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IN VITRO SCREENING FOR INSECTICIDAL ACTIVITY OF NATURAL PLANT PROTECTION PRODUCTS AGAINST *TROPINOTA (EPICOMETIS) HIRTA* (PODA) (COLEOPTERA: CETONIIDAE)

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Abstract

The insecticidal activity of newly created naturally friendly plant protection products based on plant essential oils emulsified with plant triglyceride oil-based soap were tested against adult individuals of *Tropinota (Epicometis) hirta* (Poda) under *in vitro* conditions.

Four different plant protection products (ready to be used) on the base of lavender, tobacco, coriander and fennel oil were tested.

The products were created at the University of Food Technologies - Plovdiv, Bulgaria. The trials revealed strong insecticidal action of the coriander and lavender oil-based products. Surprisingly, the formulation based on tobacco oil had no effect on *T. hirta* and neither did the fennel oil-based product.

The products were tested for phytotoxicity on two cultivars (*Golden Delicious* and *Jonagold*) of five-year-old apple trees using *in vitro* insecticidal trial concentrations according to standard № 227 of the OECD. There were no phytotoxic effects of the concentrations used.

Keywords: *Tropinota (Epicometis) hirta* (Poda), essential oils, lavender oil, tobacco oil, coriander oil, fennel oil, soaps, natural pesticides.

INTRODUCTION

Tropinota (Epicometis) hirta (Poda), sometimes called the apple blossom beetle or hairy beetle, is an important pest attacking fruit trees and ornamental plants in the Europe and the Mediterranean region (Aydin, 2011).

The adults feed on the reproductive parts of the flowers, including the pistil, stamens, and pollen. During non-blossoming periods they subsist on leaves. The larvae live in the soil, feeding on decomposing organic matter.

The adults can change their color (i.e. host) preferences during the season, and can thus locate and exploit host plants that blossom later in the year. The geographical distribution area of the species is Europe and the Mediterranean region (Aydin, 2011).

Tropinota hirta has a wide host range, attacking many species of Rosaceae, e.g. apple (*Malus communis*), pear (*Pyrus communis*), quince (*Cydonia oblonga*), plum (*Prunus domestica*), apricot (*Prunus armeniaca*), cherry (*Prunus avium*) (Walker et al., 2016). It also capable of attacking plants from other families, e.g. Cruciferae, including canola (*Brassica napus*), mustard (*Sinapis alba*), cabbage (*Brassica oleracea*) and cauliflower (*Brassica oleracea botrytis*); the Gramineae including wheat (*Triticum aestivum*) and rye (*Secalecereale*), and also some Asteraceae such as dandelion (*Taraxacum officinale*) and coltsfoot (*Tussilago windbag*) (Walker et al., 2016).

In Bulgaria, the species is widespread throughout the country and has been recorded feeding on 37 host plants belonging to seven families (Popova, 1962).

Control of *T. hirta* is difficult and risks harming beneficial insects, because the pest attacks during flowering, when pollinators are at their most active.

As a result, selective insecticides or alternative biological methods have to be used (Schmera et al., 2004). There is thus the need for the development of new and ecofriendly control methods to reduce the damage caused by *T. hirta* while preserving the pollinators and other beneficial insects.

Water and alcohol soluble plant extracts have long been used in traditional ethnic medicine, or as homemade plant protection remedies (Isman, 2000). Plant essential oils are one of the major groups that have been the focus of commercial development as natural insecticides.

Some essential oils have used against many pests, particularly in greenhouses and against household pests and veterinary insects and diseases. Botanical pesticides including essentials oils are characterized by low mammalian toxicity, reduced effects on non-target organisms and short persistence in the environment making them potentially preferable for incorporation in integrated pest management programs (Isman, 2006).

The increasing popularity of organic agriculture has increased the demand for pesticides derived from natural raw-materials, and in particular, the use of plant extracts has increased (Seiber et al., 2014). There is, however, as yet, no satisfactory product available for use against *T. hirta* as insecticides (Al-Alawi, 2014) although plant-derived products are used as attractants (Vuts et al., 2010).

The current study evaluated the insecticidal activity of four essential plant oils for their toxicity against the hairy beetle *T. hirta.*

MATERIALS AND METHODS

The experiments were carried out in 2015 in the laboratory of Phytopharmacy and Ecotoxicology, at the Agricultural University of Plovdiv. The insecticidal activity of created natural soaps prepared from plant oils (sunflower and olive) against *T. hirta* were evaluated *in vitro*.

Adults of *T. hirta* were collected from apple orchards in the Experimental Filed Station of Agricultural University, Plovdiv. The beetles were kept in glass jars covered with fine mesh for aeration until used in the bioassay. Essential oils were extracted from tobacco, coriander, fennel, and lavender.

The experimental essential oil products were created at the University of Food Technologies - Plovdiv, Bulgaria by extraction of the active substance (oil) via a steam atmospheric hydrodistillation unit with volume 30 l. The only reagent used for essential oil production was water.

The production procedure includes loading of raw material in perforated bottom basket, contact of raw material with superheated water, steam and evaporation of volatile compounds, condensing of water, steam and volatile compounds by means of cooling, decanting of oil (volatile compounds) from distillate by means of density differences, removing of decanted oil, dewatering of oil by potassium sulphate, filtering of oil.

After that, the active substance (essential oil) was emulsified with soap prepared from coconut oil, potassium hydroxide and water by the standard warm process (Schumann and Siekmann, 2013).

Beetles were placed in 12 cm high glass jars (10 cm diameter base). Each treatment was replicated three times; each replicate consisted of ten *T. hirta* per jar. Each replicate was treated with tested concentrations of products, and the control was treated with sterile distilled water. To allow comparison with conventional insecticides a further treatment used Karate Zeon® (active ingredient Lambda-cyhalothrin).

The number of surviving individuals was recorded after 24 hours. Efficacy was estimated according to Henderson and Tilton formula (1955). Ten different concentrations ranging from were tested to determine LC_{05} (NOEL), LC_{25} (LOAEL) LC_{50} and LC_{90}.

The data were statistically analyzed with R language for statistical computing (R Development Core Team, 2011) and drc R language package (Ritz and Streibig, 2005).

Also, the products were tested for phytotoxicity using two apple cultivars ("Golden Delicious" and "Jonagold"). Five-year-old apple trees were used and phytotoxicity tests conducted according to the standard №227 of OECD (OECD Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test, Adopted: 19 July 2006).

RESULTS AND DISCUSSION

Surprisingly, the products based on tobacco and fennel essential oils showed no insecticidal activity against *T. hirta*. The lack of effectiveness was also observed towards the soap used as the emulsifying agent in the preparations. The other tested products based of coriander essential oil and lavender essential oil were effective against *T. hirta* (Figure 1, 2).

The Dose – Response Modeling revealed relatively low effective concentrations (LC_{90}) of tested products (except the products based on tobacco and fennel essential oils) towards *T. hirta*. This is especially effective for plant protection based on plant-derived oils. AICs of the Dose – Response Models of coriander oil product and lavender oil product, were relatively low which proved the goodness of fit of used statistical models.

The figure below show the Dose - Response Modeling for the effectiveness of the coriander oil product SLGEL1521 towards *T. hirta* (Fig. 1).

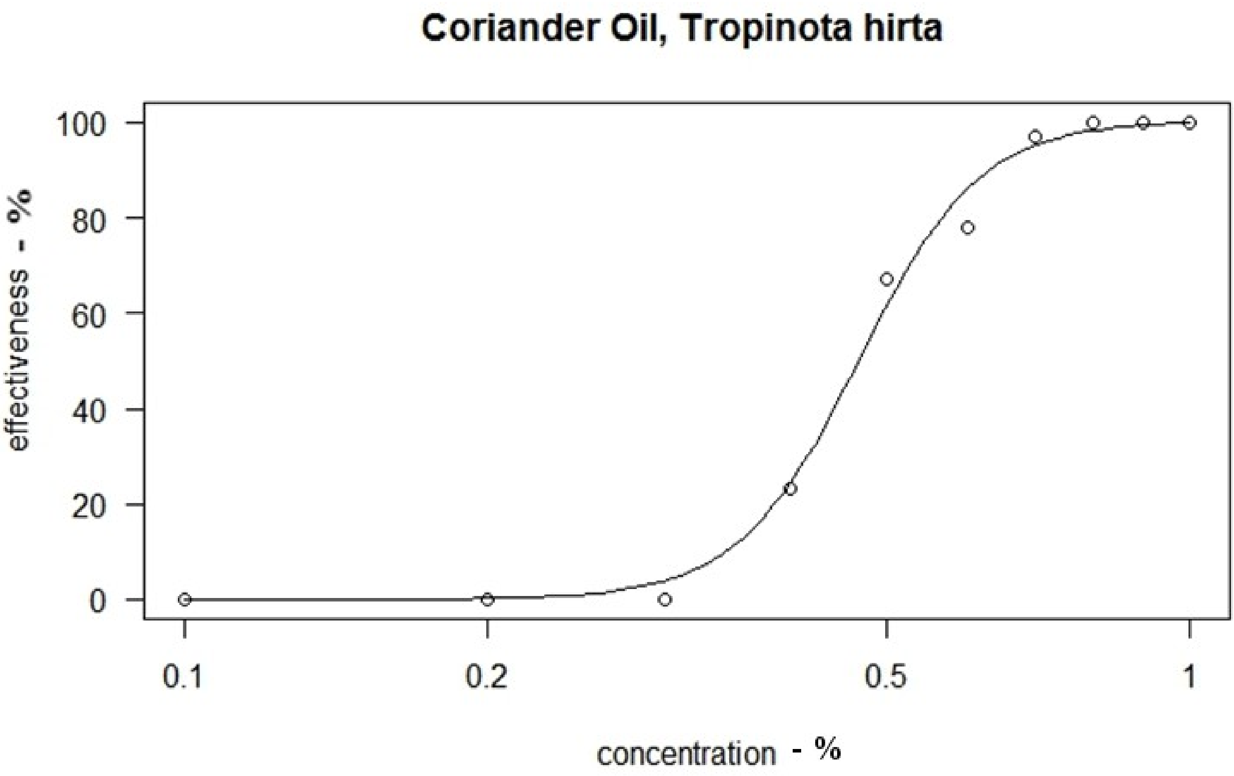

Fig. 1. *Dose - Response Curve coriander oil preparation – Tropinota hirta*

The toxicological data are:
NOEL (LC_{05}) = 0.3 % (m/v)
LOAEL (LC_{25}) = 0.4 % (m/v)
LC_{50} = 0.46 % (m/v)
LC_{90} = 0.63 (m/v)
AIC of the model = 42.8

The figure below shows the Dose-Response Curve of the lavender oil preparation towards *T. hirta* (Fig. 2).

The toxicological data are:
NOEL (LC_{05}) = 0.17 % (m/v)
LOAEL (LC_{25}) = 0.27 % (m/v)
LC_{50} = 0.34 % (m/v)
LC_{90} = 0.57 (m/v)
AIC of the model = 55.6

The test for acute phytotoxicity with two cultivars ("Golden Delicious" and "Jonagold") of five-year-old apple trees show that all tested products possess no acute phytotoxic activity towards tested plants.

During the trials, there were absolutely no phytotoxic manifestations on the treated plants.

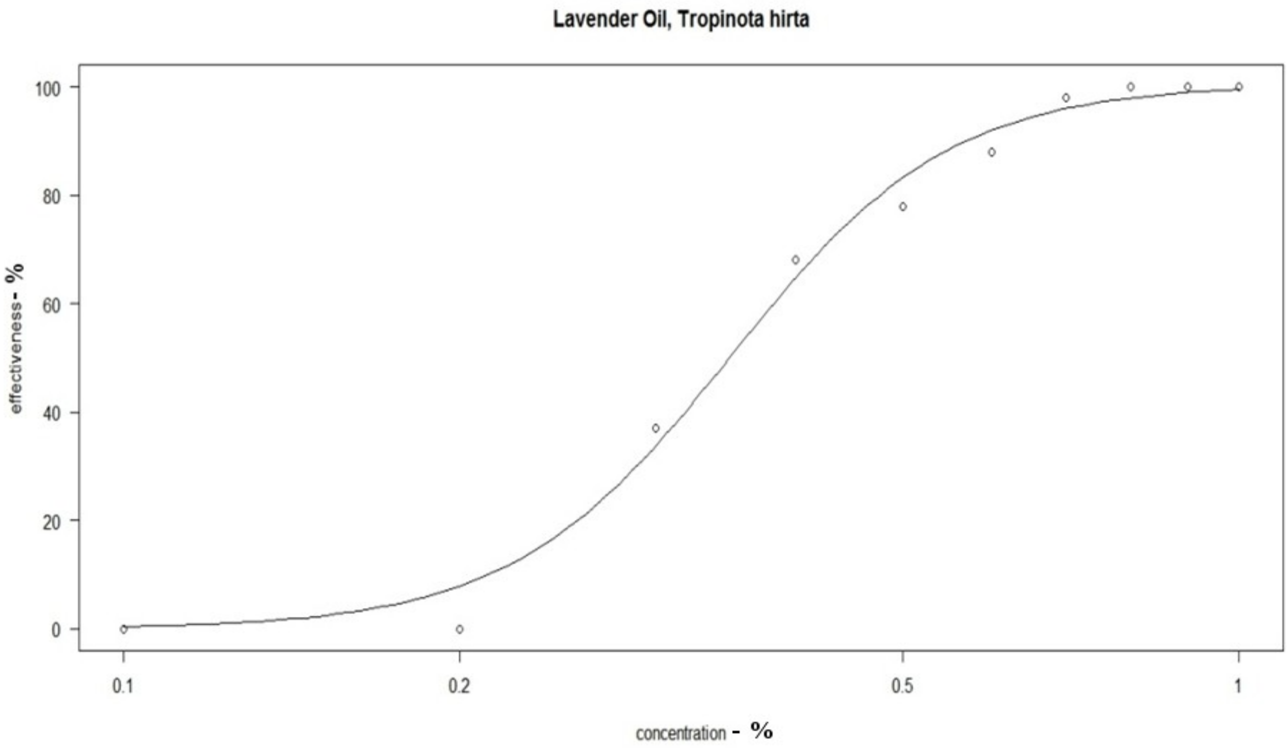

Fig. 2. *Dose- Response Curve lavender oil preparation – Tropinota hirta*

CONCLUSIONS

1. The trial results showed that two of the tested products, coriander oil and lavender oil, had strong insecticidal activity against *T. hirta* in concentrations only a little bit higher that most of the commercial plant protection products. Both these products were effective at approximately the same concentrations (LC_{90} = 0.63 (m/v) for coriander oil and LC_{90} = 0.57 (m/v) for lavender oil.

2. These *in vitro* trials reveal the promising insecticidal action of plant essential oil from coriander and lavender against *T. hirta* and can be the basis for recommending the future introduction of this type of product as an alternative to conventional insecticides. The additional positive was that products based on coriander and lavender essential oils do not cause any acute phytotoxic effects.

3. In the future, phytotoxic trials for chronic phytotoxicity will be conducted to be sure that the evaluated products are completely safe.

4. Evaluation of insecticidal activity is one of the primary steps in discovery and development of botanical insecticides. Developing botanical insecticides that are effective against the pest but safe for humans, pollinators and other useful organisms are crucial for sustainable and integrated pest management and organic plant protection practices.

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