BIOACTIVITY OF 1,8-CINEOLE AGAINST RED FLOUR BEETLE TRIBOLIUM CASTANEUM (HERBST)

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BIOACTIVITY OF 1,8-CINEOLE AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST), PUPAE

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SUMMARY

Red flour beetle Tribolium castaneum (Herbst) is a major pest of stored products. The aim of this study was to assess the potential fumigant effects of 1,8-cineole, essential oil component, on the T. castaneum pupae. The compound was tested in 6 doses; in two treatments (fumigation without grain and with wheat grain), exposed for 48 h, in 4 repetitions, for each gender. The compound 1,8-cineole had lethal effect on the treated pupae at both genders and in the both treatments. Total proportion of the normally developed beetles was decreased. In addition, 1,8-cineole had also a growth regulator effect, producing adultoids and deformed units, with males more susceptible. In the treatment with the grain there were significant lower dead pupae, normally developed live male beetles and also deformed female units in the stage 2. In general, compound 1,8-cineole has multiple effect against T. castaneum in pupal stage.

Key-words: botanical insecticide, 1,8-cineole, Tribolium castaneum, pupal stage, fumigation

INTRODUCTION

For stored pest control, synthetic insecticides and fumigant are mostly in use today. In consequence of numerous side effects (toxic residues in cereals, environmental pollution, insect resistance etc.) large number of active components which had been in use as fumigants, are in the process of withdrawal from insecticide market. All things considered, there is a need for new cognitions of pest control methods which would be effective and without harmful influence on human environment. Among them, botanical insecticides represent notable place. These are natural extracts that provide chemical protection of plants against harmful organisms.

Plants produce different secondary metabolites with varied influence on pests: repellency, antifeedant effect, negative effect on egg hatching, inhibitory effect on growth, development and reproduction. Presently there are commercial botanical insecticides such as end products or extracted plant isolates as azadirachtin – an isolate from the seed of *Azadirachta indica,* with market name „Rakshak Gold“ and „Plasma Power“. One of the advantages of botanicals compared with synthetic insecticide applications is a low toxicity for mammals and fast degradability. The compound 1,8-cineole, a constituent of essential oil extracted from eucalyptus leaves, has low toxicity for mammals and is in regularly use for many assessments of toxicity to stored pests. Prates et al. (1998) determined that monotherpenes 1,8-cineole and limonene (derivates from the essential oils of lemon) have significant insecticidal effect on two stored pests (*Rhyzopertha dominica* Fab. and *Tribolium castaneum* Herbst), with contact, fumigant and antifeedant effect. But, in spite of all the mentioned,

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botanical insecticide presents just 1% of the world insecticide use (Rozman et al., 2006).

In general, it is known that diapause in insect life cycle is served as a mechanism for surviving during the unfavourable conditions. Clearly that period enables higher resistance compared with insects without state of arrested development. Moreover, pupal stage is high tolerant on toxic compounds and other methods that are in use for stored products protection (Bell, 1994). Testing monoterpenes influence on pupal stage, revealed one more effect of those natural compounds. Thus, monoterpenes toxicity has all characteristics of juvenile hormone. By the influence on the morphogenesis process in the pupa; appearance of adultoids as well as deformed adults, monoterpenes directly affect insects hormonal system similar to effect of insect growth regulators (Bowers, 1969, Schwarz et al, 1970). The aim of this study was to test fumigant effect of 1,8-cineole on pupae of *Tribolium castaneum* (Herbst), for both genders, in fumigant treatments in empty space and in space filled with wheat grain.

MATERIAL AND METHODS

Insects were reared in controlled conditions under 30±1 °C; 70-80% RH; in darkness (Liu et al., 1999), on food mixture of wheat flour and dry yeast (10:1).

Pupae rearing: population of *T. castaneum* adults, mixed genders, were placed in jars with food mixture for three days in controlled conditions with the purpose of copulation and laying eggs. After that period, adults were moved while flour with oviposited eggs was left 20-25 days under the same condition until pupae developed. Pupae (1-3 days old) were separated by gender with stereo zoom loupe with digital camera and software Olympus SZX12.

Fumigation treatments: fumigation was conducted as two types of treatments: treatment in empty space (without wheat grain) and treatment in space filled with wheat grain (up to 50% capacity). Twenty pupae separated by gender were placed into silk mesh cages, in 4 repetitions. Cages were placed into glass jars of 350 ml volume, empty for treatment without grain or filled with grain. The compound 1,8-cineole was tested in 6 doses (30, 60 and 120 μl 350 ml^{-1}vol. for treatment in empty space and 120, 300 and 600 μl 350 ml^{-1}vol. for treatment in filled space). The tested compound was applied with Kartell micropipette on filter paper attached to the lids of the glass jars which were tightly sealed during the fumigation process and kept under controlled conditions for 48 h. Fumigant effect of 1,8-cineole was determined by mortality and insect growth activity according to the scale provided by Mandava (1985) (Table 1). According to the scale, a number of insect individuals were counted as: dead pupae, adultoids (deformed adults which developed from treated survived pupae), and normally developed adults without any deformities. Transition forms of pupae-adult or so called adultoids are expressed by the fore body parts like adult, pigmented, with spread forewings and hindwings (if they are developed), while abdomen looks like typical pupae and unpigmented.

Table 2. Scale for assesment of *Tribolium castaneum* (Herbst) pupa development stage

Tablica 2. Skala za ocjenu stadija razvoja kukuljice Tribolium castaneum (Herbst)

Development stage description mark	Explanation
0	Dead pupa
1	Transition stage from pupa into adult (adultoid)
2	Adult with deformities
3	Normally developed adult

Statistical analysis: results of fumigant efficiency of 1,8-cineole were reached in SAS/STAT Software 9.1.3. (2002-2003). Within SAS, Interactive Data Analysis module Kolmogor-Smirnov test was used. One-way analysis of variance was conducted in SAS Analyst module by using ANOVA procedure.

Differences were considered as significant at the level of 0.05 and Tukey's Studentized Range (HSD) test was used.

RESULTS AND DISCUSSION

Fumigation treatment in empty space

Male pupae: The compound 1,8-cineole was lethal, thus stopping development of treated male pupae (significant more dead individuals at stage 0, compared to control; Tukey's test, $\alpha=0.05$). In addition, percentage of normally developed adults (stage 3), which developed from treated pupae, were significant decreased (2.5% - 120 μl 350 ml^{-1} vol.; $F=38.30$; $df=3$; $p<0.05$), compared to the control (83.75%) (Table 2).

Table 2 Fumigant efficiency of 1,8-cineole on male pupae of *T. castaneum* in the treatment without grain (scale, according to Mandava, 1985)

Tablica 2. Fumigantna učinkovitost 1,8-cineola na kukuljice T. castaneum muškoga spola u tretmanu bez zrna (skala po Mandavi, 1985.)

Dose μl 350 ml^{-1}vol. ♂	Developmental stage of male pupae *T. castaneum* (%)*						
	Stage 0	Stage 1		Stage 2		Stage 3	
	dead	live	dead	live	dead	live	dead
Control 0	5.00b	3.75a	1.25a	6.25a	0.00a	83.75ac	0.00b
30	16.25b	12.50a	13.75a	5.00a	2.50a	36.25b	13.75a
60	48.75a	17.50a	10.00a	5.00a	1.25a	16.25cb	1.25b
120	70.00a	10.00a	11.25a	3.75a	2.50a	2.50ca	0.00b

*means in the same column followed by the same letters in superscript are not significantly different ($p<0.05$)

Female pupae: The compound 1,8-cineole was efficacy in female control (significant more dead individuals at stage 0, compared to control; Tukey's test, $\alpha=0.05$). Further, 1,8-cineole interfered in metamorphose of female pupae into adults. More percentage of adultoid individuals (live and dead) as well as deformed individuals described as stage 2 (live and dead) were observed on fumigation treatments compared with the control. After fumigation, the percentage of normally developed live adults (27.50% – 120 μl 350 ml^{-1}vol.; $F=32.41$; $df=3$; $p<0.05$) was significantly lower compared to the control (93.75%) (Table 3).

Table 3 Fumigant efficiency of 1,8-cineole on female pupae of *T. castaneum* in the treatment without grain (scale, according to Mandava, 1985)

Tablica 3. Fumigantna učinkovitost 1,8-cineola na kukuljice T. castaneum ženskoga spola u tretmanu bez zrna (skala po Mandavi, 1985.)

Dose μl 350 ml^{-1}vol. ♀	Developmental stage of female pupae *T. castaneum* (%)*						
	Stage 0	Stage 1		Stage 2		Stage 3	
	dead	live	dead	live	dead	live	dead
Control 0	1.25b	0.00b	2.50b	2.50b	0.00b	93.75a	0.00a
30	13.75ab	2.50ab	17.50ab	3.75b	5.00ab	55.00b	2.50a
60	33.75a	1.25b	26.25a	3.75b	17.50a	16.25c	2.50a
120	27.50ab	7.50a	7.50b	25.00a	5.00ab	27.50c	0.00a

*means in the same column followed by the same letters in superscript are not significantly different ($p<0.05$)

Comparison by gender: 48 h after the exposition by 1,8-cineole, a higher susceptibility of male pupae of *T. castaneum* was recorded, expressed through more number of dead male pupae (stage 0) at the dose of 120 μl 350 ml^{-1}vol. (70.0% of male and 27.50% of female pupae) and through the less number of normally developed live individuals (stage 3) at the same dose (2.5% of male and 27.5% of female pupae). Furthermore, females showed higher appearance of deformed individuals (stage 2), compared with males (dead at the dose of 60 μl 350 ml^{-1}vol. and live with 120 μl 350 ml^{-1}vol.) (Table 4).

Table 4. Fumigant efficiency of 1,8-cineole on pupae of *T. castaneum* according to the gender in the treatment without grain (scale according to Mandava, 1985)

Tablica 4. Fumigantna učinkovitost 1,8-cineola na kukuljice T. castaneum, po spolu, u tretmanu bez zrna (skala po Mandavi, 1985.)

Gender	Developmental stage of pupae T. castaneum (%)*						
	Stage 0	Stage 1		Stage 2		Stage 3	
	dead	live	dead	live	dead	live	dead
Dose 30 μl 350 ml^{-1}							
♂	16.25a	12.50a	13.75a	5.00a	2.50a	36.25a	13.75a
♀	13.75a	2.50a	17.50a	3.75a	5.00a	55.00a	2.50a
Dose 60 μl 350 ml^{-1}							
♂	48.75a	17.50	10.00a	5.00a	1.25a	16.25a	1.25a
♀	33.75a	1.25a	26.25a	3.75a	17.50b	16.25a	2.50a
Dose 120 μl 350 ml^{-1}							
♂	70.00a	10.00a	11.25a	3.75a	2.50a	2.50a	0.00a
♀	27.50b	7.50a	7.50a	25.00b	5.00a	27.50b	0.00a

*means in the same column followed by the same letters in superscript are not significantly different ($p<0.05$); comparison is for each dose

Fumigation treatment with grain

Male pupae: all three doses of 1,8-cineole were efficient to male pupae, stopping their development (significantly more dead individuals of stage 0, compared to the control; Tukey's test, $\alpha=0.05$). The 1,8-cineole decreased the percentage of normally developed adults (stage 3), particularly doses 300 and 600 μl 350 ml^{-1} vol. (31.25 and 18.75%, $F=25.00$; $df=3$; $p<0.05$), compared to the ontrol (81.25%). Besides that, 1,8-cineole affected male pupae development, which was expressed by higher percentage of adultoids marked as stage 1 (at the dose of 600 μl 350 ml^{-1} vol.) and deformed individuals of stage 2 (at the dose of 300 μl 350 ml^{-1} vol.) compared to the control for each relevant stage (Table 5).

Table 5. Fumigant efficiency of 1,8-cineole on male pupae of *T. castaneum* in the treatment with grain (scale, according to Mandava, 1985)

Tablica 5. Fumigantna učinkovitost 1,8-cineola na kukuljice T. castaneum muškoga spola u tretmanu sa zrnom (skala po Mandavi, 1985.)

Dose μl 350 ml^{-1}vol. ♂	Developmental stage of male pupae *T. castaneum* (%)*						
	Stage 0	Stage 1		Stage 2		Stage 3	
	dead	live	dead	live	dead	live	dead
Control 0	3.75b	3.75a	3.75b	7.50b	0.00a	81.25c	0.00a
120	30.00a	0.00a	6.75b	3.75b	3.75a	56.25b	0.00a
300	23.75a	0.00a	12.50b	22.50a	6.25a	31.25a	3.75a
600	27.50a	0.00a	37.50a	6.25b	8.75a	18.75a	1.25a

*means in the same column followed by the same letters in superscript are not significantly different ($p<0.05$)

Female pupae: the highest dose of 1,8-cineole was lethal for treated female pupae, whereas a higher percentage of dead individuals, marked as stage 0, was observed compared to the control (31.25%: 0% respectively). All three doses of 1,8-cineole decreased the percentage of normally developed individuals of stage 3. Even the dose of 600 μl 350 ml^{-1} vol. was more efficient than two lower doses. The highest fumigant dose also affected higher appearance of deformed adults (stage 2), ($F=5.89$; $df=3$; $p=0.0104$), compared to the lowest dose and the control treatment (Table 6).

Table 6. Fumigant efficiency of 1,8-cineole on female pupae of *T. castaneum* in the treatment with grain (scale, according to Mandava, 1985)

Tablica 6. Fumigantna učinkovitost 1,8-cineola na kukuljice T. castaneum ženskoga spola u tretmanu sa zrnom (skala po Mandavi, 1985.)

Dose μl 350 ml^{-1}vol. ♀	Developmental stage of female pupae *T. castaneum* (%)*						
	Stage 0	Stage 1		Stage 2		Stage 3	
	dead	live	dead	live	dead	live	dead
Control 0	0.00b	0.00a	2.50a	3.75a	0.00b	93.75c	0.00a
120	18.75ab	1.25a	7.50a	3.75a	3.75b	63.75b	1.25a
300	18.75ab	0.00a	8.75a	10.00a	6.25ab	55.00b	1.25a
600	31.25a	0.00a	11.25a	8.75a	17.50a	28.75a	0.00a

*means in the same column followed by the same letters in superscript are not significantly different ($p<0.05$)

Comparison by gender: 48 h after the exposition to 1,8-cineole, a significant (Tukey's test, $\alpha=0.05$) higher sensibility of *T. castaneum* male pupae was observed in the treatment with wheat grain. Thus higher percentage of deformed male individuals of stage 2 and lower percentage of normally developed male adults, marked as stage 3, were recorded within the treatment with fumigant dose of 300 μl 350 ml^{-1} vol. Furthermore, a higher percentage of dead male adultoids (stage 1) were observed at dose of 600 μl 350 ml^{-1} vol (Table 7)

Table 7. Fumigant efficiency of 1,8-cineole on pupae of *T. castaneum* according to gender in the treatment with grain (scale, according to Mandava, 1985)

Tablica 7. Fumigantna učinkovitost 1,8-cineola na kukuljice T. castaneum po spolovima u tretmanu sa zrnom (skala po Mandavi, 1985.)

Gender	Developmental stage of pupae T. castaneum (%)*						
	Stage 0	Stage 1		Stage 2		Stage 3	
	dead	live	dead	live	dead	live	dead
Dose 120 µl 350 ml^{-1}							
♂	30.00a	0.00a	6.25a	3.75a	3.74a	56.25a	0.00a
♀	18.75a	1.25a	7.50a	3.75a	3.75a	63.75a	1.25a
Dose 300 µl 350 ml^{-1}							
♂	23.75a	0.00a	12.50a	22.50a	6.25a	31.25a	3.75a
♀	18.75a	0.00a	8.75a	10.00b	6.25a	55.00b	1.25a
Dose 600 µl 350 ml^{-1}							
♂	27.50a	0.00a	37.50a	6.25a	8.75a	18.75a	1.25a
♀	31.25a	0.00a	11.25b	8.75a	17.50a	28.75a	0.00a

*means in the same column followed by the same letters in superscript are not significantly different ($p<0.05$); comparison is for each dose

In general, fumigant activity of 1,8-cineole on both genders of pupae *T. castaneum pupae* was expressed in two ways. Firstly, 1,8-cineole was lethal to the pupae stage, so the development into adult was stopped. Secondly, tested compound interfered with metamorphosis of pupae which survived fumigation treatment. As a result of the aforesaid, some of the survived treated pupae developed into adultoids and adults (both genders) with deformations on their body, more or less expressed on the thorax and wings. Among deformed adults, live individuals were observed, but predictably deformed adults would have lower reproduction compared with normally developed adults. Based on the study of pyriproxyphen, a juvenile hormone and its effect on cockroaches, Fathpour et al. (2007) pointed on strong positive correlation between morphogenetic anomalies on the adults wings and their sterility. The appearance of adultoids and deformed adult individuals could be explained by direct influence on hormonal system similar to influence of insect growth regulators (Bowers, 1969; Stall, 1975). That kind of effect was also noticed by Amos et al. (1974) after mixing monoterpenes with food for *T. castaneum* and *T. confusum,* as well as other authors working with hidroprene (Bell and Edwards, 1999; Arthur, 2003; Arthur and Dowdy, 2003).

According to the results in this investigation we can conclude that fumigant activity of 1,8-cineole considerably depends on storage fulfilment with stock. As a result, its activity was greater in empty space than in space 50% filled with wheat grain. The effect of space fulfilment on essential oils efficiency was also noted by other authors (Shaaya et al., 1997; Lee et al., 2004; Rozman et al., 2008). The causes of lower efficiency are: weaker vapour penetration of 1,8-cineole, as well as other essential oil components, into seed interspace, and partly grain absorption of the vapour, which lead to reduction of available amounts of active substance sufficient for high lethal effect on pest. Apparently, these are limiting factors for application of natural compounds in larger amounts of stored products.

CONCLUSION

According to the specified results, 1,8-cineol has high potential for the red flour beetle *T. castaneum* control. It was confirmed that the tested compound had certain lethal effect and influence on morphogenesis, even on pupa, the most resistant insect development stage.

In order to solve a lack of lower efficiency of 1,8-cinole in filled storages, the higher concentrations of 1,8-cineole should be applied in practice.

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BIOAKTIVNOST 1,8-CINEOLA NA KUKULJICE KESTENJASTOGA BRAŠNARA *TRIBOLIUM CASTANEUM* (HERBST)

SAŽETAK

Kestenjasti brašnar Tribolium castaneum (Herbst), je značajan štetnik uskladištenih poljoprivrednih proizvoda. Cilj rada je ispitati fumigantnu učinkovitost 1,8-cineola na kukuljice T. castaneum. Izolat je testiran u 6 doza; u dva tretmana (fumigacija bez zrna i sa zrnom pšenice) s ekspozicijom od 48 sati, u 4 ponavljanja, za svaki spol. Izolat 1,8-cineol je djelovao letalno na tretirane kukuljice kod oba spola i u oba tretmana. Smanjen je ukupni udio normalno razvijenih odraslih brašnara. Također, 1,8-cineol djelovao je i kao regulator rasta kukuljica, stvarajući adultoid i deformirane jedinke, s većom osjetljivošću muškoga spola. U tretmanu sa zrnom značajno je smanjen postotak uginulih kukuljica, normalno razvijenih živih muških jedinki, kao i deformiranih ženskih jedinki u stadiju 2. Može se zaključiti da je izolat 1,8-cineol višestruko učinkovit za suzbijanje T. castaneum u stadiju kukuljice.

Ključne riječi: botanički insekticid, 1,8-cineol, Tribolium castaneum, stadij kukuljice, fumigacija

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