Contact and fumigant activity of 1,8-cineole, eugenol and camphor against *Tribolium castaneum* (Herbst)

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Abstract

The red flour beetle, *Tribolium castaneum* (Herbst), holds a significant place in Croatia by causing considerably damages on stored products. This study was initiated in order to test contact and fumigant activity of three essential oil compounds (1,8-cineole, camphor and eugenol) for the control of adults of this stored-pest species. Contact toxicity of compounds was tested at four doses (0.2, 1.0, 5.0 and 10.0 µL/adult) and mortality was recorded every 2, 4 and 24 h after the application. The most effective compound was 1,8-cineole by its fastest action (2 h after application), with maximum mortality at the lowest dose (0.2 µL/adult). With its prolonged effect of 4 h, eugenol resulted in mortality of 87.5% at the dose of 0.2 µL/adult, while camphor obtained the highest mortality (78.5%) just after 24 h, and at the highest tested dose (10.0 µL/adult). Fumigant toxicity of compounds was tested at three doses (30, 60 and 120 µL/350 mL vol.) and after 48-h of exposure time; mortality was recorded every 24 h until the "end point mortality" (when no time-dependent changes in mortality occurred). The highest mortality (98.5%) had 1,8-cineole at the lowest dose (30 µL/350 mL vol.), followed by camphor (93.5%) at the highest dose (120 µL/350 mL vol.), while eugenol had no statistical significance in the control of *T. castaneum* adult by application of this fumigation method. Such investigations make positive contribution to new possible alternatives to conventional insecticides and fumigants used in protection of stored cereals.

Keywords: *Tribolium castaneum,* 1,8-cineole, Camphor, Eugenol, Contact and fumigant activity

1. Introduction

Synthetic insecticides and fumigants are being commonly used in stored products protection today. However, by reason of numerous side-effects such as toxic residue on cereals, environment pollution, pests' resistance, etc., many active compounds that have been used as fumigants are being withdrawn from the insecticide market. Since 2005, methyl bromide, a fumigant with broad range of activity, has merely been licensed for control of the pinewood nematode *Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle and the Asian long-horned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambyeidae) in wooden packaging for export purposes (Korunić, 2009); until 2015 its application will be permanently suspended (Montreal Protocol on Substances that Deplete the Ozone layer) (UNEP, 1994). Phosphine gas is another fumigant that is applied worldwide, mainly in fumigation process of cereals, dried fruit, nutmeg, cacao, rice and coffee, and presently being reassessed according to the regulations of EU and USA (Bell, 2000).

All these necessitate further findings on pest control methods that are effective and harmless for environment. Among them, botanical insecticides take an important role. Moreover, plant extracts contain substances that have varied influence on insects: ovicidal, repellent, toxic; they can act as sterilants or exhibit antifeedant effect (Nawrot and Harmatha, 1994). Many authors have reported their research studies into the insecticidal efficacy of the number of essential oils and plant extracts in the control of the major stored pests (Regnault-Roger et al., 1993; Pascual-Villalobos and Robledo, 1999., Huang et al., 2000; Papachristos and Stamopoulos, 2002).

One constituent of essential oil obtained from leaves of eucalyptus plant which can vary in quantity is 1,8 cineole (Gibson et al., 1991). As a natural plant extract it is of low toxicity for mammals and regularly used in many assessments of toxicity to stored pests. Prates et al. (1998) have proved that monoterpenes, 1,8 cineole (one of compounds of eucalyptus essential oil) and limonene (one of compounds of lemon essential oil) have significant insecticidal effect against *Rhyzopertha dominica* F. (Coleptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) by exhibiting contact and fumigant activity, and an antifeedant effect.

Owing to the low toxicity to warm-blooded mammals and high volatility, essential oils represent one of the alternatives to the fumigants that are presently used in stored cereal protection (Shaaya et al., 1991; Shaaya et al., 1997; Li and Zou, 2001). The aim of present study was to test contact and fumigant effects of the three essential oil compounds, 1,8-cineole, camphor and eugenol on the adults of red flour beetle, *T. castaneum.*

2. Materials and methods

2.1. Test insects

In this study we tested 2- to 4-wk-old *T. castaneum* adults of mixed sex. Test insects were reared on the medium of wheat rough flour and dried yeast at the ratio of 10:1, in a growth chamber at 30±1 ºC; 70-80% r.h., in darkness, following the method of Liu et al. (1999).

2.2. Contact toxicity

Determination of contact toxicity of essential oil compounds was done by the method of Huang et al. (2000). Compounds (1,8-cineole, eugenol and camphor) were purchased from “Sigma-Aldrich” (Export Division Grünwalder Weg 30 D-82041 Deisenhofen, Germany) and “Fluka“ (Industriestrasse 25, CH-9471 Buchs, SG Switzerland); and tested at four doses (0.2; 1.0; 5.0 and 10.0 µL/adult). The compounds were applied with “Kartell” micropipette to the thorax of the adults. The insects were placed into Petri dishes and transferred in clime chamber at 30±1 ºC, and 70-80% r.h.; mortality was recorded every 2, 4 and 24 h after the application. Each treatment with 100 adults was replicated 4 times. Suspension of camphor was prepared by mixing camphor in crystal form with 96 % alcohol (ethanol) at the ratio of 1:1 and applied at 4 previously mentioned doses. Control samples relevant for the four suspension doses were 0.2; 0.5; 2.5 and 5.0 µL/adult, as follows. Control relevant for 1,8-cineole and eugenol was a sample without treatment.

2.3. Fumigant toxicity

Determination of fumigant activity of 1,8-cineole, camphor and eugenol was done by somewhat modified method described by Huang et al. (1997). Fifty *T. castaneum* adults per sample, 2 to 4 wks old, and of mixed sex, were placed into silk mesh cages containing mixture of some flour and yeast, and put into glass jars of 350-mL capacity. Each isolate was applied at three doses (30, 60 and 120 µL/350 mL vol.) with “Kartell“ micropipette on to filter paper attached to the lids of the glass containers; and applied in 4 replications. After application tightly sealed containers were kept 48 h under controlled conditions at 30±1 ºC and 70-80% r.h., in darkness. Single adults of *T. castaneum* were placed in jars on new medium for 7 d under the same conditions and recorded for total mortality.

2.4. Statistical analysis

Statistical data analysis was done by analysis of variance (ANOVA) following the GLM model for test-insect mortality per compounds tested, and to test doses. Significant differences were identified by LSD tests at 5% level in SPSS 11.0 programme for Windows.

3. Results

3.1. Contact toxicity

Two hours after application at the lowest dose of 0.2 µL/adult, 1,8-cineole exhibited 100% mortality of *T. castaneum* adults, which proved the fastest activity at the lowest dose. Second by efficacy was eugenol (74.7%) with significantly higher differences in comparison to the control (0%) and camphor (5.0%). By dose increase of eugenol from 0.2 to 1.0 µL/adult 100% mortality was obtained, while camphor exhibited significantly lower mortality (7.7%) in comparison to the first two compounds, and significantly higher mortality in comparison to the control (0%). By dose increase of camphor to 5.0 and 10.0 µL/adult, after

2-h application no significant changes in mortality (7.2% and 6.2% respectively) were recorded in comparison to the 1,8-cineole and eugenol (Table 1).

Table 1 Mortality (%) of *T. castaneum* adults 2 h after contact toxicity test at four doses of 1,8-cineole, eugenol, and camphor.

Treatments	Average mortality* (%) ± SE			
	0.2 µL /adult	0.2 µL /adult	0.2 µL /adult	0.2 µL /adult
1,8-cineole	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Eugenol	74.7 ± 5.1b	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Camphor	5.0 ± 1.2c	7.7 ± 0.8b	7.2 ± 0.4b	6.2 ± 1.3b
Control 0	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
Control 1**	0.0 ± 0.0d	0.5 ± 0.2c	0.0 ± 0.0c	0.5 ± 0.5c

*Means in the same column followed by the same letters are not significantly different (P>0.05) as determined by LSD test. **Control relevant for suspension of camphor at the following ethanol doses: 0.2, 0.5; 2.5 and 5.0 µL/adult

Four hours after application camphor exhibited significantly higher mortality at all four doses (13.2%, 20.2%, 19.0 and 18.0%, respectively) in comparison to the control, but was insufficiently effective to control adults of the test-species (Table 2).

Table 2 Mortality (%) of *T. castaneum* adults 4 h after contact toxicity test at four doses of 1,8-cineole, eugenol, and camphor.

Treatments	Average mortality* (%) ± SE			
	0.2 µL /adult	0.2 µL /adult	0.2 µL /adult	0.2 µL /adult
1,8-cineole	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Eugenol	87.5 ± 5.6^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Camphor	13.2 ± 3.4^{b}	20.2 ± 1.1^{b}	19.0 ± 0.7^{b}	18.0 ± 0.0^{b}
Control 0	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
Control 1**	0.2 ± 0.2^{c}	1.2 ± 0.6^{c}	0.0 ± 0.0^{c}	0.7 ± 0.7^{c}

*Means in the same column followed by the same letters are not significantly different (P>0.05) as determined by LSD test. **Control relevant for suspension of camphor at the following ethanol doses: 0.2, 0.5; 2.5 and 5.0 µL/adult

After 24 h contact activity of camphor the mortality values were obtained by 68.0, 74.7, 74.2 and 78.5% at the dose of 0.2, 1.0, 5.0 and 10.0 µL/adult, respectively (Table 3). The mortalities for camphor were significantly lower at all applied doses than those for 1,8-cineole and eugenol with 100% mortality. High contact activity against *T. castaneum* adults for camphor would be possible only with prolonged exposure time (after 24 h), and at the dose of 10.0 µL/adult or higher.

Table 3 Mortality (%) of *T. castaneum* adults 24 h after contact toxicity test at four doses of 1,8-cineole, eugenol, and camphor.

Treatments	Average mortality* (%) ± SE			
	0.2 µL/adult	0.2 µL/adult	0.2 µL/adult	0.2 µL/adult
Cineole	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Eugenol	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Camphor	68.0 ± 2.1^{b}	74.7 ± 6.3^{b}	74.2 ± 3.3^{b}	78.5 ± 6.8^{b}
Control 0	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{c}	0.0 ± 0.0^{d}
Control 1**	2.7 ± 1.1^{c}	4.75 ± 1.1^{c}	2.0 ± 1.0^{c}	3.0 ± 0.5^{c}

*Means in the same column followed by the same letters are not significantly different (P>0.05) as determined by LSD test. **Control relevant for suspension of camphor at the following ethanol doses: 0.2, 0.5; 2.5 and 5.0 µL/adult

3.2. Fumigant toxicity

Results of fumigant activity of three tested compounds were shown as total mortality of *T. castaneum* adults after 7-d observation. At the lowest dose (30 µL/350 mL vol.) the highest mortality was recorded with 1,8 cineole (98.5%) with significant differences in comparison to the control after 48 h (Table 4), while the treatments with eugenol and camphor at the same dose showed no significant differences in comparison to the control. This proved application of 1,8-cineole at the dose of 30 µL/350 mL vol. valid for use by this method of fumigation.

Table 4 Total mortality (%) of *T. castaneum* adults resulting from 48-h laboratory fumigation at three doses of 1,8-cineole, eugenol, and camphor.

Treatments	Total average mortality* (%) ± SE		
	30 µL/350mL vol.	**30 µL/350mL vol.**	**30 µL/350mL vol.**
1,8-cineole	98.5 ± 1.5[a]	100.0 ± 0.0[a]	100.0 ± 0.0[a]
Eugenol	0.5 ± 0.5[b]	0.0 ± 0.0[c]	0.5 ± 0.5[b]
Camphor	3.5 ± 2.2[b]	7.0 ± 0.5[b]	93.5 ± 3.2[a]
Control 0	0.5 ± 0.5[b]	0.5 ± 0.5[c]	0.5 ± 0.5[b]
Control 1**	0.0 ± 0.0[b]	0.0 ± 0.0[c]	2.0 ± 0.8[b]

*Means in the same column followed by the same letters are not significantly different (*P*>0.05) as determined by LSD test. **Control relevant for suspension of camphor at the following ethanol doses: 0.2, 0.5; 2.5 and 5.0 µL/adult

By increasing dose of camphor from 30 to 60 µL/350 mL vol. mortality of 7.0% was recorded with significant differences in comparison to eugenol and the control, but still not effective enough in comparison to 1,8-cineole (100%). High fumigant activity of camphor on *T. castaneum* adults (93.5%) was obtained at dose of 120 µL/350 mL vol. while it was not significantly different from that for 1,8-cineole. At all the three doses tested mortality for eugenol was not significantly different from that for the control. This result indicated that eugenol had a weak fumigant activity on *T. castaneum* adults.

4. Discussion

Essential oils can consist of hundreds of different compounds, but only a few can be found in greater quantity in single oil, or in different oils. Certain compounds can exhibit significantly higher activity than the essential oil in its entirety (Tunç et al., 2000). Thus Rozman et al. (2006), on the basis of the results obtained by gas chromatography, came to the conclusion that 1,8-cineole was the principal active compound in four oils tested (oils of lavender, rosemary, thyme and laurel). By studying fumigant toxicity of plant essential oils from Myrtaceae family, Lee et al. (2004) also reported that the majority of the oils showing potential fumigant toxicity were rich in 1,8-cineole.

By examining fumigant toxicity of essential oil compounds from Lamiaceae and Lauraceae families Rozman et al. (2007) have proved *T. castaneum* adults as highly tolerant of the tested compounds. Not sooner than 7 d of exposure only 1,8-cineole at the highest dose (100 µL/720 mL) obtained acceptable efficacy (92.5%), against *T. castaneum* species, followed by camphor (77.5%) and linalool (70.0%).

In our studies by fumigating adults with 1,8-cineole, we obtained mortality of 98.5% even with the lower dose (30 µL/350 mL vol.).

Quintai and Yongcheng (1998) proved contact efficacy of camphor in the control of R. *dominica, Sitophilus zeamais* Motschulsky and *T. castaneum*, reporting that the isolate responded only as a repellent with no insecticidal effect against *T. castaneum*. Our investigations proved that camphor by its contact activity could exhibit insecticidal effect against the adults of the species, inducing 78.5% mortality, but only at the highest dose (10.0 µL) and prolonged exposure time to 24 h.

In general, based on above obtained results of contact and fumigant activity of the three compounds tested against *T. castaneum* adults the most effective one was 1,8-cineole. The other compounds were effective only in one method: eugenol in contact, and camphor in fumigant application.

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