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Bioactivity of 1,8 – cineole, camphor and carvacrol against rusty grain beetle (*Cryptolestes ferrugineus* Steph.) on stored wheat

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Abstract

The rusty grain beetle is the most prevalent pest in silos and mills in Croatia. These investigations aimed to determine bioactivity of 1,8-cineole, camphor, and carvacrol against rusty grain beetle under laboratory and storage conditions. Seven-day laboratory tests of fumigant activity in 5 rates (0.1; 1; 10; 50; 100 ml/720 ml vol.) obtained 100 % mortality with 1,8-cineole and camphor even at the lowest dose, while carvacrol obtained 100 % mortality at the dose of 10 ml/720 ml vol. Under storage conditions during 24-day exposure and dose of 200 ml/5 kg of wheat 100 % mortality was obtained in16 days with 1,8-cineole, and with camphor in 24 days, while carvacrol obtained 65 % mortality at the end of the testing. Tested isolates did not affect rheological properties of the flour. However, in treatment with carvacrol flour retained certain scent residues. Such investigations enable new alternatives to be found as substitutes for fumigants applied so far.

Key words: Cryptolestes ferrugineus, 1,8-cineole, camphor, carvacrol, fumigant activity, stored wheat.

Introduction

Cryptolestes ferrugineus (Stephens) is a secondary species of storage pests whose population in silos and mills in Croatia has been rising rapidly in the past five years and in comparison to the other main storage pests it takes first place (Kalinovic and Rozman, 2002). *C. ferrugineus* does not belong to the group of economically most harmful pests, such as pests of *Sitophilus* genus. However, their growing population and presence of all developmental stages of the species affect directly qualitative properties of the stored goods. Present measures of the pest control are mainly based on application of synthetic insecticides and fumigants. However, their non-selective and uncritical application in storage facilities brings up some serious issues, such as toxic effects to the wheat that is used in diet for people and livestock (Fishwick, 1988), and contamination of the environment (WMO, 1995). In practice in Croatia phosphine is presently in use in storage pests' control. However, fumigation with phosphine should be also limited for evident increase in the resistance of some pest species to a phosphine compound, which has been observed in more than 45 countries (Bell and Wilson, 1995). The cause of the resistance is often non-professional application (short exposure, insufficient hermetization at raised temperature, lowered gas concentration) that support thesis that this above all efficient fumigant should be replaced in the near future. This means that phosphine in use can and has to be limited on the global level. Therefore, there is a necessity to find safe alternatives to the conventional insecticides and fumigants that would protect grain and its products. One alternative are monoterpenes isolated from essential oils of aromatic plants from

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the Mediterranean area of Croatia. The objective of these investigations is to detect bioactivity of 1,8-cineol, camphor and carvacrol isolated from essential oils of local aromatic plants of *Lamiaceae* and *Lauraceae* genus, as follows, *Lavandula angustifolia* Ch**.**, *Rosmarinus officinalis* L**.**, *Thymus vulgaris* L**.** and *Laurus nobilis* L., to control *C. ferrugineus* in stored wheat.

Materials and methods

Essential oils, compounds, gas chromatography

The essential oils of the aromatic plants *Lavandula angustifolia* Ch**.**, *Rosmarinus officinalis* L**.**, *Thymus vulgaris* L**.** and *Laurus nobilis* L. were purchased from"Ireks aroma", Zagreb, Croatia. To determine the three main compounds present, we applied a method described by Andronikashvili and Reichmuth (2002) and Regnault-Roger et al. (2004). Oils were analyzed by gas chromatography, using a "Perkin-Elmer" (USA) GC – type 8,700, fitted with a "Supelco" (USA) SP-2380 capillary column 30 m, i.d. 0.32 mm, 0.20 ì m film thickness.

Chemicals (Compounds)

The three main compounds: 1,8-cineole - (eucalyptol, $C_{10}H_{18}O$, EC No: 207-431-5); camphor - ((1R)-(+) camphor 99 %, $C_{10}H_{16}O$, EEC No: 207-355-2) and carvacrol – (98 % 5-isopropyl-2-methylphenol, $C_{10}H_{14}O$, EC No:207-889-6) discovered in oils of the aromatic plants analyzed were purchased from "Sigma-Aldrich" and used for tests in bioassay.

Insects

Culture of the rusty grain beetle – *C. ferrugineus* was collected from the granaries in the eastern part of Croatia. They were reared in the laboratory on mixture of whole wheat flour and maize flour at the rate of 1:1 in glass containers containing 0.5 kg of the mixture with 12 % m.c. The species was reared at 29 ± 1 °C and 70 ± 5 % r.h. in darkness to obtain the first generation (F1) for use in bioassays.

Bioactivity of 1,8-cineole, camphor and carvacrol

Bioactivity of 1,8 – cineole, camphor and carvacrol was assessed by evaluation of fumigant activity against *C. ferrugineus* under laboratory and storage conditions.

Fumigant activity – 7 days laboratory test

To determine fumigant activity of the compounds tested a modified procedure described by Prates et al., 1998 was used. In silk mesh cages that were put into separate glass jars of 720 ml capacity containing 250 g of wheat of 12 % m.c. four replicates of ten adults of *C. ferrugineus* species were placed. 1,8-cineole, camphor and carvacrol were tested at 5 dose rates (0.1; 1; 10; 50 and 100 ì l/720 ml volume) and over exposure periods of 1, 2, 3, 4, 5, 6 and 7 days under controlled conditions (temp. 29 ± 1 °C, humidity 70 ± 5 %, in darkness). The compounds were applied with a "Hirschman" digital micropipette on filter paper attached to the lids of the tightly sealed glass containers. To determine fumigant activity all the samples were observed daily over the exposure of 1-7 days or until 100 % mortality of test insects, and compared to the untreated control samples.

Fumigant activity – 24 days test in storage conditions

Analyses under storage conditions were done at IPK "Croatia" d.o.o. Silos and Mill, Osijek. Tested market wheat was dried to 12-13 % r.h. in the storage and cooled at the temperature of 10 °C. All the samples each of them weighing 5 kg were packed into two-layered paper bags and infested with 10 *C. ferrugineus* adults that were previously placed into silk mesh cages. 1,8-cineol, camphor and carvacrol were applied at the dose of 200 ml to impregnated porous strips 10 cm in length and incorporated into the prepared wheat samples. Control was infested wheat samples with no treatment. Bags with samples were then sealed with adhesive tape and placed on wooden pallets under storage conditions (temp. 5 – 10 °C; r. h. 65-75 %). Control and observation of the samples to 100 % mortality were done every fourth day by lens on lighted, heated background during 24 hours storage. Bioassay was set in 4 replications.

Qualitative analyses of tested wheat flour

Rheological analyses

Rheological analyses of wheat flour were done at technological laboratory of IPK "Croatia"d.o.o. Silos and Mill, Osijek. Treated samples of wheat flour and control samples weighing 2 kg were analyzed by farinograph and extenzograph according to the standard methods. Analyses were done on 60 % laboratory flour (after cleaning, wheat samples were prepared for grinding by soaking in two phases: I – soaking at 13,5 % m.c. and left aging for 24 h; II phase – soaking at 14 % m.c. ½ h before grinding). Flour samples were then left 5 days for aging and tested for rheological properties. Dough properties from flour obtained in the grinding process (water absorption, dough progress, dough stability, resistention, soften level, quality number, and quality group) were determined by farinograph. Dough elasticity properties (energy, resistance, elasticity, and their relation) were determined by extenzograph. The samples were tested in 4 replications. The results obtained were compared to the control with no treatment.

Scent analyses

To determine scent in the samples tested we used standard organoleptic method described by technologists at «Ireks aroma» d.o.o., Zagreb. The method implies scent evaporation by stirring wheat flour and hot water at the rate of 1: 1 with inhalation of the vapours of each sample during the first 30 seconds, and comparison to the scent vapours of the control with no treatment. Analyses were done at the Department for plant protection, Faculty of Agriculture in Osijek.

Statistical analysis

Mortality data were subjected to a multi-factorial analysis of variance (ANOVA) for each day of the exposure period according to the GLM (general linear model). Significant differences were shown by LSD test (least significant difference) and entered in the tables. Data processing was conducted by the SAS System for Windows 98 and SPSS 11.0 for Windows.

Results

Chemical compounds of the essential oils

Chemical compounds of the essential oils of each aromatic plant were determined by gas chromatography. The nine compounds identified in the essential oil of *L. angustifolia*, *R. officinalis, T. vulgaris and L. nobilis* were entered in Table 1. In this paper 1,8-cineol, camphor and carvacrol were chosen to be presented in the bioactivity analysis.

Bioactivity of 1,8-cineole, camphor and carvacrol

Fumigant activity - 7 days laboratory test

Fumigant activity (Table 2) of 1,8 – cineole and camphor was observed to 100 % mortality of *C. ferrugineus* after the 24 h exposure, at the dose levels of 10; 50 and 100 µl/ 720 ml volume and 50 and 100 µl/ 720 ml with carvacrol (Figure 1). However, 7 days laboratory test of fumigant activity in 5 dose rates (0.1; 1; 10; 50; 100 ml/ 720 ml vol.) obtained 100 % mortality with 1,8-cineole and camphor even at the lowest dose (after 5 and 6 days, as following), while carvacrol obtained 100 % mortality at the dose of 10 ml/720 ml vol. at the same time.

Table 1. Main compounds identified in the essential oils.

Compound	*Lavandula angustifolia* Chaix. lavender	*Rosmarinus officinalis* Linn. rosemary	*Thymus vulgaris* Linn. thyme	*Laurus nobilis* Linn. laurel
1,8-cineole	+	+	+	+
camphor	+	+		
eugenol	+		+	+
linalool	+		+	+
carvacrol			+	
thymol			+	
borneol	+	+	+	
bornyl-acetate	+	+	+	
linalyl-acetate	+			

Table 2. Results of fumigant activity in 7 days laboratory test on *C. ferrugineus.*

Exposure time	dose µl/720ml)	% mortality *C. ferrugineus*[1] Compound 1.8 cineole	camphor	carvacrol
1 day	0.1	75.0[b]	22.5[c]	0.0[d]
	1	95.0[ab]	62.5[b]	45.0[c]
	10	100.0[a]	100.0[a]	82.5[b]
	50	100.0[a]	100.0[a]	100.0[a]
	100	100.0[a]	100.0[a]	100.0[a]
2 day	0.1	80.0[b]	42.5[c]	15.0[d]
	1	97.0[a]	70.0[b]	47.5[c]
	10	-	-	85.0[b]
	50	-	-	-
	100	-	-	-
3 day	0.1	85.0[b]	70.0[b]	55.0[c]
	1	100.0[a]	80.0[b]	60.0[c]
	10	-	-	90.0[b]
	50	-	-	-
	100	-	-	-
4 day	0.1	92.5[ab]	85.0[b]	72.5[b]
	1	-	92.5[ab]	75.0[b]
	10	-	-	97.5[a]
	50	-	-	-
	100	-	-	-

Continue...

Table 2. Continue.

Exposure time	dose µl/720ml)	% mortality *C. ferrugineus*[1] Compound 1.8 cineole	camphor	carvacrol
5 day	0.1	100.0[a]	97.5[a]	80.0[b]
	1	-	100.0[a]	85.0[b]
	10	-	-	100.0[a]
	50	-	-	-
	100	-	-	-
6 day	0.1	-	100.0[a]	90.0[b]
	1	-	-	90.0[b]
	10	-	-	-
	50	-	-	-
	100	-	-	-
7 day	0.1	-	-	95.0[ab]
	1	-	-	97.5[a]
	10	-	-	-
	50	-	-	-
	100	-	-	-

[1] means in the same column followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test.

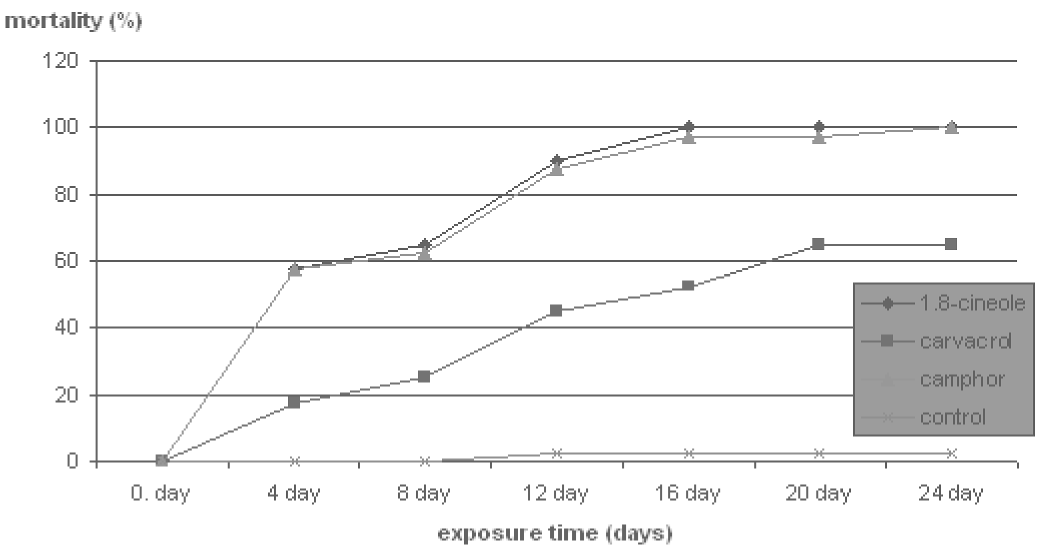

Figure 1. Fumigant activity of 1,8 –cineole, camphor and carvacrol in storage conditions against *C. ferrugineus*.

Fumigant activity – 24 days test under storage conditions

Results of fumigant activity of 1,8 – cineole, camphor and carvacrol against *C. ferrugineus* and under storage conditions proved their bioactivity requiring significantly longer exposure period if comparing to the laboratory test. Significant differences were observed in the first four days in treatments with 1,8 – cineole and camphor (57.5 %

mortality) in comparison to carvacrol and to the control (17.5 % and 0 % mortality, as follows) (Table 3). Under storage conditions, in general, applications of 1,8 – cineole and camphor were similarly effective, without significant differences between them, while application of carvacrol showed as twice as weaker effect then the remaining two compounds. Under storage conditions during 24-day exposure and dose of 200 ml/5 kg of wheat 100 % mortality was obtained in16 days with 1,8-cineole, and with camphor in 24 days, while carvacrol obtained 65 % mortality at the end of the testing. In comparison to the control with no treatment all the three compounds showed significant differences in the mortality of *C. ferrugineus*.

Results of qualitative analyses of tested wheat flour

Rheological analyses

Laboratory analyses of the wheat quality tested

Table 3. Results of fumigant activity in storage condition test on *C. ferrugineus.*

Exposure time	% mortality *C. ferrugineus*[1] Compound			
	1.8 cineole	camphor	carvacrol	Control
	dose 200 µl/ 5 kg of wheat			
	$\bar{x} \pm$			
4 day	57.5 ± 7.0[c]	57.5 ± 7.0[c]	17.5 ± 2.4[e]	0 ± 0.0[f]
8 day	65 ± 6.0[c]	62.5 ± 2.9[c]	25 ± 2.7[e]	0 ± 0.0[f]
12 day	90 ± 3.4[b]	87.5 ± 4.2[b]	45 ± 4.3[d]	0 ± 0.0[f]
16 day	100 ± 0.0[a]	97.5 ± 1.2[a]	52.5 ± 4.6[d]	2.5 ± 0.9[f]
20 day	100 ± 0.0[a]	97.5 ±1.2[a]	65 ± 6.1[c]	2.5 ± 0.9[f]
24 day	100 ± 0.0[a]	100 ± 0.0[a]	65 ± 6.1[c]	2.5 ± 0.9[f]

[1] means in the same column and row followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test.

Table 4. Results of farinograph and ekstenzograph on tested stored wheat.

Treatment	Farinogram						
	Water absorb %	Dough progress min.	Dough stability min.	Resistention min.	Soften level FJ	Quality No.	Quality group
1,8 - cineole	62.7	1.7	0.2	1.9	98	48.1	B2
Camphor	62.7	1.7	0.3	2.0	83	53.3	B2
Carvacrol	63	1.8	0.3	2.1	86	50.9	B2
Control	62.7	1.7	0.3	1.9	80	51.6	B2

Treatment	Ekstenzogram			
	Energy cm^2	Resistence EJ	Elasticity mm	Relation EJ/mm
1,8 - cineole	55.1	307	122	2.52
Camphor	61.4	324	128	2.53
Carvacrol	66.4	320	130	2.46
Control	61.5	325	135	2.44

showed that tested isolates did not affect rheological properties of the flour. Results of the ekstenzograph analyses (Table 4) show that the treatments with 1,8 – cineole, camphor and carvacrol did not exhibit negative effect on the dough quality during the elasticity process, and did not have negative effect on dough properties tested by farinograph. Such results were expected as wheat quality is a complex feature that is genetically inherited, and could be simultaneously exposed to the external factors, in our case, application of essential oil compounds.

Scent analyses

Results of scent analyses show that in treatment with carvacrol flour retained certain scent residues (Table 5), while in the treatments with 1,8 – cineole and camphor no scent residues remained in tested wheat flour.

Table 5. Results of scent analyses in tested wheat flour.

Treatment	Scent
1,8 - cineole	-
Camphor	-
Carvacrol	+
Control	-

Discussion

C. ferrugineus as presently most prevalent pest species in Croatian silos and storages showed very high susceptibility to fumigant activity of 1,8 – cineole, camphor and carvacrol isolated from essential oils of local lavender, laurel, rosemary and thyme. The species showed positive reaction to the treatment under storage conditions, particularly to the application of 1,8 – cineole and camphor. Very few foreign authors investigated about the influence of terpenoids to the rusty grain beetle. Some authors emphasised that the species was susceptible to thymol (Suya et al., 1998).In our studies, as a whole, 9 compounds were isolated from essential oils of lavender, laurel, rosemary and thyme, and examined. However, for extensive data obtained, we were forced to present three most effective compounds. Our results placed thymol on the fifth place regarding its efficiency, but it does not mean that the compound is not effective against the pest species.

By comparing the fumigant activities against *C. ferrugineus* of 3 essential oil compounds, 1,8-cineole was arguably the most effective, followed by camphor and carvacrol. Also, results of the analyses by using a gas chromatograph (Table 1) proved 1,8-cineole was present in all essential oils (lavender, rosemary, thyme and laurel) which strongly suggested that it was one of the principal active substances in these oils.

On the basis of the positive results of the analyses, new preparation based on the particular compounds would be patented as a carrier of the toxic effect observed. In general, aromatic plants contain essential oils only in concentrations of 1 – 3 % (Çakir, 1992). Large quantities of plant material would need to be processed to gain enough essential oil for commercial-scale tests. However, certain compounds in the oils exhibit much stronger activity than others. Plant varieties should be sought that produce these compounds in larger quantities, or synthetic production methods should be explored as an option to gain enough material for full-scale use. The preparation would be applicable in the practice of stored pests’ control, as well as in the ecologically acceptable plant protection.

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