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Fumigant Effect of Oxygenated Monoterterpenes on adults of *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)

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Abstract
The Red flour beetle, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) is a common insect that attacks stored grains and foods. This insect has a world wide distribution. In this study, fumigant effect of oxygenated monoterpenes (Geranyl acetate, Bornyl acetate, Neryl acetate) was investigated on adults of *T.castaneum*. The differences of applications were found statistically significant ($P \leq 0.05$). Results show that Neryl acetate had highest fumigant effect on adults of *T.castaneum*, compared with the negative control. Mortality rate of *T. castaneum* adults was increased significantly, depending on the concentration levels and exposure times. At 72, 96 hours and 20 (µl/petri) concentration, the highest mortality rates of Neryl acetate, Geranyl acetate and Bornyl acetate were determinated as 70% and 96%, 40% and 83%, 31% and 53%, respectively. Mortality rate of positive control (DDVP) was 100% at least concentration and exposure time. LC_{50} and LC_{90} values of Geranyl acetate and Bornyl acetate were calculated as 0.808-1.522, 1.155-2.391 and 1.804- 8.794 (µl/petri) after 96 hours, respectively. Research results suggested that Neryl acetate from the monoterpenes might have potential to be used as fumigant on adults of *T. castaneum.*

Key words: *Tribolium castaneum,* Fumigant Effect, Oxygenated monoterpenes

INTRODUCTION

The confused flour beetle *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) is a major pest of stored products such as flour, cereals, pasta, biscuits, beans and nuts. Economic losses consists of reduced weight and quality, difficulties in baking, reduced marketability of infested products and an accompaying unpleasent smell[1].

Fumigation is one of the most effective methods in protection of stored products. Fumigants such as methyl bromide, phosphine and residual insecticides were the most effective for the protection against insect infestation of stored foods [2]. Presently, because of methyl bromide prohibited, only phosphine is used for fumigant at stored products. However, using of phosphine limitations are also reported [3]. Recent researches were focused on natural product alternatives for pest control in developing countries. Recently, there has been a growing interest in reseach concerning the possible use of plant extracts, essential oil and secondary compounds in plants as alternatives to synthetic insecticides. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects [4], [5], [6], [7] and [8]. Several studies have demonstrated different bio activities of essential oils. For example, insecticide, ovicide, attractant, repellent, antifeedant, growth and reproduction inhibitor [9], [10], [11], [12], [13], [14], [15], [16], [17] and [18]. antibacterial and antifungal effects [19], [20], [21] and [22]. The aim of the study was to evaluate the fumigant effect of oxygenated monoterpenes (Neryl acetate, Geranyl acetate, Bornyl acetate) on adults of *T. castaneum* under laboratory conditions.

MATERIALS AND METHODS

Insects and Rearing Conditions

Tribolium castaneum adults were obtained from laboratory stock cultures maintained on wheat at 25±5^{0}C, %65±5 relative humidity and a completely dark conditons. Wheat took from the department of field crops. Its variety was bezostaja. For *Tribolium castaneum* adult tests, 1-7 days old adults were used. In order to receive adults of the same age, some wheat grains that included larvae of the beetles were placed in 2 liters plastic container. Adults were collected after emergence maximum populations. The compounds tested were Bornyl acetate, Neryl acetate, Geranyl acetate.

Bioassays

In order to test the toxicity of the monoterpenes against *T. castaneum* adults, 20 inviduals with 5 grain of wheat were placed into plastic petri dishes (9 cm diameter). 10 and 20 ml of pure oxygenated monoterpenes were applied with an automatic pipette on a filter paper (2 × 2 cm) attached to the undersurface of cover of petri dish. After that, around of the petri dishes wrapped with parafilm. Control insects were kept under the same conditions. Mortality rate of the adults was determined after 12, 24, 48, 72 and 96 hours of treatment. Sterile pure water was used as negative control while, DDVP was used positive control. Three replicates of each treatment were set up.

Data Analysis

Firstly obtained % values were transformed to arcsine transformation. Differences among the fumigant toxicities of the monoterpenes tested were determined according to analysis of varience (ANOVA) test by using SPSS 17 software package. Differences between percents were tested through Duncan tests and values with $p \leq 0.05$ were

considered significantly different. The median lethal concentration (LC_{50} and LC_{90}) values were calculated according to the method of Finney (1971)[23]. Probit analysis of concentration-mortality data was conducted to estimate the $LC_{50,90}$ values and associated 95 % confidence limits for each treatment (EPA Probit Analysis).

EXPRIMENTAL RESULTS AND DISCUSSION

Fumigant effect of the Neryl acetate, Geranyl acetate, Bornyl acetate was calculated for 12, 24, 48, 72 and 96 hours. The results showed that the oxygenated monoterpenes had fumigant effect on *T. castaneum* adults in comparision with control. Analysis of varience summarized in Table 1. Mortality rate of *T. castaneum* was highly significant depending on dosage level and exposure time ($p \leq 0.05$). As paralel increase of exposure times and applied doses, a significant increase was observed in death of adults. Higher dose and longer exposure time demonstrated the maximum toxcitiy on adults. Neryl acetate had highest fumigant effect from oxygenated monoterpenes on adults of *T. castaneum.* The mortality percentages after 48, 72 and 96 h treatments with maximum dose (20 µl/petri) of Neryl acetate, Geranyl acetate, Bornyl acetate were obtained as 26-70-96%, 33-63-83% and 10-35-53%, respectively (Table 1). Mortality rate of control was remained below 10% even at 96 h. Highest mortality percentages of the monoterpenes were achieve 96 h post treatments.

Table 1. Percentage mortality of oxygenated monoterpenes on adults of *Tribolium castaneum* (Herbst, 1797) at different doses and exposure times under laboratory conditions

Oxygenated monoterpenes	Doses (µl/petri)	Mortality (%) Exposure time (h)				
		12 h	24 h	48 h	72 h	96 h
Neryl Acetate	10	0.0 ± 0.0 b	1.66 ± 1.66 b	25.0 ± 8.66 cb	50.0 ± 2.88 dc	66.6 ± 6.0 b
	20	1.66 ± 1.66 b	3.33 ± 1.66 b	26.6 ± 6.00 cb	70.0 ± 2.88 b	96.6 ± 3.33 a
Bornyl Acetate	10	0.0 ± 0.0 b	0.0 ± 0.0 b	6.66 ± 3.33 d	15.0 ± 5.77 e	31.6 ± 3.33 d
	20	1.66 ± 1.66 b	5.0 ± 2.88 b	10.0 ± 7.6 dc	35.0 ± 5.77 d	53.3 ± 6.66 cb
Geranyl Acetate	10	0.0 ± 0.0 b	3.33 ± 1.66 b	6.66 ± 3.33 d	33.3 ± 4.40 d	40.0 ± 7.63 dc
	20	3.33 ± 3.33 b	6.66 ± 6.66 b	33.3 ± 16.4 b	63.3 ± 20.2 cb	83.3 ± 16.6 a
Positive Control (DDVP)	10	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
	20	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
Negative Control (Steril water)	-	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 d	0.0 ± 0.0 e	1.66 ± 1.42 e

Values followed by different letters in the same column differ significantly at $P \leq 0.05$ according to Duncan Multiple test.

Mean±SE of three replicates, each set up with 20 adults.

Neryl acetate was the most effective monoterpene according to LC_{50} and LC_{90} values 0.808 and 1.522 µl/petri, respectively at 96 h. LC_{50} and LC_{90} values of geranyl acetate and bornyl acetate were 1.155-2.391 and 1.804-8.794 µl/petri, respectively (Table 2).

Table 2. LC_{50} and LC_{90} values (µl/petri) of oxygenated monoterpenes on adults of *Tribolium castaneum* (Herbst, 1797) at 96 h under laboratory conditions

Treatments	LC_{50} [a]	LC_{90} [b]	X^2 [c]	Slope ± SE
Neryl Acetate	0.808	1.522	6.088	4.661 ± 1.177
Bornyl Acetate	1.804	8.794	2.759	1.863 ± 0.777
Geranyl Acetate	1.155	2.391	26.917	4.055 ± 0.840

[a] The lethal concentration causing 50% mortality after 96 hours

[b] The lethal concentration causing 90% mortality after 96 hours

[c] Chi square value

Results of previously conducted studies with this study are similar. The most of the monoterpenes have significant insecticidal effect on adults of *Sitophilus zeamays*. Insecticidal effects of oxygenated monoterpenes were found to be more effective than monoterpene hydrocarbons. Oxygenated monoterpenes have a potential action for control of *S. zeamays* adults [24]. Five monoterpenoids (terpinen-4-ol, 1,8-cineole, linalool, R-(+)-limonene and geraniol) were tested in vapour form against different stages of *Tribolium confusum.* Terpinen-4-ol (LC_{50} values ranging between 1.1 and 109.4 ml/l) was the most toxic to all stages tested, while the least toxic monoterpenoid tested was geraniol with LC_{50} values ranging between 607 and 1627 ml/l [25]. The toxic activity of some characteristic monoterpenoids (*p*-cymene, α-pinene, camphor, linalool, terpineol, cuminaldehyde, cinnamaldehyde, anethole, carvacrol, thymol, estragole and eugenol, was tested against *Acanthoscelides obtectus* (Say). All monoterpenes revealed a more or less vapour toxicity and significantly inhibited beetle reproduction. Oxygenated monoterpenoids (carvacrol, thymol, eugenol, linalool and terpineol were identified as the most efficient compounds in terms of both respects and the structure-activity [26]. The toxicity of a number of essential oils and their components, monoterpenes has been evaluated against stored product insects [27], [28], [29] and [30].

CONCLUSION

In recent years, the intensive use of synthetic pesticides in the control of stored product pests, has come to threaten human health and wildlife by disturbing the ecological balance. So that, researching of alternative methods is speeding up in recent years. The development of natural or biological insecticides will help to decrease the negative effects (residues, resistance and environmental pollution) of

synthetic chemical insecticides. In this respect, bio-insects may be also effective, selective, biodegradable and associated with little development of resistance in the pest population. Among the applied oxygenated monoterpenes, Neryl acetate is could be potential fumigant agent for controlling the adults of *T. castaneum* in stored food products.

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