Evaluation of potential insecticidal activity of essential oil and entomopathogenic fungus against *Diabrotica virgifera virgifera* LeConte

Teodora Toshova1, Dimitar Velchev2, Marek Bartu3, Danail Takov4, Ivoiylo Todorov5, Daniela Pilarska5, Miklos Toth6, Shrinko Berker7, Milena Nikolova8

1 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria
2 Institute for Forest Research, Agricultural Academy, 5355 Kestlya, Bulgaria
3 Institute of Forest Ecology, Slovak Academy of Sciences, 24, 949 01 Nitra, Slovakia
4 Plant Protection Institute, Centre for Agricultural Research, Herman O. u. 15, H-1022 Budapest, Hungary
5 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 23 Acad. Georgi Bonchev Street, 1113, Sofia, Bulgaria
6 e-mail: teodora_toshova@yahoo.com

Abstract

The Western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) (*VRC*), is an important pest of maize (*Zea mays*) and an invasive species in Europe. In this study, the insecticidal activity of *Oregano vulnerub* subsp. *Arnica essential oil (Dimiento of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (Greek-oregano EO) and entomopathogenic fungus *Metarhizium anisopliae* (a strain from the Institute of Forest Ecology, Slovak Academy of Sciences) against *WCR* adults was evaluated in laboratory bioassays. In August 2021, the test insects were attracted to *CALLODORIS* pheromone and floral lures in a maize field in Kestlya (Bulgaria), and captured by hand-collaring or by means of an aspirator. The test substance was applied on a filter paper disc on the bottom of Petri dish, and groups of *D. v. virgifera* adults were introduced into the Petri dishes. Each bioassay was repeated three times and the number of dead beetles was counted in both test and control variants for a period of ten days. In a preliminary study, the *OE* concentration range was *0*–*1% Tween 80* varied with the concentration from 4% to 10%. The mortality caused by the EO dissolved in distilled water with 0.01% Tween 80 varied with the concentration from 4% to 10%. The mortality caused by the EO dissolved in distilled water with 0.01% Tween 80 varied with the concentration from 4% to 10%. The mortality caused by the EO dissolved in distilled water with 0.01% Tween 80 varied with the concentration from 4% to 10%.

**Biocides with entomopathogenic fungus (EPF) Metarhizium anisopliae**

- The strain of *M. anisopliae* was from the fungal collection of the Institute of Forest Ecology, Slovak Academy of Sciences.
- Aerial-dispersion of *M. anisopliae* was prepared in distilled water with 0.01% Tween 80 in follows: 2 × 107, 2 × 108, 2 × 109, 2 × 1010 and 2 × 1011 conidia/ml.
- A filter paper impregnated method was used to examine the insecticidal activity of the EPF and the controls contained 0.01% Tween 80 in distilled water. One milliliter of each suspension/solution was applied into a Petri dish and test insects were introduced for observations.

**Conclusions**: The essential oil from *O. vulnerub* subsp. *Arnica* possesses strong insecticidal activity against *D. v. virgifera* adults while the mortality rates caused by the entomopathogenic fungus *M. anisopliae* was moderate. Pure *EO* evoked highest insecticidal effect reaching 98 – 100% in ten days while the mortality rates caused by the ED dissolved in distilled water with 0.01% Tween 80 varied from the concentration from 4% to 10%. The mortality caused by the *EO* dissolved in distilled water with 0.01% Tween 80 varied with the concentration from 4% to 10%. Our results are a base for possible utilization of *Greek oregano* ED as a botanical insecticide to be used in IPM programmes for WCR control.

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1 Insects for laboratory bioassays. *Diabrotica v. virgifera* adults were collected from a maize field [1 ha] belonging to the Main Research Institute, Kestlya, Bulgaria (47°20'05.20" N; 24°72'32.57" E) at the beginning of August, 2021. Phoretal and floral lures (products of Callodis). Plant Protection Institute, C.A.R, Budapest, Hungary, "www.callodis.com") were used to attract males and females beetles in the hours between 9 am – 12 am. Adults were captured by hand-collaring or by means of insect aspirator and kept in plastic boxes with ventilation.

2 Bioassays with *O. vulnerub* subsp. *hirtum* essential oil.

- O. vulnerub subsp. *hirtum* essential oil (Greek-oregano EO) was a product from the collection of the Institute of Biodiversity and Ecosystem Research. It contains carvone, β-pinene, α-phellandrene and β-terpinene as main components.
- In a preliminary test, different amounts of the pure essential oil [0.5 and 1%] were applied individually at the center of a piece of filter paper (10 mm diameter) placed at the bottom of a Petri dish. Insects in control groups were untreated.
- In a subsequent bioassay, a serial dilution of *EO* was prepared in distilled water with 0.01% Tween 80 as follows: 0.01, 0.1, 0.3, 1%, and 10%. One milliLitre of each concentration was applied separately on the white filter paper disc covering the bottom of a Petri dish. Adults in control variants were treated with 1 ml of distilled water with 0.01% Tween 80.
- The experiments were conducted at the Main Research Institute, Kestlya (Bulgaria) under controlled conditions - temperature of 24 ±1°C, 65-70% relative humidity, and normal photoperiod.
- All experiments were repeated three times with the average of three groups in each variant of 20 insects (n = 20).
- Insect mortality was recorded at 24 h intervals during 10 days after treatment. The mortality data were corrected using *Schumacher-Teel's formula*, where:

\[ \frac{L(T) - L(0)}{1 - L(0)} = \frac{1}{1 - \exp(-rT)} \]

- Probit analysis was used to estimate the lethal concentrations (LC50 and LC95) and the median lethal time (LT50) (time necessary to kill 50% of the tested individuals) and the 95% confidence intervals of these features.

3 Bioassays with *O. vulnerub* subsp. *hirtum* essential oil and *Metarhizium anisopliae*.

- The strain of *M. anisopliae* was from the fungal collection of the Institute of Forest Ecology, Slovak Academy of Sciences.
- Aerial-dispersion of *M. anisopliae* was prepared in distilled water with 0.01% Tween 80 in follows: 2 × 107, 2 × 108, 2 × 109, 2 × 1010 and 2 × 1011 conidia/ml.
- A filter paper impregnated method was used to examine the insecticidal activity of the EPF and the controls contained 0.01% Tween 80 in distilled water. One milliliter of each suspension/solution was applied into a Petri dish and test insects were introduced for observations.