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BIOINSECTICIDAL EFFECT OF *ORIGANUM VULGARE* SUBSP. *HIRTUM* (LINK) ESSENTIAL OIL VAPOURS ON *MYZUS PERSICAE* SULZER IN TOBACCO AND *APIS MELLIFERA* L.

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Abstract

The peach leaf aphid *Myzus persicae* Sulzer is a polyphagous species which causes economic damages to many agricultural crops in Bulgaria and worldwide. The aim of the study was to test the bioinsecticidal effect of the essential oil (EO) vapours of *Origanum vulgare* subsp. *hirtum* (Link) on *M. persicae* in tobacco and *Apis mellifera* L., as nontarget organism. The determination of the main EO contents was carried out on gas chromatograph. The chemical composition of the *O. vulgare* subsp. *hirtum* EO includes the terpenoid carvacrol – 67.4%, ρ-cymene – 13.7%, cineole + γ-terpinene – 8.3%, and α-pinene + β-pinene – 4.5%. The study was conducted in laboratory conditions. The study established efficacy of the oregano EO vapours against the peach leaf aphid. The findings indicated 100% efficacy of the *O. vulgare* subsp. *hirtum* EO vapours against *M. persicae* at a dose of 2 µl/l^1 air and 3 µl/l^1 air; 63.6% efficacy at 1 µl/l^1 air and 31.2% at 0.5 µl/l^{-1} air. No phytotoxicity of the tobacco plants was found at any of the tested doses. The minimum dose with the highest efficacy tested on honey bees was also toxic to them.

Key words: aromatic plants, essential oil, toxicity, aphids, honey bees

Introduction. Pest control of agricultural crops is of great importance to reduce losses and increase production yields. Changing climate conditions on a global scale can lead to complications in the fight against pests. Temperature

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is the most important meteorological factor affecting insect populations, and has great influence on agricultural pests [1]. Therefore, pest populations will respond to changing temperatures, and pest control will need alternatives. Globally today chemical pesticides are widely used for pest control. Their permanent use has led to resistance in many insect species and can affect nontarget organisms [2]. Use of pesticides can impact the environment [3].

In recent years, alternatives of chemical pesticides have been sought. Plants can be used as a source of bioactive raw materials. The chrysanthemum extract has insecticidal activities against pests [4]. Biopesticides have advantages over synthetic pesticides i.e. rapid biodegradation and low toxicity to nontarget organisms. Plant species can produce volatile compounds as essential oils (EOs) that are released to the environment. EOs contain a mixture of monoterpenes, phenols, and sesquiterpenes [5]. Monoterpenes form the richest group with biocidal activities [6].

EOs contain bioactive compounds with effective bioinsecticidal activity against different agricultural pests [7]. The most frequently used commercial EOs include eugenol, anise, pine, thyme, and eucalyptus oils [8]. It was found that *Origanum vulgare* L. EO indicated biocidal activities [9]. In laboratory conditions RADEV [10] used *Salvia officinalis* L. EO against *Myzus persicae* Sulzer (Hemiptera; *Aphididae*) in potatoes and also studied its effect on *Apis melliferra* L. (Hymenoptera; *Apidae*). *M. persicae* is a polyphagus that infests a large number of plant species and can destroy the quality of important agricultural, horticultural and greenhouse crops [11].

The aim of the present study was to test the bioinsecticidal effect of *Origanum vulgare* subsp. *hirtum* (Link) EO vapours on *Myzus persicae* Sulzer in tobacco and *Apis mellifera* L.

Materials and methods. This study was conducted in laboratory conditions at the Tobacco and tobacco products institute, Markovo (Bulgaria). The EO of *O. vulgare* subsp. *hirtum* (Link) was produced at the Institute for roses and aromatic plants, Kazanlak (Bulgaria).

The *O. vulgare* subsp. *hirtum* plants were grown in the territory of the Institute for roses and aromatic plants, Kazanlak. The EO was obtained by water-steam distillation in a Clevenger-type apparatus for 2 hours. The determination of the main EO content was carried out on a PYE UNICAM gas chromatograph with flame ionization detector (FID) under the following operating conditions: capillary column – EKONO – CAP™EC™–1, length 30 m; internal diameter 0.32 mm; oven temperature: temperature programming from 60 °C to 200 °C at a rate of rise of 8 °C/min; injector temperature: 300 °C; detector temperature: 300 °C; carrier gas: hydrogen; injected volume: 0.05 µl; carrier gas speed: 1.2 ml/min. For accurate results, when determining the peaks, pure substances were used.

The effectiveness of *O. vulgare* subsp. *hirtum* EO vapours against *M. persicae* on tobacco was studied under laboratory conditions applying methodology analogous to [12]. Tobacco plants were infested with aphids. After 3 days, the plants with a noted number of aphids were placed in 10 l air-proof containers. Doses of 0.5, 1, 2 and 3 µl/l^{-1} air and one control group (treated only with distilled water) were studied in three replicates for each dose. After 24 h, the effectiveness was calculated using the method of ABBOTT [13]: $E(\%) = (T - t)100/T$, where T is the number living aphids in control; t is the number living aphids after the treatment. Aphids which did not respond when touched were counted as dead.

The lowest dose with maximum effectiveness was tested on 3-day-old newly hatched *A. mellifera* honey bees fed on 50% sugar syrup in wooden cages $10 \times 10 \times 10$ cm. Three repeats and a control group (untreated) were used for the test. The honey bees were treated with EO for 24 h under the same conditions as the aphids. Each repeat contained twenty bees.

The data was statistically processed by using one-way ANOVA in Excel at $P \leq 0.05$.

Results and discussion. The chemical composition of *O. vulgare* subsp. *hirtum* EO was determined. In the EO 6 main components with retention times (t_R) were established: carvacrol with $t_R = 11.74$, ρ-cymene with $t_R = 6.21$, cineole + γ- terpinene with $t_R = 6.43$ and 6.97, α-pinene + β-pinene with $t_R = 5.35$. The major chemical component in the oregano EO is the terpenoid carvacrol (Table 1), as confirmed by MANCINI et al. [14].

Table 1

O. vulgare subsp. *hirtum* EO chemical composition (%)

Components	means ± std
Terpenoide	
carvacrol	67.4 ± 0.1
Terpenes	
ρ-cymene	13.7 ± 0.2
Cineole + γ-terpinene	8.3 ± 0.3
α-pinene+β-pinene	4.5 ± 0.2

The research results provided information that the EO aromas of *O. vulgare* subsp. *hirtum* showed bioinsecticidal effectiveness against *M. persicae*. The mortality of the peach aphids rose as the dose increased. Using the method of Abbott [13], the linear trendline results $y = 27.159x + 29.566$, $R^2 = 0.8263$ proved the 100% effectiveness against aphids at doses of 2 and 3 µl/l^{-1} air. At 0.5 µl/l^{-1} air the effectiveness was 31.2% and at 1 µl/l^{-1} air it was 63.6% (Fig. 1). No aphid mortality was reported in the control group. There were significant differences when comparing the results with different doses ($F > F_{\text{crit}}$, $P = 0.00$).

No phytotoxicity of the tobacco plants was detected in any of the tested

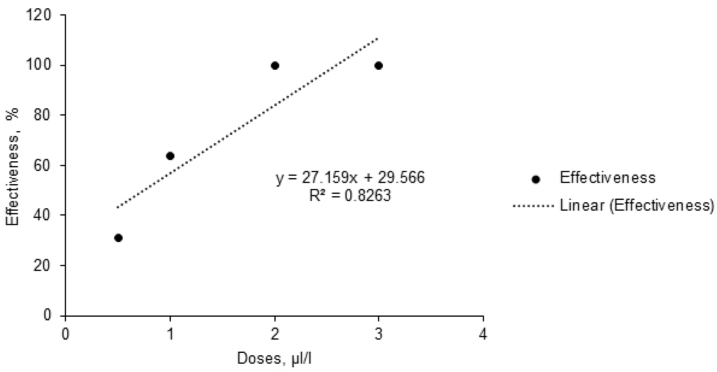

Fig. 1. Bioinsecticidal effectiveness of *O. vulgare* subsp. *hirtum* EO against *M. persicae* at different doses

doses, and it can be assumed that tobacco plants are resistant to *O. vulgare* subsp. *hirtum* EO vapours. Effectiveness results for the doses 0.5 µl/l^{-1} air and 1 µl/l^{-1} air showed lower efficacy compared to those of Digilio et al. [12]. In another research a full toxic effect on *M. persicae* was found by applying a solution with 3 µl/ml concentration of *O. vulgare* subsp. *hirtum* EO [15].

Present data confirmed the laboratory experiments with *Origanum vulgare* L. EO, having an insecticidal effectiveness against *Acyrthosiphon pisum* Harris [16] and *Aphis gossypii* Glover [17]. There are repellent and toxic effects of EO and pure compounds against aphids [18].

The minimum dose of *O. vulgare* subsp. *hirtum* EO aromas with highest effectiveness of 2 µl/l^{-1} air against *M. persicae* was applied for bioinsecticidal toxicity on honey bees *A. mellifera*. The results indicated mortality of 100% (means $\pm$ SD 100 ± 0.0) on the tested honey bees and no mortality in the control group. EOs are natural products but contain a high concentration of components. Also, the way in which EOs are applied is of significance [19].

The lifespan of honey bees depends on various factors, one of them being the influence of chemical substances used in agriculture. The search for and use of substances safe to honey bees is a major future task, because the extensive use of products for pest control in agricultural crops has resulted in the reduction or even disappearance of pollinators [20].

Conclusions. This research proved the bioinsecticidal effectiveness of *O. vulgare* subsp. *hirtum* (Link) essential oil vapours against *M. persicae*. Additionally, the essential oil vapours of the tested doses showed no phytotoxicity of the tobacco plants. It was also found that the minimum dose with the highest efficacy against aphids tested on *A. mellifera* was also toxic to honey bees.

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