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Insecticidal contact toxicity of several essential oils against stored product pests

Petr A. Iakovlev*

Department of Disinfection, All-Russian Plant Quarantine Center, 32 Pogranichnaya st. 140150, Bykovo, Moscow region Russian Federation
*Corresponding author: petro8710@gmail.com
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Abstract

Results of laboratory bioassays in Petri dishes on evaluation of contact toxicity of *Illicium verum*, *Artemisia absinthium* and *Abies sibirica* essential oils (EOs) against larvae of khapra beetle, *Trogoderma granarium* Ev., adults of grain weevil, *Sitophilus granarius* L., and rice weevil, *Sitophilus oryzae* L., and confused flour beetle, *Tribolium confusum* Duv., and larvae and adults of the lesser mealworm, *Tenebrio molitor* L., are presented. EOs commercial samples from retail pharmacy were tested at doses 0.01, 0.25, 0.50, 0.75 and 1.00 µl/cm^2. A treated Petri dish surface treted with acetone was used as a control. The experiment was carried out in triplicate. Mortality of insects was assessed after 1, 3, 6 and 24 hours post exposure. After exposure insects were placed into untreated Petri dishes for 3 days. The main components of the *A. absinthium* EOs are thujil alcohol (19.65%), phellandrene (16.71%), borneol (12.1%) and thujone (11.55%) was found. The major component of *I. verum* EOs was anethole (98.64%). Isobornyl acetate (57.25%), α-pinene (13.55%) and limonene (10.62%) were found as the main components of *A. sibirica* EOs. *S. oryzae* and *S. granarius* were most sensitive to each EO. *I. verum* EOs was the most effective and caused 100% mortality of each insect at the dose 0.25 µl/cm^2.

Keywords: essential oils, stored product pests, contact toxicity, *Illicium verum*, *Artemisia absinthium*, *Abies sibirica*

Introduction

Aluminium and magnesium phosphides formulations for phosphine fumigation and chemical protectants based on deltamethrin and pirimiphos-methyl for spraying are allowed for grain disinfection in Russian Federation. Regular and non-alternative application these formulations could cause development of resistance in major stored product pests (Nakakita & Winks, 1981; Mordkovich, 2003, Holloway et al., 2016) and also accumulation of pesticide residues in food commodity. Therefore, it is necessary to apply the same effective but more environmental friendly not chemical synthetic formulations for stored product protection. Essential oils (EOs) could be used as alternative formulation for management of stored product pests.

Materials and Methods

Test Insects

The insects used in bioassays were adults of *S. granaries*, *S. oryzae*, *T. confusum*, larvae and adults of *T. molitor* and larvae of *T. granarium*. *S. granaries* and *S. oryzae* were reared on *Triticum aestivum* whole grain in 25±1°C and 70±5% relative humidity (r.h.). *T. confusum* and *T. granarium* were reared on *T. aestivum* crushed grain in 27±1°C and 70±5% r.h. *T. molitor* was reared on *T. aestivum* crushed grain with *Zea mays* whole corn grain in 25±1°C and 70±5% r.h. The rearing conditions were darkness.

Essential oils and GC/MS spectrometry analysis

Commercial samples of *I. verum*, *A. absinthium* and *A. sibirica* EOs from retail pharmacy were tested. The obtained oils were subjected to GC/MS analysis using an Agilent 7890A gas chromatograph (Agilent Technologies, USA) equipped with a Agilent 5975C mass spectrometer, fitted with a DB17MS capillary column (30 m × 0.25 mm; 0.25 µm film thickness). Temperature was kept at 50 °C for 2 min and programmed to reach 200 °C (a rate of 10 °C/min), then to reach 260 °C (a rate of 20 °C/min) and held at this temperature for 4 min. Helium was used as the carrier gas at the rate of 1.18 mL/min. The samples were injected at the injector temperature of 240 °C.

Experimental procedure

A direct contact application assay (Qi and Burkholder, 1981; Broussalis et al., 1999) was used to evaluate the insecticidal activity of the tested essential oils. Five concentrations (640, $16{\cdot}10^3$, $32{\cdot}10^3$, $48{\cdot}10^3$ and $64{\cdot}10^3$ ppm) of each EO were prepared using acetone as a solvent. Aliquots of 1 ml of each concentration correspond to the respective 0.01, 0.25, 0.50, 0.75 and 1.00 µl/cm^2 were placed on the surface of Petri dish (9 cm diameter, ~ 64 cm^2). After evaporation of the solvent, 20 adults of *S. granaries*, *S. oryzae*, *T. confusum* and 20 larvae of *T. granarium* and 10 adults and larvae of *T. molitor* were separately introduced on the treated Petri dish's surface. The experiment was carried out in 25±1°C and 70±5% r.h. and a photoperiod of 10/14 L/D during the entire period. Control sets were made, where the same number of insects were placed in Petri dishes with surface treated with acetone only. The experiment was carried out in triplicate. The mortality was assessed after 1, 3, 6, and 24 hours (h) of exposure under a Zeiss stereomicroscope (Stemi-2000, Carl Zeiss Microscopy GmbH, Germany). Then, after exposure, insects were moved to untreated Petri dishes and were hold there during 3 days. The viability of insects was estimated every day during this period. The data were subject to a two-way ANOVA. A 5% probability level was used for individual pairwise comparisons by the made Tukey-Kramer's HSD test.

Results

Chemical composition of the test oils

Data of the chemical composition analysis (Table 1) revealed that the *A. absinthium* EO contains, mainly thujil alcohol (19.65%), phellandrene (16.71%), borneol (12.1%) and thujone (11.55%).

Isobornyl acetate (57.25%), α-pinene (13.55%) and limonene (10.62%) were found as the main components of *A. sibirica* EO, while anethole (98.64%) was the main compound in the essential oil of *I. verum*.

Tab. 1 Chemical composition of the tested essential oils

Compound	RT	Concentration (%)		
		A. absinthium	***I. verum***	***A. sibirica***
α-Pinene	4.091	4.29	-	13.55
Camphene	4.558	1.76	-	2.22
Phellandrene	5.117	16.71	-	0.80
Carene	5.634	7.01	-	8.91
d-Limonene	6.033	3.98	-	10.62
p-Cymene	6.480	7.80	-	0.91
1,8-Cineole	6.733	1.74	-	0.41
Terpinolene	7.250	-	-	3.68
Linalool	7.577	1.75	-	-
Thujil alcohol	8.111	19.65	-	-
β-Thujone	8.256	11.55	-	-
Isoborneol	8.950	1.11	-	-
Borneol	9.172	12.10	-	-
Camphor	9.285	3.58	-	-
Estragole	10.146	-	1.36	-
Isobornyl acetate	10.826	5.14	-	57.52
Anethole	11.780	-	98.64	-
Total, %	-	98,17	100	98,62

Contact Activity

Results show (Tables 2-4) that the test essential oils showed variant degrees of toxicity, where the *I. verum* EOs was the most toxic, followed by *A. absinthium* and *A. sibirica*. Essential oil of *I. verum* at the dose 0.25 µl/cm^2 caused 100% mortality of *S. oryzae* and *T. confusum* and also significant mortality (96,7%) of *S. granaries* after 24 h exposure. In addition, the total mortality of adults and larvae of *T. molitor* were found on 1 and 3 days during post-exposure period. *Trogoderma granarium* larvae were also sensitive to *I. verum* at the dose 0.25 µl/cm^2, where percentage of killed insects 98% was recorded on third day during post-exposure period. Essential oil of *A. absinthium* was the less toxic, where 100% mortality of tested pests were found after application at the dose 0.5 µl/cm^2. *A. sibirica* displayed the least contact activity. In this case, total mortality of insects was found to the dose 0,75 µl/cm^2. *S. oryzae* and *S. granarius* were the most sensitive to each tested essential oil. Larvae of *T. molitor* were the most resistance to *A. absinthium* and *A. sibirica* EOs. Larvae of *T. granarium* were the most resistance to *I.verum* EO.

Tab. 2 Mean ±SE mortality (%) of insects exposed to *A. absinthium* EO at different doses after 24 h exposure. For all Tables, means within columns followed by different lower-case letters represent significant differences between doses, means followed by capital letters represent significant differences between species ($P < 0.05$).

Dose (µl/cm^2)	Stored product pest					
	S. granarius	***S. oryzae***	***T. confusum***	***T. molitor* (larvae)**	***T. molitor* (adults)**	***T. granarium***
0.01	0.0 ± 0.0bA	0.0 ± 0.0bA	3.3 ± 5.8cA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA
0.25	95.0 ± 8.7aA	96.7 ± 5.8aA	40.0 ± 20.0bC	13.3 ±11.5bD	66.7 ± 23.1bB	40.0 ± 10.0bC
0.50	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	86.7± 11.5aA	100.0 ± 0.0aA	83.3 ± 11.6aA
0.75	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	73.3± 30.5aB	100.0 ± 0.0aA	100.0 ± 0.0aA
1.00	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	93.3± 11.5aA	100.0 ± 0.0aA	100.0 ± 0.0aA
Control	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA

Tab. 3 Mean ±SE mortality (%) of insects exposed to *I. verum* EO at different doses after 24 h exposure

Dose (µl/cm²)	Stored product pest *S. granarius*	*S. oryzae*	*T. confusum*	*T. molitor* (larvae)	*T. molitor* (adults)	*T. granarium*
0.01	0.0 ± 0.0bA	0.0 ± 0.0bA	6.7 ± 11.6bA	0.0 ± 0.0cA	0.0 ± 0.0cA	0.0 ± 0.0cA
0.25	96.7 ± 5.8aA	100.0± 0.0aA	100.0± 0.0aA	46.7 ± 25.2bC	73.3 ± 5.8bB	46.7 ± 20.2bC
0.50	100.0± 0.0aA	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	90.0 ± 0.0abA	86.7 ± 10.4aA
0.75	100.0± 0.0aA	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	93.3 ± 5.8aA	100.0 ± 0.0aA
1.00	100.0± 0.0aA	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA
Control	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA

Tab. 4 Mean ±SE mortality (%) of insects exposed to *A. sibirica* EO at different doses after 24 h exposure

Dose (µl/cm²)	Stored product pest *S. granarius*	*S. oryzae*	*T. confusum*	*T. molitor* (larvae)	*T. molitor* (adults)	*T. granarium*
0.01	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA
0.25	100.0± 0.0aA	100.0± 0.0aA	31.7 ± 17.6bB	0.0 ± 0.0bC	26.7± 30.6cBC	43.3 ± 28.9bB
0.50	100.0± 0.0aA	100.0± 0.0aA	91.7 ± 7.6aA	93.3± 11.6aA	60.0 ± 20.0bB	100.0 ± 0.0aA
0.75	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	93.3± 11.6aA	73.3 ± 23.1abA	100.0 ± 0.0aA
1.00	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	100.0± 0.0aA	93.3 ± 11.6aA	100.0 ± 0.0aA
Control	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA

Discussion

The composition of tested *I. verum* and *A. sibirica* essential oils in the present study showed a similarity to that reported in the literature (Tuan and Ilangantileke, 1997; Singh et al., 2006; Matsubara et al., 2011). For example, according to Singh et al. (2006), major component of *I. verum* EOs was trans-anethole (85-90%). Some differences were observed in the composition and abundant compounds of *A. absinthium* EO (Lawrence, 2006; Gandomi Nasrabadi et al., 2012). For example, myrcene (21.5 %), thujyl alcohol (18.9 %), sabinene (17.3 %), α-thujone (7.4 %) and camphor (5.5 %) were found to be the main components in *A. absinthium* essential oil growing in Russia (Lawrence, 2006). These differences in essential oil compositions might arise from several environmental (climatical, seasonal, geographical) or genetic differences and different chemotypes and the nutritional status and the extracted part of the plant.

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Toxicity of extracts derived from different parts of cassava plant, *Manihot esculenta* Crantz to four major coleopteran pests of stored-products

Arumughan Jayaprakas Cheruvan *, L. Ragesh

Central Tuber Crops Research Institute, Sreekariyam, 695 017, Thiruvananthapuram, Kerala, INDIA
*Corresponding author: prakashcaj@gmail.com
DOI 10.5073/jka.2018.463.183

Abstract

Fumigant toxicity of insecticidal principles extracted from tuber rind, fresh leaf, fresh leaf with petiole, and dried leaf of cassava (var. M4) was studied against four major stored-product insect pests *viz. Sitophilus oryzae (*L.*)*, *Rhyzopertha dominica* (F.*)*, *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* (L.) under laboratory conditions (28±2ºC, Rh. 75±5%). Mortality of the test insects varied with respect to extracts collected from different parts of the plant, and time of exposure. Extract collected from cassava rind recorded the highest toxicity. *Callosobruchus chinensis* was highly susceptible and showed immediate knockdown effect to the active principles extracted from tuber rind, fresh leaf, fresh leaf with petiole, twig and semi-dried leaf. The extract collected from various parts of plant caused 100% mortality of *R. dominica* at 1 hour after treatment (HAT), but the same collected from tuber and dried leaves did not show any toxic effect. Mortality of *S. oryzae* was 100% at 1 HAT with tuber rind extract, but no response was observed from the extract collected from semi-dried leaf, twig, and leaf with petiole. No fumigant action was observed in all the four coleopteran pests exposed to the extract collected from dried leaves. The study revealed that fresh leaf and tuber rind are good sources for the extraction of biofumigant against major coleopteran pests, however dried leaves are unfit for same purpose.

Key words: Cassava, extracts, Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum, Callosobruchus chinensis.

Introduction

Insect pest infestation is a major problem in the storage of cereals and pulses. Realising the fact that indiscriminate application of synthetic pesticides has created major challenges to man and ecosystem, there is a global concern to contain pests using non-chemical methods, particularly to lessen the pesticide residues in food (Flinn and Hagstrum, 2001). Fumigation is an effective method to protect stored-products from insect infestation. The commonly used fumigant like aluminium phosphide, ethylene dibromide and methyl bromide are associated with health and environmental pollution, and also many of the stored- product pests have developed resistance against synthetic fumigants (Zettler, 1982). Phillips et al. (2001) opined that exposure to low or sublethal doses pose an increased risk in phosphine resistance. Opit et al. (2012) reported high levels of resistance in several strains of major storage pests to phosphine in the USA.

The use of natural compounds in place of synthetic insecticides is an alternative strategy to reduce environmental pollution, and to preserve non-target organisms. Phytochemicals have been suggested as the alternatives to synthetic insecticides as they are the storehouse of a wide range of bioactive chemicals (Wink, 1993). Plant products are inexpensive products for the management of stored-grain pests (Mishra et al., 2012b), and are potentially suitable as vital components in integrated pest management strategies (Saxena, 1989; Schmutterer, 1992). The insecticidal activity of many plant derivatives against several stored-product pests has been demonstrated (Malik and Mujtabe Naqvi, 1984; Singh, S. 2017).

Cassava (*Manihot esculenta* Crantz), originally from Amazonia, is a woody shrub extensively cultivated as an annual crop in tropical and subtropical regions that provides the staple food of an estimated 800 million people worldwide. Although the leaves of cassava are rich in proteins, minerals, and vitamins, the presence of antinutrients and cyanogenic glucosides are the major drawbacks to human consumption. However, cyanogen can play a pivotal role in pest management.