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Effect of Different Quantities of Wheat on the Effectiveness of the Essential Oil Cineole against Stored Grain Insect Pests

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Abstract: These investigations aimed to determine the effect of wheat grain mass on the effectiveness of the essential oil cineole against adults of *Tribolium castaneumn* (Herbst.), *Rhyzopertha dominica* (F.) and *Cryptolestes ferrugineus* (L.) in an empty space, 50% and 95% spaces occupied with wheat. Concentration of cineole of 50g/m^3 in empty space induced 100% mortality in all three tested insect species. However, fumigation in space 50% occupied with wheat was absolutely effective against *C. ferrugineus*, with 89.5% efficacy against *R. dominica*, and only 11% against *T. castaneum*. In space 95% occupied with wheat mortality of *C. ferrugineus* was 88%, *R. dominica* 64% and *T. castaneum* 4.5% only. The price of natural cineole may be a significant barrier to adoption as a grain fumigant.

Key words: fumigation, cineole, *Tribolium castaneumn*, *Rhyzopertha dominica*, *Cryptolestes ferrugineus*

Introduction

The primary cause of food contamination and environmental pollution arising from agriculture are chemical pesticides[1]. Also, the pesticide residues in grain arising from postharvest treatments[2] come from their non-selective and uncritical application causing the toxic effects in the food and contamination of the environment[3]. With the growing evidence regarding detrimental effects of many of the conventional pesticides on health and environment, require for safer means of pest management has become very crucial[4].

The use of botanical pesticides is now emerging as one of the safer and prime means to protect crops and their products[5,6]. Among botanicals the plant volatile essential oils (EO) are the most frequently studied as pesticides for pests and diseases management [6,7,8]. However, EO, besides needing a large scale demonstration of their efficacy and penetration, need a lot of research in order to determine their toxicological and safety data prior to the registration[9]. Also, as with other groups of insecticides, the potential use of the natural EO in stored grain insect pest management depends on many factors. Some of the factors that may greatly prevent the adoption and use of the natural EO in stored grain fumigation are their relatively high concentrations needed for the effective protection of stored grain against insect pests[8,10], a great difference in the sensitivity of various insect species[10] and current prices of natural essential oils on the market (Korunic and Rozman, unpublished manuscript).

One of effective and safe EO is cineole, the active component of many natural EO such as eucalyptus. It is a cyclic ether with empirical formula $C_{10}H_{18}O$ and systematic name 1,3,3 – trimethyl – 2 – oxabicyclo[2,2,2]octane. Sometimes is traded commercially as "eucalyptol". It is readily biodegradable, un-reactive and relatively non-toxic[11]. Also, it inhibits the enzyme acetylcholinesterase[12], it interferes with sonic communication and mating in leafhoppers[13], and it is a mosquito feeding and ovipositional repellent[14].

Several researchers determined a good fumigant activity of cineole against stored-product insects[15,16,17,8,18,19,20]. Significant effect of grain on the effectiveness of cineole and other EO as well, has been determined by Shaaya et al.[21], Lee et al.[8] and Rozman et al.[22]. They found out that cineole was significantly less effective in a space occupied with wheat grain in the comparison with the effectiveness in an empty space.

The main objective of this research was a determination of the effectiveness of EO cineole in fumigation vessels filled with wheat to 0, 50 and 95% of capacity, against against adults of three stored grain insects. The species tested were the rusty grain beetle, *Cryptolestes ferrugineus* (L.), the lesser grain borer, *Rhyzopertha dominica* (F.), and the red flour beetle, *Tribolium castaneum* (Herbst).

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Materials and Methods

The essential oil used in the experiment was 99% cineole ($C_{10}H_{18}O$) purchased from "Sigma-Aldrich" (Export Division Gr nwalder Weg 30 D – 82041 Deisenhofen, Germany, EC No: 207 – 431 – 5).

Cultures of *C. ferrugineus*, *R. dominica* and *T. castaneum* were reared in the laboratory under controlled conditions (30 ± 1℃, 70% ± 5% r. h.) in darkness. *C. ferrugineus* and *R. dominica* were reared on whole wheat grain and *T. castaneum* on wheat flour containing 10% broken wheat kernels and 5% un-activated yeast.

- The commodity in the experiment was Canadian Western Hard red wheat, clean, with 14% m. c.
- The following combinations had been set up.
- Empty jars 450 mL in volume with 0.5 g of wheat flour and 10 wheat kernels at the bottom with introduced test insects (control).
- Uninfested grains 200 g in 450 mL jars (50% full) with introduced test insects (control).
- Uninfested grain 360 g in a 450mL jar (95% full) with introduced test insects (control).
- Empty jars with 0.5 g of flour and 10 wheat kernels at the bottom with introduced test insects. A piece of treated filter paper with 0.05 g of cineole (50 g/m³) was put on the bottom of the jar. The jar was tightly closed with metal lid.
- Uninfested 200 g of grain in a 450 mL jar (50% full) with introduced test insects. A piece of treated filter paper with 0.05 g of cineole (50 g/m³) was put on the surface of grain. The jar was tightly closed with metal lid.
- Uninfested 360 g of grain in a 450 mL jar (95% full) with introduced test insects. A piece of treated filter paper with 0.05 g of cineole (50 g/m³) was put on the surface of grain. The jar was tightly closed with metal lid.

Each combination was repeated four times. One hundred unsexed adults (2 – 4 wk old) were introduced into each a replicate and each insect species was run separately.

The experiment was carried out under controlled laboratory conditions (30 ± 1℃, 70% ± 5% r. h.) in darkness. The results of the experiment were assessed after 2 days exposure.

All data were subjected to one-way analysis of variance (ANOVA) according to the GLM (general linear model) and LSD test entered in the table. Data processing was conducted by the SAS System for Windows 98. The figures that represent mean values were made by Microsoft Excel 2003.

Results and Discussion

In comparison to the control, fumigation with cineole at the dose of 50 g/m³ proved to be absolutely effective in empty space with achieved mortality of 100% in all three insect species. Fumigation in space 50% occupied with grain was absolutely effective against *C. ferrugineus*, with obtained mortality for *R. dominica* of 89.50%, and for *T. castaneum* 11% only. *C. ferrugineus* had very good response to cineole fumigation in 95% occupied space (88% mortality), *R. dominica* showed mortality of 64%, whilst application to *T. castaneum* proved to be ineffective (4.5%) (Tables 1 – 3).

Table 1. Mortality (%) of *Cryptolestes ferrugineus* adults after 48 h exposure to an application of 50 g/m³ cineole.

Space	Mortality (%) *			
	Control		Cineole 50 g/m³	
	Mean	SD.	Mean	SD.
Space empty	0.50c	0.57	100.00a	0.00
Space 50 % full with wheat grain	1.50c	1.73	100.00a	0.00
Space 95 % full with wheat grain	1.50c	1.73	88.00b	4.96

Table 2. Mortality (%) of *Rhyzopertha dominica* adults after 48 h exposure to an application of 50 g/m³ cineole.

Space	Mortality (%) *			
	Control		Cineole 50 g/m³	
	Mean	SD.	Mean	SD.
Space empty	1.00d	0.81	100.00a	0.00
Space 50 % full with wheat grain	2.50d	1.29	89.50b	3.69
Space 95 % full with wheat grain	0.25d	0.50	64.75c	4.50

* means followed by the same letters are not significantly ($P > 0.05$) different as determined by the LSD – test.

Shaaya et al. [21] assessed the fumigant activities of a large number of essential oils extracted from various spices and herb plants against *T. castaneum*, *Sitophilus oryzae* (L.), *R. dominica* and *Oryzaephilus surinamensis* (L.). The most active was *Labiatae* sp. oil ZP51, at a concentration of

Table 3. Mortality(%) of *Tribolium castaneum* adults after 48 h exposure to an application of 50 g/m³ cineole.

Space	Mortality(%)*			
	Control		Cineole 50 g/m³	
	Mean	SD.	Mean	SD.
Space empty	0.00d	0.00	100.00a	0.00
Space 50 % full with wheat grain	0.00d	0.00	11.00b	1.41
Space 95 % full with wheat grain	0.00d	0.00	4.50c	1.29

* means followed by the same letters are not significantly ($P > 0.05$) different as determined by the LSD – test.

1.4 – 4.5 μL/L air(1.4 – 4.5 g/m³) and exposure time of 24 h causing 90% kill of all insects in space tests. However, in columns 70% filled with wheat, a concentration of 50 μL/L and 7 d exposure were needed to obtain 94 100% kill of the insects.

Lee et al.[8] studied the fumigant toxicity of 42 essential oils and found that six of them extracted from *Eucalyptus nicholi* (Maiden & Blakely), *E. codonocarpa* (Blakely & McKie), *E. blakelyi* (Maiden), Callistemon sieberi (F. Muell.), *Melaleuca fulgens* (R. Br.) and *M. armillary* (R. Br.) were toxic to *S. oryzae*, *R. dominica* and *T. castaneum*. The fumigant toxicity of five oils in the space 50% filled up with wheat was 3 – 5 times lower and in a case of EO extracted from *E. codonocarpa*, nine times lower than in an empty space.

Rozman et al.[22] determined the bioactivity of cineole essential oil against *S. oryzae* on stored wheat in spaces with or without wheat (empty space, 50 and 95% full). A concentration of 50 g/m³ cineole in empty space induced nearly 100% mortality of *S. oryzae*. However, with the fumigation in space 50% filled with wheat there was 57.5% mortality and in space 95% filled with wheat mortality was 34% only.

Our results are in a good agreement with the results of Shaaya et al.[21], Lee et al.[8] and Rozman et al.[22].

Although, we didn't study the reasons for such significant effect of wheat grain on the effectiveness, we believe that the probable cause is a considerable sorption of cineole in wheat grains and poor permeability of cineole vapours into seed inter – space which largely decreased the fumigation effect. According to Korunic and Rozman[10] to gain as similar results as obtained with phosphine and methyl – bromide cineole concentrations should range from 200 – 250 g · m³. Champ and Dyte[23] analyzed the concentrations of phosphine and methyl bromide and phosphine dose of 0.03 g/m³ and methyl – bromide dose of 1 g/m³, if applied in airtight space, were found to be enough to gain LD_{95} for *S oryzae*, while Lee et al.[8] reported required cineole dose of 42 g/m³ to gain LD_{95} for *S. oryzae* in the space 50% full up with the grain. The cost of 1 kg of phosphine pellets is approximately US $41.00, whilst 1 kg of cineole in packages of 100 g reaches about US $236.00. When the highest dosage of phosphine pellets is applied(30 pellets/t) with 1 kg of phosphine it is possible to fumigate approximately 55 t of grain. It means the cost of phosphine to fumigate 1 t of grain is about US $0.74. With 1 kg of 1,8 – cineole it is possible to fumigate 4 tons[10] to about 10 tons of grain[8]. It means the cost of 1,8 – cineole to fumigate 1 t of grain is US $23.60 to US $59.00. Such a considerable effect of grain on the effectiveness of cineole and relatively high price of cineole and other EO (Korunic and Rozman, unpublished manuscript) may greatly increase the cost of the grain fumigation with cineole and other natural EO and make them to expensive to be adopted for wider use.

Conclusions

At the applied dose of 50 g/m³ cineole proved to have varying fumigant effect against adults of the three species tested. It was most effective against *C. ferrugineus*, effective to a lesser degree against *R. dominica*, and least effective against *T. castaneum*. Very good results were gained in a fumigation of an empty space, but results of fumigation of a space occupied with wheat(50 or 95%) tended to be less successful or acceptable. Probable cause could be found in considerable sorption of cineole in wheat grains and poor permeability of cineole vapours into seed interspace and into grains, which largely lessen fumigation effect.

The effect of grain on significant decreasing of the effectiveness of cineole and relatively high price of cineole and other natural EO make them too expensive to be adopted for wider use.

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