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Mediterranean Wild Herbs As Grain Protectants †

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Abstract: In this study we explored the pesticide properties of several essential oils (EOs), yielded from aromatic plants of the Mediterranean Basin, as wheat protectants. For this purpose, laboratory bioassays were conducted with two major stored-product insect pests, the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Regarding *T. granarium* adults, the LD_{50} values 7 d after the application were 440.6, 501.3, 506.9, 530.7, 619.1, and 745.3 ppm for the *Origanum majorana*, *Juniperus phoenicea*, *Laurus nobilis*, *Origanum vulgare* ssp. *hirtum*, *Echinophora tenuifollia* ssp. *sibthropiana* and *Citrus limon* EOs, respectively. The LD_{50} values for *T. castaneum* larvae 14 d after the application were 416.1, 424.1, 454.5, 490.5, 539.4 and 649.4 ppm for the *C. limon*, *E. tenuifollia* ssp. *sibthropiana*, *O. majorana*, *O. vulgare* ssp. *hirtum*, *L. nobilis* and *J. phoenicea* EOs, respectively. The generated results could be useful for the management of the target stored-product insect pests by using reduced-risk plant protection products.

Keywords: *Trogoderma granarium*; *Tribolium castaneum*; pest management; micro emulsions; stored products

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1. Introduction

Numerous agricultural products are damaged by pests and diseases during their development and maturation in the field [1–3]. These products are stored in facilities, where gradual manipulations are taking place, such as processing, industrialization, packaging and transportation [4]. Infestations by insects, mites, fungi and rodents are particularly common in these places [3]. Stored products are infested by insect pests that cause qualitative and quantitative losses up to 10% of the world production in developed countries and more than 20% in developing countries [5].

The essential oils (EOs) of several plants are known to serve as efficient insecticides [6-8]. In Greece, sufficient quantities of various wild herbs are available for extraction. A review of the literature showed that little is known on the application of EOs as grain protectants [6–8]. In this context, it is challenging to investigate the insecticidal properties as grain protectants of EOs derived from wild herbs of the Mediterranean Basin. It is worth noting that in recent years the bioactivity of EOs from plants originated in the Mediterranean Basin has been reported, indicating their insecticidal potential [9,10] Therefore, the aim of this study was to investigate the efficacy of *Citrus limon, Echinophora tenuifollia* ssp. *sibthropiana*, *Juniperus phoenicea*, *Laurus nobilis*, *Origanum majorana* and *Origanum vulgare* ssp. *hirtum*, commonly found in Mediterranean, as grain protectants, against two key stored-product insect pests, i.e. the khapra beetle, *Trogoderma granarium*

Everts (Coleoptera: Dermestidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae).

2. Materials and Methods

Trogoderma granarium and *T. castaneum* were cultured on wheat at 30 °C, 65% relative humidity and continuous darkness. The EOs of *C. limon*, *E. tenuifollia* ssp. *sibthropiana*, *J. phoenicea*, *L. nobilis*, *O. majorana* and *O. vulgare* ssp. *hirtum* were tested as wheat protectants. All the EOs were formulated to micro emulsions, with food grade emulsifier [11] and were applied as wheat coating. A stock solution of EO and Tween 20 (1:1) was prepared from each plant. The EOs stock solutions were tested at the concentrations of 250, 500 and 1000 ppm, where water with 0.05% Tween 20 served as control. Sprays were performed on plates. Quantities of 0.20 kg of wheat were sprayed each with 1 mL of the test solution as thin layers. The sprayed wheat quantities were inserted separately in plastic canisters and were shaken for 10 min to realize the balanced distribution of the EOs on the entire wheat masses. Three subsamples of 10 g were obtained and placed in Petri dishes (9 cm diameter, 1.5 cm height). The covers of the dishes bore a circular hole (1.50 cm diameter). The holes were covered by muslin cloth that allowed the ventilation of the inside space of the dishes. Larvae of *T. castaneum* (2−4 mm length) and adults of *T. castaneum* (< 1 week old), were used in the experiments. The mortality of *T. castaneum* larvae was determined after 14 d of exposure, while the mortality of *T. granarium* adults was determined after 7 d of exposure due to short adult longevity of this species.

Mortality data obtained from the dose-response trials were subjected to probit analysis and LD_{50}, values, i.e., the minimal inhibition dose of an EO to cause 50% mortality on the studied population, were estimated. The statistical analysis was conducted using Polo Plus [12].

3. Results

Mortality in the control treatments was low (<5%) for both species, therefore no correction was considered necessary for the mortality values. The results of the laboratory toxicity bioassays are shown in Table 1.

Table 1. Mean LD_{50} values and 95% confidence intervals (ppm) for *Trogoderma granarium* adults and *Tribolium castaneum* larvae 7 and 14 days after application with several essential oils, respectively.

Treatment	*Trogoderma granarium*	*Tribolium castaneum*
Citrus limon	745.3 a (597.1−861.5)	416.1 ac (368.6−466.4)
Juniperus phoenicea	501.3 b (429.6−586.4)	649.4 b (542.4−815.9)
Laurus nobilis	506.9 b (447.7−574.9)	539.4 abc (456.9−643.8)
Echinophora tenuifollia ssp. *sibthropiana*	619.1 ab (525.7−752.3)	424.1 ac (368.4−482.5)
Origanum majorana	440.6 b (369.9−517.4)	454.5 ac (396.9−517.9)
Origanum vulgare ssp. *hirtum*	530.7 ab (468.2−604.5)	490.5 abc (431.0−558.7)

Means in the same column followed by different letters are significantly different.

For *T. granarium* adults, the mean LD_{50} values of *J. phoenicea*, *L. nobilis* and *O. majorana*, i.e., 501.3, 506.9, and 440.6, respectively, were significantly lower compared to *C. limon* (745.3 ppm). The mean LD_{50} values of *E. tenuifollia* ssp. *sibthropiana* (619.1 ppm) and *O. vulgare* ssp. *hirtum* (530.7 ppm) did not significantly differ in comparison to the mean LD_{50} values of all other EOs.

Concerning *T. castaneum* larvae, the mean LD_{50} values of *C. limon*, *E. tenuifollia* ssp. *sibthropiana* and *O. majorana*, i.e., 416.1, 424.1 and 454.5 ppm, respectively, were

significantly lower compared to the mean LD_{50} value of *J. phoenicea* (649.4 ppm). In addition, the mean LD_{50} values of *J. phoenicea*, *L. nobilis* and *O. vulgare* ssp. *hirtum*, i.e., 649.4, 539.4 and 490.5 ppm, respectively, did not significantly differ compared to the mean LD_{50} values of all the other EOs.

4. Discussion

T. granarium is considered one of the most important quarantine insects in various countries of the world [13]. Quarantine insects have potential economic importance in an area where they are not yet present or where they are present but are not widespread and are not controlled by phytosanitary authorities [14,15]. Quarantine insects disrupt the marketing of agricultural stored products between countries and geographical areas within countries (14). In addition, *T. castaneum* is widely spread in agricultural and food storage worldwide [16].

The preservation of nutritional and commercial value of stored agricultural products is highly based on their protection from insect pests [3]. Our study clearly shows the effectiveness of the EOs of six Mediterranean wild herbs against two notorious stored-product insect species. The insecticide activity of EOs as grain protectants has only recently been studied. For example, the EOs obtained from *Mentha longifolia*, *Dysphania ambrosioides*, *Carlina acaulis* and *Pimpinella anisum* were efficient against the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) and *T. granarium* [6]. The potential of *Hazomalania voyronii* essential oil-to protect wheat infestations by *T. castaneum*, *T. confusum* and *Tenebrio molitor* has also been highlighted [7]. *Trogoderma granarium* suffered high mortality after the application of EOs obtained from *Ferula assa-foetida* and *F. gummosa* [8].

We revealed that EOs can exhibit a significant range of pesticidal activities. Treatments with synthetic contact insecticides is a common practice against stored-product insect pests [17–19]. However, food safety is linked with integrated pest management, aiming at the reduced use of synthetic pesticides [20,21]. Our results are towards this direction, as we showed that the EOs of several Mediterranean wild herbs have the potential to serve as efficient tools against the tested major stored-product insect pests.

We expect our results to have bearing on the management of these notorious stored-products insect species. Further research on the insecticidal activity of several Mediterranean wild herbs as grain protectants will gather more information towards an efficient, eco-friendly management of several storage pests.

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References

1. Leesutthiphonchai, W.; Vu, A.L.; Ah-Fong, A.M.V.; Judelson, H.S. How does *Phytophthora infestans* evade control efforts? Modern inside into the late blight disease. *Phytopathology* **2018**, *108*, 916−924.
2. Biondi, A.; Guedes, R.N.C.; Wan, F-H.; Desneux, N. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present, and future. *Annu. Rev. Entomol.* **2018**, *63*, 239−258.
3. Hagstrum, D.W.; Phillips T.W. Evaluation of stored-product entomology: protecting the world food supply. *Annu. Rev. Entomol.* **2017**, *62*, 379−397.
4. Hill, D.S. *Pests of Storage Foodstuffs and their Control;* Kluwer Academic Publishers: New York, NY, USA, 2003.
5. Mason, L.J.; McDonough, M. Biology, behavior, and ecology of stored grain and legume insects. In Stored Product Protection; Hagstrum, D.W., Phillips, T.W., Cuperus, G., Eds.; Kansas State University: Manhattan, KS, USA, 2012; pp. 7–20.
6. Kavallieratos, N.G.; Boukouvala, M.C.; Ntalli, N.; Skourti, A.; Karagianni, E.S.; Nika, E.P.; Kontodimas, D.C.; Cappellacci, L.; Petrelli, R.; Cianfaglione, K.; Morshedloo, M.R.; Tapondjou, L.A.; Rakotosaona, R.; Maggi, F.; Benelli, G. Effectiveness of eight essential oils against two key stored-product beetles, *Prostephanus truncatus* (Horn) and *Trogoderma granarium* Everts. *Food Chem. Toxicol.* **2020**, *139*, 111255.
7. Kavallieratos, N.G.; Nika, E.P.; Skourti, A.; Ntalli, N.; Boukouvala, M.C.; Ntalaka, C.T.; Maggi, F.; Rakotosaona, R.; Cespi, M.; Perinelli, D.R.; Canale, A.; Bonacucina, G.; Benelli, G. Developing a *Hazomalania voyronii* essential oil nanoemulsion for the eco-friendly management of *Tribolium confusum*, *Tribolium castaneum* and *Tenebrio molitor* larvae and adults on stored wheat. *Molecules* **2021**, *26*, 1812.
8. Pavela, R.; Morshedloo, M.R.; Lupidi, G.; Carolla, G.; Barboni, L.; Quassinti, L.; Bramucci, M.; Vitali, L.A.; Petrelli, D.; Kavallieratos, N.G.; Boukouvala, M.C.; Ntalli, N.; Kontodimas, D.C.; Maggi, F.; Canale, A.; Benelli, G. The volatile oils from the oleo-gum-resins of *Ferula assa-foetida* and *Ferula gummosa*: a comprehensive investigation of their insecticidal activity and eco-toxicological effects. *Food Chem. Toxicol* **2020**, *140*, 111312.
9. Pavela, R.; Maggi, F.; Lupidi, G.l Vianfaglione, K.; Dauvergne, X.; Bruno, M.; Nenelli, G. Efficacy of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils against *Culex quinquefasciatus* Say and *Spodoptera littoralis* (Boisd.). *Ind. Crops Prod.* **2017**, *109*, 603–610.
10. Badalamenti, N.; Ilardi, V.; Bruno, M.; Pavela, R.; Boukouvala, M.C.; Kavallieratos, N.G.; Maggi, F.; Canale, A.; Benelli, G. Chemical composition and broad-spectrum insecticidal activity of the flower essential oil from an ancient Sicilian food plant, *Ridolfia segetum*. *Agriculture* **2021**, *11*, 304.
11. Evergetis, E.; Michaelakis, A.; Papachristos, D.P.; Badieritakis, E.; Kapsaski Kanelli, V.N.; Haroutounian, S.A. Seasonal variation and bioactivity fluctuation of two *Juniperus* sp. essential oils against *Aedes* (*Stegomyia*) *albopictus* (Skuse 1894). *Parasitol. Res.* **2016**, *6*, 2175–2183.
12. LeOra Software. *Polo Plus 1.0 Probit and Logit Analysis.* 2002−2014, Petaluma, CA.
13. Kavallieratos, N.G.; Athanassiou, C.G.; Guedes, R.N.C.; Drempela, J.D.; Boukouvala, M.C. Invader competition with local competitors: displacement or co-existence among the invasive khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), and two other major stored-grain beetles? *Front. Plant Sci.* **2017**, *8*, 1837.
14. Myers, S.W.; Hagstrum, D.W. Quarantine. In Stored Product Protection; Hagstrum, D.W., Phillips, T.W., Cuperus, G., Eds.; Kansas State University: Manhattan, KS, USA, 2012; pp. 297–304.
15. Follett, P. A.; Neven, L.G.; Current trends in quarantine entomology. *Annu. Rev. Entomol.* **2006**, *51*, 359–85.
16. Aitken, A.D. *Insect Travelers, I: Coleoptera.* Technical Bulletin 31; HMSO: London, UK, 1975.
17. Skourti, A.; Kavallieratos, N.G.; Papanikolaou, N.E. How is fitness of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) affected when different developmental stages are exposed to chlorfenapyr? *Insects* **2020**, *11*, 542.
18. Skourti, A.; Kavallieratos, N.G.; Papanikolaou, N.E. Exposure of *Tribolium castaneum* (Herbst) females to pyrimiphos-methyl alters the fitness of their progeny. *Environ. Sci. Poll. Res.* **2021**, 7, 7893−7900.
19. Kavallieratos, N.G.; Athanassiou, C.G.; Barda, M.S.; Boukouvala, M.C.; Efficacy of five insecticides for the control of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) larvae on concrete. *J. Stored Prod. Res.* **2016**, *66*, 18–24.
20. Campbell, J.F.; Perez-Mendoza, J.; Weier, J. Insect pest management decisions in food processing facilities. In Stored Product Protection; Hagstrum, D.W., Phillips, T.W., Cuperus, G., Eds.; Kansas State University: Manhattan, KS, USA, 2012; pp. 219–233.
21. Abrol, D.P.; Shankar, U. Pesticides, food safety and integrated pest management. In Integrated Pest Management; Pimentel, D., Peshin, R., Eds.; Springer; Dordrecht, 2014; pp. 167−199.