecticide and repellent effect of essential oils from 9 plant species on *M. aeneus* adults.

2. Materials and methods

2.1. Insects

Post-diapause adult pollen beetles *M. aeneus* (Fabricius) (Coleoptera: Nitidulidae) were collected from a winter oilseed rape crop between May and July. In the laboratory, the sex of each captured beetle was determined. Beetles were placed individually on their dorsal side on a microscope slide, and gentle pressure was exerted on a glass coverslip placed over the beetle. This action exerted the ovipositor tip of females or the tegmen of males, which became visible under the low magnification (50×) of a binocular microscope. A similar procedure was described by Ruther and Thiemann (1997). Single sex groups of beetles were maintained until required for experiments on flowering racemes of glasshouse grown spring oilseed rape, caged within ventilated, transparent plastic boxes lined with filter paper moistened with distilled water. Beetles were starved for 24 h before experiments to increase their motivation to search for food.

2.2. Essential oil extraction and analysis

Plant material from 9 plant species (Table 1) was obtained from our own collection cultures. Individual parts of the plants were dried at 40 °C. The dried samples were subjected to hydrodistillation for 2 h using a Clevenger-type apparatus.

The oil obtained was separated from water and dried over anhydrous Na_2SO_4. The identification of the major chemical components of the oil samples was done in a complete HP 6890 gas chromatograph using a mass selective detector HP 5973, equipped with Chemstation software and Wiley 275 spectra data. A HP-Innowax fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used.

The chromatographic conditions were: column temperature 60 °C (8 min), 60–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min), interface 180 °C, split ratio 1:100, carrier gas, He (55.4 kPa), flow rate 1.0 ml/min, ionization energy 70 eV, mass range 40–350, volume injected 0.5 μl, solvent cut, 3.5 min.

GC analysis was performed on a HP 5973 gas chromatograph with FID detector using a HP-Innowax fused silica capillary column (30 m × 0.25 mm, 0.50 μm film thickness). The chromatographic conditions were: column temperature 40 °C (8 min), 40–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min), injector temperature 250 °C, split ratio 1:50, detector temperature 250 °C, carrier gas hydrogen (34 kPa), flow rate 1.0 ml/min, volume injected 0.2 μl.

2.3. Experimental

2.3.1. Acute toxicity

The tarsal test was chosen for determining the insecticidal effect of essential oils on mortality of adults; this test mostly corresponds to practical utilization of the contact of the beetles in treated vegetation.

Considering the size of the beetles and their high mobility, special boxes were made of filter paper, sized 15 cm × 10 cm and 3 cm high (to ensure tarsal contact and to prevent the fumigation effect of the essences). Acetone solution (dosage 10 μl cm^{-2}) was applied on the inside surface of the containers, in which an appropriate dose of the essential oil was dissolved, corresponding to successive dilution values (5000, 4000, 3000, 2000, 1000, 500, 250, 125 and 50 μg cm^{-2}). The control was treated with pure acetone. The boxes stood still at 25 °C for 15 min after application to let the solvent evaporate. Afterwards, always 20 adults of *M. aeneus* were introduced in each box and the boxes were closed and placed in a ventilated room at 25 °C and RH 75% for 3 h. After the exposure time, the adults were placed in clean plastic containers (6 cm × 5 cm × 6 cm). Ambient conditions were identical to the former ones. Oilseed rape flowers were presented as food. The experiment was repeated 4 times.

Mortality was evaluated 6 and 24 h from the beginning of exposure. Doses causing 50% (ED_{50}) and 90% (ED_{90}) mortality, including corresponding values within a 95% confidence limit (CI_{95}), were estimated using probit analysis applied to the found FDI values (Finney, 1971). Before undertaking the analysis, the percentages were transformed by arsin $\sqrt{(x/100)}$.

2.3.2. Repellent effect

Acetone 2 ml containing always 20 μl of dissolved essential oils was applied using an electronic atomizer on flowering sprouts of oilseed rape 15 ± 3 cm high with 10 yellow buds. Control plants were treated with acetone only. Upon treatment, the sprouts were stuck into decorator material soaked with water so that the sprouts would not wither. Afterwards, they were inserted in growing cages made of a wired structure sized 20 cm × 30 cm × 30 cm, coated with transparent fabric. Always 20 adults were then introduced in every cage. Numbers of adults sitting on the treated sprouts were determined 1, 6, 24 and 48 h from introduction. The experiment was located in a room at 24 ± 1 °C, RH 75 ± 5% and 16L:8D. The whole experiment was repeated 6 times.

Repellent index was calculated as

$$RI = \left(\frac{C - T}{C + T}\right) \times 100,$$

where C is the number of adults on control plants and T is the number of adults on treated plants.

The determined data was subjected to ANOVA statistical analysis and followed by least significant difference (LSD) test ($P < 0.05$).

Lethal concentrations causing 50 and 90% repellency 48 h from application were determined for those essential oils which exhibited more than 50% RI 48 h after application, using the method of successive dilution and the identical procedure.