Insecticidal effect of oregano (*Origanum vulgare* L. ssp. *hirtum* Ietswaart) on bean weevil (*Acanthoscelides obtectus* Say)

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Summary

The effectiveness of drug plant and of essential oil of oregano (*Origanum vulgare* L. ssp. *hirtum* Ietswaart) against the adults and egg hatching of bean weevil (*Acanthoscelides obtectus* Say) was studied in laboratory trials in order to screen for a new potential insecticide in the control of this granary pest of beans. The adult mortality as well as oviposition and hatching capabilities were evaluated after 6 days of treatment of beans cv. 'Berggold' (55 g) in Petri dishes by powdered drug (0.33 g, 0.66 g, 1.0 g and 2.0 g) and by equivalent amounts of essential oil (5 µl, 10 µl, 15 µl and 30 µl). Oregano essential oil showed both fumigant and contact toxicities to bean weevil, the later being more potent. When considering fumigant toxicity, insecticidal effect (mortality rate 82.5%) was observed 6 days after application of high concentrations of oregano essential oil (150 µl per 55 g of beans). When considering contact toxicity, both oregano drug plant and essential oil at all tested concentrations significantly increased the bean weevils' mortality rates with comparison to the controls. Also, egg laying and hatching were inhibited after treatment of bean weevil with powdered drug plant or with essential oil at all tested concentrations. Insecticidal effect of drug plants issuing from two different stocks depended on their essential oil contents. Essential oils (5 µl, 15 µl and 30 µl per 55 g of beans) induced 100% mortality of bean weevil population when applied directly to the surface of beans (55 g) in Petri dishes. The content of essential oil in oregano and oregano-based preparations for insecticidal use is proposed as a parameter for quality control purposes.

Introduction

Bean weevil (*Acanthoscelides obtectus* Say) is a typical cosmopolitan species due to its broad acclimatization capabilities. Egg laying and development of bean weevil are continuous at the temperature range of 14 to 35 deg C (*Regnault & Hamraoui*, 1993). Hence, this granary pest can survive for 16 days (at temperature range 23 to 25 deg C) or even 42 days (at temperature range 19 to 21 deg C) whithout any feed. This is the reason why bean weevil is one of the most common granary pests all over the world.

Higher plants, especially medicinal and aromatic plants (MAP), are potential source of new insecticides and many of research groups try to prove their activity against noxious pests. Some natural compounds, isolated from these MAP (like rotenon, pyretrins, azadirachtin) are already commercially available on the insecticide preparations' market. Investigations on activity of aromatic plants against stored product insects gave diverse results when considering different insect species and/or their developmental stages (eggs and adults) (*Shaaya* et al., 1993, *Regnault-Roger & Hamraoui*, 1993a; *Regnault-Roger & Hamraoui*, 1995; *Kalinović* et al., 1997; *Mateeva* et al., 1997; *Rakowski & Ignatowicz*, 1997). The insecticidal activity (against *Drosophila melanogaster*) of essential oil of oregano (*Origanum vulgare* L. susbsp. *hirtum* Ietswaart) with high concentration of carvacrol was reported also by *Karpouhtsis* et al. (1998). Oregano essential oil was found to have an ovicidal activity against eggs of stored product insects *Tribolium confusum* and *Ephestia cautella* (*Shaaya* et al., 1993). The exposure to vapours of essential oil from oregano resulted in 77% and 89% mortality of the eggs of the confused flour beetle (*Tribolium confusum*) and the Mediterranean flour moth (*Ephestia kuehniella*), respectively (*Tunç* et al., 2000). Lypophilic extracts of thyme (*Thymus vulgaris* L., *Thymus serpyllum* L.), of chamomille (*Chamomilla recutita* Rausch.), of peppermint (*Mentha x piperita* L.), of tarragon (*Artemisia dracunculus* L.) and of sweet clover (*Melilotus officinalis* Lam.) inhibited development of next generation of bean weevil and egg laying (*Rakowski & Ignatowicz*, 1997). The highest

inhibitory effect on egg laying was achived by extracts of peppermint (91%) and of tarragon (83,4%). On the contrary, the extracts of yarrow (*Achillea millefolium* L.) and of buckbean leaves (*Menyanthes trifoliata* L.) stimulated the reproductivity of bean weevil. It was also found, that extracts of some aromatic plant species (*Tussilago farfara* L., *Thymus vulgaris* L., *Solidago virgaurea* L., *Humulus lupulus* L., *Cymbopogon citratus* /DC./ Stapf.) prolong longevity of bean weevil, while other species (*Ocimum basilicum* L., *Thymus serpyllum* L.) shorten its lifetime. Among different aromatic species, the plant essential oils from Labiatae family have the best insecticidal effects against *Acanthoscelides obtectus (Regnault-Roger & Hamraoui,* 1993a). Oregano (*Origanum vulgare* L.) is one of the plants, used traditionally in southern France, to control bean weevil (*Acanthoscelides obtectus* Say) in stored kidney beans (*Phaseolus vulgaris* L.) *(Regnault & Hamraoui,* 1993b, *Bernath,* 1996). The main biocide compounds in oregano were proved to be terpenoids, flavonoids, tannins, essential oils and their compounds like carvacrol, the most potent being carvacrol with LD_{50} 810 mg/kg (*Bernath,* 1996).

Bean weevil endangers bean crops also in Slovenia. Since we endeavour to replace synthetic chemicals by effective, environment- and user- friendly natural substances for pest control, the objective of our study was to test the toxicity of powdered oregano as well as its essential oil against this bean storage insect. The hypothesis presumed, that the essential oil of oregano was the main active principle of the drug plant's drug insecticidal effect on bean weevil and thus an useful quality parameter in official control of plant drug *Origani herba* for insecticidal use.

Material and methods

Test organisms

The adults (approximately even amount of males and females) of a bean weevil beetles (*Acanthoscelides obtectus* Say), obtained from a laboratory collection at the Department of Agriculture, Biotechnical Faculty of University of Ljubljana, were regenerated in glass vessels (V= 150 ml). Beans cv. 'Berggold' was preliminary found to be a favourable regenerating subtrate for a bean weevil (not published data). Several glass vessels filled with regenerating substrate were covered by perforated parafilm and put into the growth chamber (T = 30 °C, dark) for 1 month until the critical mass of test organisms developed. Immediately after hatching was observed in each of the glass vessels, 10 randomly chosen beetles (1 to 6 days old) were transferred on the surface of beans (55 g) in test Petri dishes (V = 100 cm^3) for treatment purposes.

Plant materials and essential oil

Two stocks of dry plants (production year 1999 and production year 1998) of the same accession (Acc. Nr. 9/50 Fab.) of oregano (*Origanum vulgare* L. spp. *hirtum* letswaart), held in Genebank for medicinal and aromatic plants at Biotechnical Faculty of Ljubljana, Slovenia, were used in the study of insecticidal effects of drug plants and/or oregano essential oil against bean weevil. Before treatment experiments dry plants were pulverised in a mortar.

To verify the research hypothesis, drug plants and essential oils had to be applied to test organisms in equivalent doses. This was the reason, why the content of essential oil in both stocks of drug plants had to be determined and isolated. The essential oil was obtained from oregano dry plants (10 g) by steam destillation in a Clevenger apparatus according to the procedure of Ph. Eur. III (Edition 2000). Plants from production years 1999 and 1998 contained 3.16% and 1.50% of essential oil, respectively.

A commercially available essential oil of oregano (*Origanum vulgare* L.) was used in the study of fumigant toxicity (i.e. respiratory activity) of oregano essential oil against bean weevil.

Carvacrol (56.04% to 70.53%) was found to be the leading compound of all 3 sources of oregano essential oils (essential oil from plants Acc. Nr. 9/50 Fab – production year 1999, essential oil from plants Acc. Nr. 9/50 Fab – production year 1998 and commercial essential oil), while only scarce amounts of thymol (less than 2%) were found after HPTLC analysis of essential oils samples.

Methods

Preliminary trials

Before two main experiments were set up, several preliminary trials were designed in order to estimate a) a range of toxicities obtained after application of different solvents (70% ethanol; 35% ethanol, acetone; dimethyl sulfoxide – DMSO; Sigma – Aldrich Chemie GmbH, Germany), b) the toxicity of drug plant and of oregano essential oil to the culture of bean weevil. Different volumes (100 µl, 300 µl and 500 µl) of each of the tested solvents were applied directly on the surface of 55 g of beans, which was thereafter infested by 10 bean weevils per Petri dish. Solvent toxicity trials were made in two replications. Also, a fumigant toxicity of the solvents was tested with reference to the controls. This was achived by application of solvents (100 µl, 300 µl and 500 µl) on the surface of cutton tampons, which were put within the culture substrate (55 g of beans, infested by 10 bean weevil) in Petri dishes. In this case, in order to prevent evaporation of solvents, Petri dished were covered by parafilm. In control treatments no solvent was added to the culture substrate. Test Petri dishes were put into the growth chamber with constant temperature control (T = 23 ± 2 °C) in the dark. The mortality of bean weevil with regard to the controls was registered for 6 days.

To estimate the potency of insecticidal effect of oregano against bean weevil, equivalent amounts of drug plant (production year 1999; 1g) and of oregano essential oil (31.6 l)

were applied directly on the surface of 55 g of beans, which was thereafter infested by 10 bean weevils per Petri dish. No oregano or its essential oil was applied in the control treatments. Each of the treatments was replicated 4-times. Test Petri dishes were handled as previously (T = 23 ± 2 °C; dark). Registration of mortality rate (no respond of the beetle to the painting-brush test) was made each 2 days in the time period of 10 days.

On the basis of results of preliminary trials the observation period in main experiments was shortened to 6 days. From the view of observation methodology the experimental design of the experiments anticipated two different approaches. a) The beetles' mortality was registered at 2 days' interval (first experiment) or at daily interval (second experiment). For observation reasons Petri dishes were opened before registration of mortality and closed immediately after that ("opened treatments"). b) The beetles' mortality was registered after opening the test Petri dishes at the end of experimental period (6 days) ("closed treatments"). After the experiments were finished, the "closed treatment " and control group of the cultures in Petri dishes were left in a growth chamber (T = 23 ± 2 °C, dark) for 1 month in order to observe egg laying and hatching abilities.

First experiment – fumigant toxicity of oregano essential oil against bean weevil

Oregano essential oil was diluted with DMSO (1 : 1) in order to enable an even distribution of essential oil all over the surface of beans. The dosage volumes of diluted essential oils referred to the volumes of essential oils in the mixture. In each of the two treatment groups (i. e. "opened" and "closed") five different volumes of diluted essential oil (5 µl, 15 µl, 30 µl, 50 µl and 150 µl) were applied on the surface of cutton tampons, which were put within the culture substrate (55 g of beans, infested by 10 bean weevil) in Petri dishes in order to study the fumigant toxicity of oregano. In control Petri dishes no essential oil was applied to cutton tampons. 4 replications of each treatment were made. Test Petri dishes and controls were put into two separate growth chambers with the same culture regimes (T = 23 ± 2 °C, dark) in order to prevent possible impact of evaporated essential oil on the control. The number of dead beetles per Petri dish was counted every 2 days in the "opened treatment" group and after 6 days in the "closed treatment" group. The whole experiment was replicated 3-times.

Second experiment – contact toxicity of oregano and its essential oil against bean weevil

In each of the two treatment groups (i. e. "opened" and "closed") different amounts (0.33 g, 0.66 g, 1.0 g and 2.0 g) of pulverised oregano (production year 1998) and equivalent amounts of its essential oils (5 µl, 10 µl, 15 µl, 30 µl) diluted in 35%-ethanol (1 : 1) were homogeneously applied to the substrate (beans cv.'Berggold, 55 g) in Petri dishes. After that, 10 randomly chosen adult beetles of bean weevil (1 to 6 days old) were colonised to the substrate-oregano mixture. Two sets of control treatments were applied: a) control (insect-colonised substrate without oregano or its essential oil) and b) control with 35% -ethanol – treated substrate. Experiment, which was replicated 3-times, comprised 20 treatments and 4 replications.

Statistical analysis

The results of the fumigant and contact insecticidal effect of oregano against bean weevil were analyzed by description statistical methods. Individual treatments were evaluated also by t-test and ANOVA (= 0.05; Statgraphics program) on the basis of statistically significant differences in the number or dead beetles per Petri dish.

Results and discussion

Preliminary trials

The results of preliminary trials showed, that none of the tested solvents was toxic to bean weevil when applied in low doses (100 µl, 300 µl) to the surface of infested substrate (mortality 10%). Also, no DMSO fumigant toxicity was observed in any of tested concentrations (mortality 30%). On the contrary, ethanol (70%) and acetone showed a high fumigant toxicity to bean weevil (100% mortality) when compared to the controls (10%).

The drug plant (*Origani herba*) as well as its essential oil (*Origani aetheroleum*) showed a potent insecticidal effect against bean weevil. Already 2 days after application of 1 g of drug plant or of 31.6 l of essential oil to test Petri dishes, 91% (drug plant) or 100% (essential oil) mortality of bean weevil was observed. At the end of the preliminary trial all oregano treated substrates caused 100% mortality of bean weevil. The controls showed low insecticidal effect (mortality rate 10%) under the laboratory test conditions. These preliminary results enabled determination of the range of concentrations of drug plants and/or essential oils in further testing of insecticidal activity.

First experiment – fumigant toxicity of oregano essential oil against bean weevil

The results of the study showed fumigant toxicity of high concentrations of oregano essential oil (EO) against bean weevil. When compared to the controls, the "opened treatment" group measurements showed significant mortality (p = 0.035) of bean weevil only when the highest concentration of essential oil (150 l per 55g of beans) was applied (figure 1). Opening of the Petri dishes in the course of the experiment reduced the fumigant toxicity of oregano essential oil. Six days after application of 30 l, 50 l and 150 l of EO to the substrate (55 g of beans cv.'Berggold') an increased percentage (85.8%, 99.2% and 100%, respectively) of dead beetles was observed in the culture

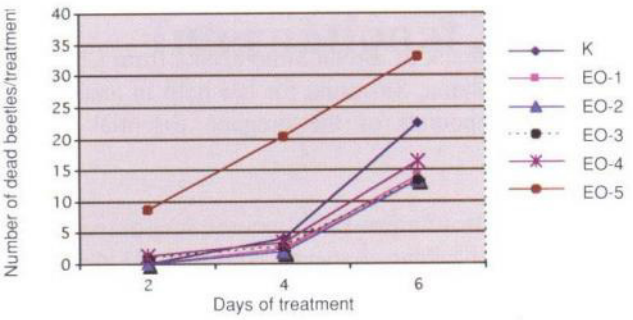

Figure 1: Fumigant toxicity of oregano essential oil against the bean weevil (*Acanthoscelides obtectus* Say) , expressed as average number of dead beetles per treatment. K = control; EO-1: 5 l of essential oil, EO-2: 15 µl of essential oil; EO-3: 30 µl of essential oil; EO-4: 50 µl of essential oil; EO-5: 150 µl.

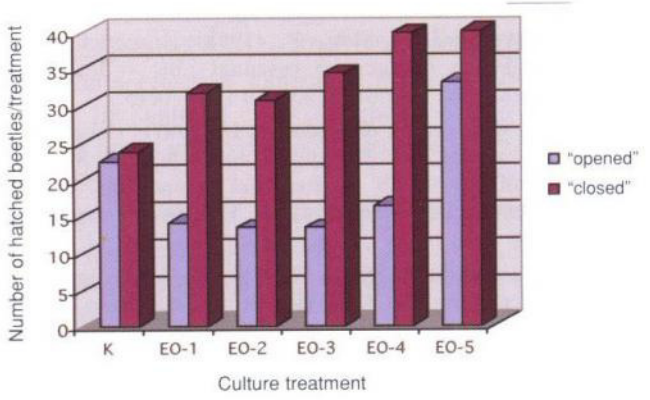

Figure 2 An average mortality of bean weevil beetles (*Acanthoscelides obtectus* Say) after 6 days of experiment on the fumigant toxicity of oregano essential oil, when considering "opened treatment" and "closed treatment " measurements.

("closed treatment"). A high variability of results was noticed, especially in control treatments, where the average mortality rate was very high (59.2%) in relation to the controls of the preliminary trials (10%). The reasons for such a high mortality rate in controls were not established. Figure 2 shows the influence of the treatment group measurements on the average number of dead beetles in test Petri dishes.

Second experiment – contact toxicity of oregano and its essential oil against bean weevil

Oregano drug plant significantly increased the average number of dead beetles per treatment at all tested concentrations when compared to the controls (p= 0.014 to 0.032). No significant differences in the insecticidal activity were observed between application doses *(Figure 3)*. Maximal mortality rates, induced by application of drug plants, were 85.0% and 86.7% in "opened treatment" and "closed treatment" group, respectively *(Table 1)*.

Oregano essential oils were proved to be highly toxic (100% mortality) to bean weevil beetles at all tested concentrations *(Figure 4* and *Table 1)*. Controls and 35%-ethanol controls did not differ significantly when considering the mortality rate (p = 0.096).

Results of ANOVA showed, that oregano essential oil, when applied directly to the beans' surface, significantly

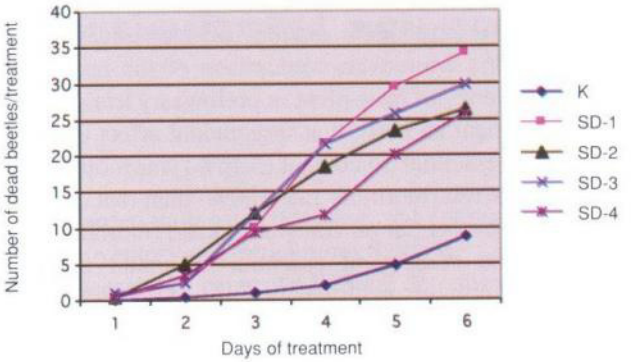

Figure 3 Mortality (average number of dead beetles per treatment) of bean weevil (*Acanthoscelides obtectus* Say) influenced by different amounts of pulverized drug plant *(Origani herba)* and under control treatments. K: control; SD-1: 0.33 g of the drug plant; SD-2: 0.66 g of the drug plant; SD-3: 1.00 g of the drug plant; SD-4: 2.00 g of the drug plant

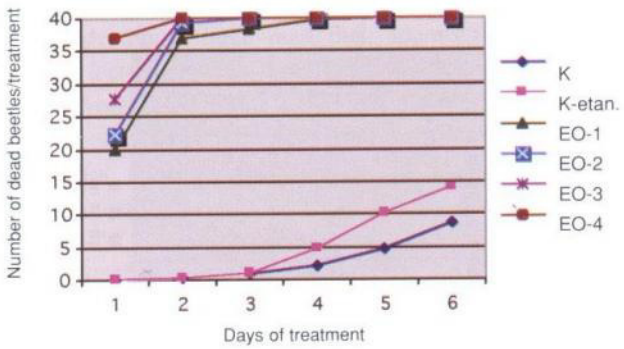

Figure 4 Mortality (average number of dead beetles per treatment) of bean weevil (*Acanthoscelides obtectus* Say) influenced by different volumes of essential oil *(Origani aetheroleum)* and under control treatments. K: control; K-EtOH: control treated with 35% of ethanol; EO-1: 5 µl of essential oil, EO-2: 10 µl of essential oil; EO-3: 15 µl of essential oil; EO-4: 30 µl of essential oil.

Table 1 The mortality rates (%) in bean weevil (*Acanthoscelides obtectus* Say) culture measured as "opened treatment" and "closed treatment" regimes in controls and after application of the different amounts of drug plant

Culture treatment Amount of the drug plant/ volume of essential oil	Mortality (%) Measurement approach	
	"opened treatment"	"closed treatment"
Control	21.7	24.2
0.33 g	85.0	86.7
0.66 g	65.8	49.2
1.00 g	74.2	67.5
2.00 g	64.2	43.3
Control	21.7	24.2
Control-EtOH (35%)	35	32.5
5 µl	100	100
10 µl	98.3	95.8
15 µl	100	100
0 µl	100	100

increased the mortality of bean weevil beetles when compared to the equivalent amount of drug plants (p = 0.0007 to 0.006). Moreover, comparison of the results on insecticidal potency of drug plant in prelimirary trials and in second experiment showed, that insecticidal effect of plant drug with high essential oil content (3.16%) was more active against bean weevil (mortality rate 100%) than that of plant drug with lower essential oil content (1.50%) (mortality rate of 85.0%). The content of essential oil could thus be assumed as important quality parameter of oregano drug plants (*Origani herba*) for insecticidal use.

The mortality rates in bean weevil culture measured as "opened treatment" and "closed treatment" regimes are presented in *Table 1*.

It was found, that the drug plant (*Origani herba*) and oregano (*Origani aetheroleum*) essential oil at any of tested concentrations completely inhibited the ability of bean weevil egg laying and hatching of beetles *(Figures 5* and *6)*.

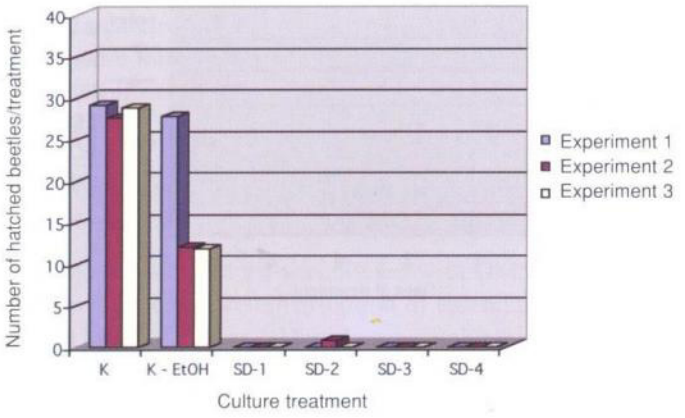

Figure 5 An average number (3 experiments) of hatched beetles of bean weevil (*Acanthoscelides obtectus* Say) in control treatments and after application of different amounts of pulverised drug plant *(Origani herba)*. K = control; K-EtOH: control treated with 35% of ethanol; SD-1: 0.33 g of the drug plant; SD-2: 0.66 g of the drug plant; SD-3: 1.00 g of the drug plant; SD-4: 2.00 g of the drug plant

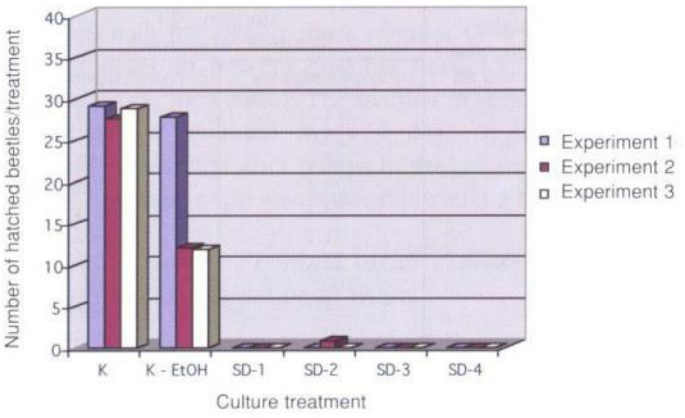

Figure 6 An average number (3 experiments) of hatched beetles of bean weevil (*Acanthoscelides obtectus* Say) in control treatments and after application of different volumes of essential oil. K = control; EO-1: 5 µl of essential oil, EO-2: 15 µl of essential oil; EO-3: 30 µl of essential oil; EO-4: 50 µl of essential oil; EO-5: 150 µl

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