

Preliminary Tests Regarding Fumigant Effect of Volatile Oils Obtained from Medicinal Plants

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Abstract

The objective of this work was testing 4 volatile oils obtained from new varieties of medicinal plants (yellow basil; red basil; marigold; hyssop), over *Beauveria brongniartii* (BbgMm1a/09) entomopathogenic fungi and *Sitophilus granarius*, deposit insect. The results obtained showed that at different concentrations, red basil volatile oil showed the strongest inhibitory effect compared to the other tested oils (yellow basil, marigold and hyssop). Concerning the insecticidal action, the preliminary tests with the 4 volatile oils obtained from new varieties of medicinal plants had no effect on the test insect - *Sitophilus granarius*.

Keywords: *Beauveria brogniartii*, fumigant, *Sitophilus granarius*

Introduction

Medicinal plants are an important source of useful compounds that can be used directly or can serve as precursors for the production of agents that can be used as potential controllers of disease and pest, insects, etc. (Planta Europa, 2010). The antimicrobial effect of extracts obtained from medicinal plants (Zarkani *et al.*, 2016), has also been studied on gram-positive and gram-negative bacteria (Semnani *et al.*, 2017).

Stored grain infestation is a very serious problem causing economic damage by deterioration of the quality of food grains and food products. The granary weevil, *Sitophilus granarius* is a major pest of stored grain capable of causing considerable losses (Rosca *et al.*, 2009). Among

alternatives to chemical treatments against store insect pests, the biological control using pathogens or natural plant products have shown promising results. Essential oils have shown insecticidal activity against field crop pests (Verma *et al.*, 2000). Among the pathogens, the entomopathogenic fungi have been most studied for stored product insect control (Kavallieratos *et al.*, 2006; Athanassiou and Steenberg, 2007; Kavallieratos *et al.*, 2008; Golnaz *et al.*, 2011). Isolates of entomopathogenic fungus *Beauveria* sp. were found to be pathogenic against *S. granaries* (Storm *et al.*, 2016). The hypothesis of simultaneously using the essential oils and entomopathogenic fungus treatments against *S. granarius* lead us to test the fumigant effect

of essential oils on the insect pests and mycelial growth of *B. brongniartii*.

Materials and methods

Determination of antifungal effects of the volatile oils on mycelial growth - the antifungal properties of volatile oils were evaluated for assessing its fumigant phase effects towards mycelial growth of *B. brongniartii*. For this, agar-mycelium plugs (7mm diameter) were cut from the edge of a colony of *B. brongniartii* after 5 days of growth on solid media. Using a microbiological loop, each cut piece was transferred to the center of a Petri plate (90x15mm) containing PDA medium (~15ml/plate). A sterile filter paper disc (5mm diameter, Filtrack 388) was glued to the inside of the Petri plate lid and was impregnated with volatile oil to obtain final concentrations of 1, 2, 5, 10, 20, 50 and 100 $\mu\text{l l}^{-1}$ air. Immediately after the volatile oil application, the boxes were sealed with parafilm and incubated at 23°C. A cuvette of water was placed in the incubator. The dilution of the oil to obtain concentrations of 1 and 2 $\mu\text{l l}^{-1}$ air was achieved by dissolving the required amount of oil in warm water-agar sterile solution (0.2%). Each concentration was tested in 3 repetitions. The size of the colonies was measured in two perpendicular directions for 7 days. The inhibition of mycelium growth was based on the size of the control colony using the formula: mycelial growth inhibition MGI (%) = $((D_c - D_s) / D_c) * 100$, where D_c (mm) = control colony diameter and D_s (mm) = sample colony diameter.

Determination of insecticidal effects of the volatile oils on *S. granarius* insect - the Granary weevil adults cultured at the Research Development Institute for Plant Protection, Romania, were used in this study. This culture was kept in darkness at room temperature (22±2°C). Round filter papers (6 cm diameter) were impregnated with 10 μl of volatile oil and placed at the bottom of a 250 ml Berzelius glass in which 100 grams of wheat grains were added over which 30 adult insects were released. The Berzelius glasses were immediately placed in 5 L glass jars and immediately capped. The treatments were tested in the dark at 20°C and 55% relative humidity. The control treatments did not include volatile oils. The number of dead *S. granarius* adults was determined at 2, 4 and 7 days after the initiation of the treatment.

Statistical analysis - The effects of volatile oil treatments and concentrations on mycelium growth and the interaction between these factors were analyzed using the two-way ANOVA followed by Bonferroni post-hoc test ($P \leq 0.05$). Statistical data interpretation was performed using the GraphPadPrism5 software.

Results and discussions

The four volatile oils tested showed a strong fungistatic effect (100%) at the concentration of 100 $\mu\text{l/l}$ air two days after inoculation and for basil oils (yellow and red) this effect was maintained at the same level even after 7 days (Figure1). It is also noted that in the first two days, all oils in concentrations of 1 and 2 $\mu\text{l l}^{-1}$ air have a mycelium growth stimulating effect. The inhibitory effect is positively correlated with the tested concentration of all oils. The two-way ANOVA test showed that treatment factors ($df=3$, $F=10.76$, $p=0.003$) and concentrations ($df=6$, $F=198.1$, $p<0.0001$) showed a highly significant influence on mycelium growth. At the concentration of 5 $\mu\text{l/l}$ air, the inhibition percentage caused by the volatile action of red basil oil is significantly higher than that of yellow basil oil ($p<0.05$) and hyssop ($p<0.05$). At the concentration of 10 and 20 $\mu\text{l/l}$ of air, there are no statistically significant differences between mycelium inhibition percentages. At 50 $\mu\text{l/l}$ air, there are significant differences between the yellow basil and marigold treatments ($p<0.05$). Also, the inhibition percentage due to the volatile action of red basil oil on fungal mycelium is very significant compared to that of marigold and hyssop oils ($p<0.01$). At the concentration of 100 $\mu\text{l/l}$ air there are insignificant differences between the treatments with yellow basil, red basil, and hyssop. Compared to these treatments, the marigold oil had the weakest effect of inhibiting the mycelium growth, the difference being very significant.

Studies on the compatibility of essential oils extracted from natural product commonly used in disease management reported various effects on spore germination and growth of *Beauveria sp.* (Mohan *et al.*, 2007; Islam *et al.*, 2010).

No volatile oil produced satisfactory fumigant activity on the adult granary weevils since no mortality was recorded during testing period. This could be related to the low concentration of oil in the air and to the low temperature of incubation.

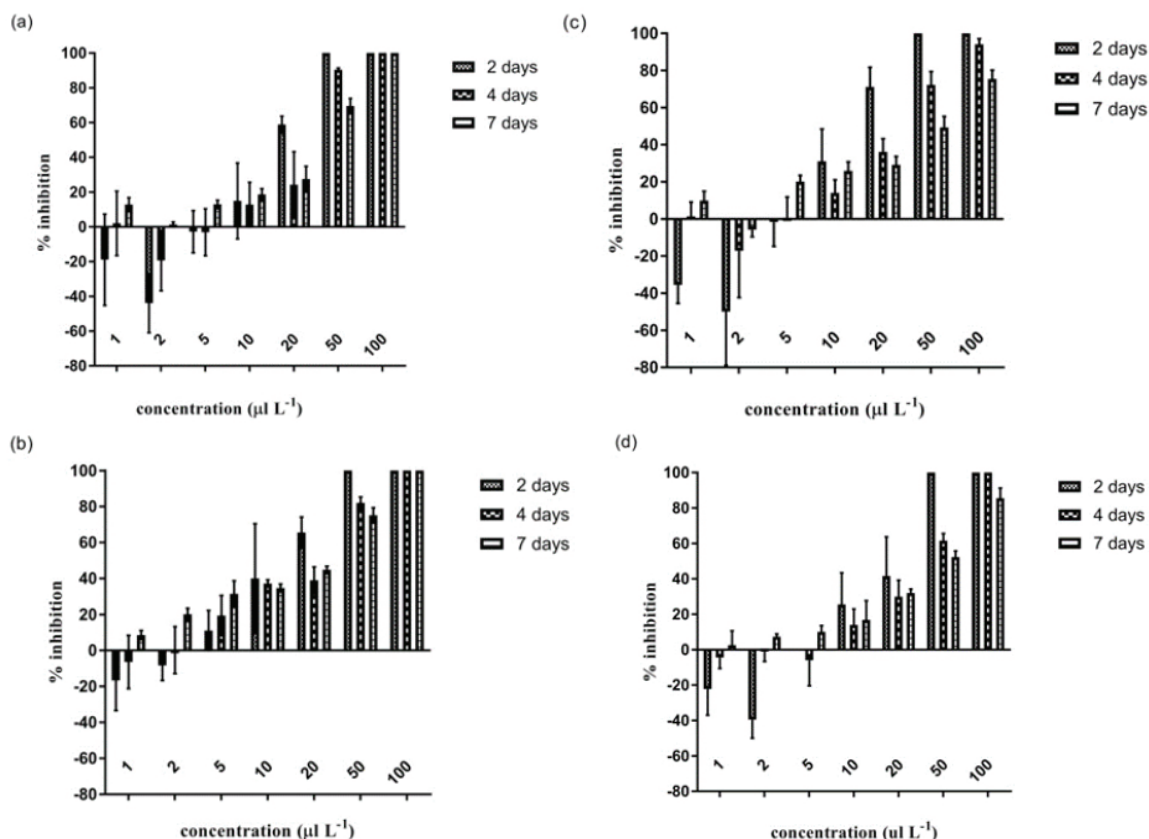


Figure 1. The fumigant effect of Yellow basil (a), Red basil (b), Marigold (c) and Hyssop (d) volatile oils on the mycelial growth of *Beauveria brongniartii*, at different concentrations, after 2, 4 and 7 days

Laznik *et al.* (2012) observed an increased fumigant toxicity of different essential oils against adult granary weevils with temperatures. In the experiment, the efficacy of the essential oils at 40°C was 95%, whereas their efficacy was considerably lower at lower temperatures (from 12 to 36%).

Conclusions

The growth of fungal mycelium was completely inhibited by volatile basil oils tested at the concentration of 100 $\mu\text{l l}^{-1}$ air. At different concentrations, volatile red basil oil showed the most potent inhibitory effect compared to the other oils tested. Concerning the insecticidal action, the preliminary tests with the 4 volatile oils obtained from new varieties of medicinal plants had no effect on the test insect, *S. granarius*. Other values of oil concentration and temperature of incubation should be tested against the pest insect to evaluate the fumigant effect. The results of this study demonstrate the potential of combining two biological control methods, based on essential oils and entomopathogenic fungi products as

an alternative to the use of fungicides in disease management.

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