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POSSIBILITY OF USING SOME MONOTERPENOID COMPOUNDS AS A FUMIGANT FOR CONTROLLING STORED-WHEAT INSECTS

Özgür Sağlam[1*], Nihal Özder[1]

[1]Namık Kemal University, Faculty of Agriculture, Department of Plant Protection Tekirdağ-Turkey

*Corresponding author's e-mail: *osaglam14@hotmail.com*

ABSTRACT

This study was carried out to determine fumigant toxicity of monoterpenoid components; α-pinene, *p*-cymene, eugenol, cuminaldehyde, linalyl acetate, linalool, α-terpinene, gamma terpinene, limonene, β-pinene, allyl isothiocyanate and diallyl disulphide against all life stages of *Tribolium confusum* du Val. and *Ephestia kuehniella* Zell. at 25±1 °C and % 65±5 r.h. In preliminary biological tests all life stages were exposed to 100 µl/l concentration of monoterpenoid compounds for 24 h. Preliminary bioassay tests indicated only allyl isothiocyanate and diallyl disulphide had high fumigant toxicity to all lifestages of *T. confusum*. Whereas, fumigant toxicity to *E. kuehniella* varied with tested monoterpenoid components. Cuminaldehyde, allyl isothiocyanate and diallyl disulphide were highly toxic to *E. kuehniella* eggs while only allyl isothiocyanate had high fumigant toxicity to *E. kuehniella* larvae. For *E. kuehniella* pupae, only allyl isothiocyanate and diallyl disulphide had high fumigant toxicity while all tested monoterpenoid compounds except linalyl acetate and eugenol were highly toxic to *E. kuehniella* adults. The toxicities of the most effective compounds (cuminaldehyde, allyl isothiocyanate and diallyl disulphide) to different life stages of both species in absent and present of commodity were varied. Cuminaldehyde was highly toxic to only egg stage in absent of the commodity, however it had a low toxicity to the eggs placed at bottom position in present of commodity. Diallyl disulphide was shown high toxicity to all life stages of *T. confusum* and *E. kuehniella* (except of larva stage) in absent of the commodity, but it had a very low toxicity in present of the commodity. In conclusion, these results indicated that allyl isothiocyanate would be a potential compound in controlling stored-product insects.

Key words: Monoterpenoids compounds, *Tribolium confusum* duVal., *Ephestia kuehniella* Zell. fumigant toxicity

INTRODUCTION

Insect control in stored products at present relies upon the use of gaseous fumigants and residual insecticides, both of which pose serious hazards to mammals and the environment (Shaaya et al., 1997; Ren et al., 2008). Fumigation is still one of the most effective methods for prevention of storage losses. Aluminum phosphide is the major compound for disinfestation of stored grains. But unlike residual pesticides where new compounds have become available and continue to do so, the development of new fumigants have not been forthcoming (Bell, 2000; Zettler and Arthur, 2000). Recent studies of attention among alternative fumigants are the biofumigants, which reflects the growing attention received by bio- pesticides or biorational pesticides (Isman, 2006; Rajendran and Sriranjini, 2008).

Monoterpenoid compounds had potent fumigant activities against the adults of various stored-product insects (Regnault-Roger and Hamraoui, 1995; Erler, 2005; Huang et al., 2000); however, only limited information is available on the efficacy of monoterpenoids against the immature stages and had been limited information in absent or present of commodity. The present study was carried out to determine the fumigant toxicity of twelve monoterpenoids with different chemical groups; α-pinene, p-cymene, eugenol, cuminaldehyde, linalyl acetate, linalool, α-terpinene, γ-terpinene, limonene, β-pinene, allyl isothiocyanate and diallyl disulphide in absent and present of commodity against all life stages (adult, egg, larva and pupa) of confused flour beetle, *Tribolium confusum* Jacquelin Du Val. and Mediterranean flour moth, *Ephestia kuehniella* Zell. and to discuss the possible use of these monoterpenoids as bio-fumigants against stored-grain insects.

MATERIALS AND METODS

Test insects

Bioassays were carried out on all stages (egg, larva, pupa and adult) of *T. confusum* and *E. kuehniella*. *Tribolium confusum* were obtained from the cultures reared on wheat flour mixed with dry brewer's yeast (17:1, wt:wt) using standard culture techniques (Donahaye, 1990) at 1 l glass jars. *Ephestia kuehniella* were collected from cultures reared on a diet of a 1:1:1 mixture of wheat flour: bran: craked maize at 14 x 20 cm plastic box. Insect rearing and all experimental procedures were carried out in room set at 65± 5 % r.h. and 25± 1 °C, under continuous darkness. The experiments of *T. confusum* were conducted with eggs (24-48 h old), old larvae (30-35 d old), pupae (1-2 d old) and adults (6-7 d old) while for *E. kuehniella;* eggs (24-48 h old), old larvae (30-35 d old), pupa (1-3 d old) and adult (1-2 d old). Eggs were separated from the flour by using a sieve with 70 mesh and old larvae were separated from the other life stages using a sieve with 1 mm openings while pupae and adults were separated by using a sieve with 25 mesh. *Ephestia kuehniella* eggs were obtained from empty oviposition jars containing 40 to 50 adults. Larvae and pupae of *E. kuehniella* were collected by fine pens and adults were collected by vacuum machine.

Tested monoterpenoid compounds

Twelve monoterpenoid compounds; α-pinene (Aldrich, 147524, 98 %), *p*-cymene (Sigma-Aldrich, C121452, 99 %), eugenol (Fluka, 46129, *Ph eur*), cuminaldehyde (Fluka, 28210, 85 %), linalyl acetate (Fluka, 45980, 95 %), linalool (Fluka, 62140, 95 %), α-terpinene (Aldrich, 223182, 85 %), γ-terpinene (Fluka, 86478, 97 %), limonene (Sigma-Aldrich, 183164, 97 %), β-pinene (Aldrich, 112089, 99 %), allyl isothiocyanate (Merck, 800260, 95 %) and diallyl disulphide (Sigma-Aldrich, 317691, 80 %) were tested against all life stages of *T. confusum* and *E. kuehniella*. After purchase, the monoterpenoid compounds were collected in sealed glass containers with frangible septum which allows easy penetration by a syringe needle to permit withdrawal of the compounds and refrigerated in the dark at 4°C until use.

Bioassay and experimental procedures

The single-dose test in absent and present of commodity was carried out to determine the effective concentrations of each compound against the all life stages of *T. confusum* and *E. kuehniella*. Each stage of *T. confusum* and *E. kuehniella* were exposed to a constant concentration of 100 µl l^{-1} air of each compounds for 24 h. For single-dose tests in present of commodity, each stage of *T. confusum* was exposed to a concentration of 100 µl l^{-1} air of monoterponoid compounds while egg and pupa stage of *E. kuehniella* were exposed to concentration of 100 µl l^{-1} and its larva and adult were exposed to concentration of 80 µl l^{-1} and 55 µl l^{-1} of tested monoterponoids respectively. The biological tests of highly toxic compounds were conducted for different life stages of both species placed into two different positions (top and bottom) in present of 2 kg wheat (*Triticum aestivum* L var Flamura 85). Tested stages of *T. confusum* and *E. kuehniella* were collected from the cultures and placed in the glass vials covered with a fine mesh to allow penetration of any volatiles emanating from the monoterpenoid compounds. Fifty eggs and twenty five larvae, pupae and adults were used in each replicate. Single-dose tests in absent and present of commodity were carried out in 3-l and 5-l glass jars respectively. Both closed with metal screw-on lids, which served to as fumigation chambers and were kept at 25±1 °C and 65±5 % r.h. An aqueous saturated magnesium nitrite solution ($Mg(NO_3)_2$) was placed in small glass petri dishes of 7 cm diameter to provide 65±5 % r.h. in the glass jar (Greenspan, 1977). Monoterpenoid compounds were applied on filter paper (2 x 5 cm) attached to the under-side of the lids of fumigation chambers by using 50 µl micropipette. Eggs, larvae, pupae and adults of each species kept in the glass vials were transferred separately into fumigation chambers. The fumigation chambers were closed with screw-on lids. Each treatment and control was replicated three times. For the control treatment, all life stages of each insect species were exposed to atmospheric conditions and were kept at 25±1 °C and 65±5 % r.h.

Data processing and analysis

After each treatment, larvae, pupae, and adults (except *E. kuehniella* adults) were transferred to 50-ml jars containing food medium and were held at 25±1 °C and 65±5 % r.h. until examined for mortality. The eggs in their Perspex slides were held under the same conditions until the oviposition sites were examined for egg hatch. Mortality counts for adults were made 4-5 d after exposure; for larvae they were based on those insects that when failed to pupate 9 d after exposure; pupal mortality was based on those pupae that failed to produce adults 9 d after exposure, and egg hatch was counted 7 d after treatment. Mortality data obtained from preliminary bioassay tests were normalized using arcsine transformation and then were analyzed using two-way analysis of variance (ANOVA). The means were separated by using the Duncan's test at the 5 % level (SPSS, 2009).

RESULTS AND DISCUSSION

In present study, the single-dose tests in absent and present of commodity indicated that tested monoterpenoids with different chemical groups (concentration of 100 µl l^{-1}) had significantly different fumigant activity on all life stages of *T. confusum* and *E. kuehnilla* (P<0.05). Allyl isothiocyanate and diallyl disulphide indicated a strong fumigant activity on all life stages of *T. confusum*, whereas other tested monoterpenoids (expect cuminaldehyde for only egg stage) had very low fumigant toxcicity (Table 1).

Compared with the investigation of Lee et al. (2003) our results indicated a similar low toxicity of limonene and linalool against red flour beetle, *Tribolium castaneum* (Herbst) adults. The high fumigant activity of allyl isothiocyanate to adults of *T. confusum* were similar to other studies with the maize weevil *Sitophilus zeamais* (Motsch.), the lesser grain borer, the book louse *Liposcelis entomophila* (Enderline) , the confused flour beetle and one other study with red flour beetle (Worfel et al., 1997; Demirel et al., 2009; Wu et al., 2009). In this study, cuminaldehyde had a high fumigant activity on *T. confusum* eggs. Isikber et al. (2009) reported that cumin essential oil of which cuminaldehyde is major monoterpenoid compound had high fumigant toxicity to *T. confusum* eggs, which it is similar to our results. The results obtained from single-dose tests in absent of commodity for *E. kuehniella* indicated that cuminaldehyde, allyl isothiocyanate and diallyl disulphide were highly toxic to *E. kuehniella* eggs while only allyl isothiocyanate had high fumigant toxicity to *E. kuehniella* larvae. On the other hand all tested monoterpenoid compounds except linalyl acetate and eugenol were highly toxic to *E. kuehniella* adults (Table 2).

Table 1. Percent mortality of *Tribolium confusum* eggs, larvae, pupae and adults exposed to 100 µl l^{-1} concentration of tested monoterpenoid compounds for 24 h in absent of commodity

Monoterpenoid compounds	Means of Mortality (%) ± S.E.				F and P value
	Egg	Larva	Pupa	Adult	
α-pinene	17.3±4.6 BCDa*	5.4±1.3 CDE b	21.7±3.89 BC a	1.3±1.33 B b	$F_{3, 8}$=12.65 P< 0.002
p-cymene	12±1.15 CD a	6.8±1.33CDE bc	9.5±1.5DEF ab	4±0 Bc	$F_{3, 8}$=9.90 P< 0.005
Cuminaldehyde	100±0 A a	4.2±2.4 E b	6.7±2.63EF b	1.3±1.33B b	$F_{3, 8}$=137.94 P< 0.0001
Linalool	27.3±1.76 B a	5.7±1.32 CDE b	4.1±2.31F b	0±0 B c	$F_{3, 8}$=24.93 P< 0.0001
β-pinene	14.7±2.85 CD a	5.3±2.66 DE bc	9.8±1.1DEF a	1.3±1.33 B b	$F_{3, 8}$=5.21 P< 0.027
Eugenol	16.7±3.52 BC a	18.7±3.52 C a	24±2.3B a	0±0B b	$F_{3, 8}$=17.64 P< 0.001
α-terpine	15.3±3.71CD a	14.1±1.63CD a	17.3±5.8 BCDa	5.3±3.52B a	$F_{3, 8}$=2.32 P=0.152
Linaly acetate	16±4.16 CD ab	5.5±1.41CDE bc	21.8±3.89 BC a	2.7±2.66 B c	$F_{3, 8}$=7.56 P< 0.01
D. disulphide	100±0 A a	92 ±4 B b	100±0 A a	100±0 A a	$F_{3, 8}$=4.00 P= 0.05
A. isothiocyanate	100 ± 0 A a	100 ± 0 A a	100 ± 0 A a	100 ± 0 A a	---**
γ- terpinen	8.7±1.33 DE bc	17.3±4.8 C a	12±2.3CDE a	2.7±2.66B b	$F_{3, 8}$=4.96 P< 0.031
Limonene	9.3±3.52 DE a	17.3±4.8 CD a	9.3±1.33DEF a	8±4 B a	$F_{3, 8}$=1.02 P=0.433
Control	2.7±0.66 E a	1.4±1.38 E a	0±0 G a	2.7±1.33 B a	$F_{3, 8}$=2.17 P= 0.16
F and P value	$F_{12, 26}$=133.03 P< 0.0001	$F_{12, 26}$=51.87 P< 0.0001	$F_{12, 26}$=129.31 P< 0.0001	$F_{12, 26}$=68.51 P< 0.0001	

*Two-way ANOVA was applied to the data. Means within a row with the same lower-case letter and a column with the same upper-case letter do not differ significantly (Duncan test at 5% level).

Single-dose test in present of commodity (100 µl l^{-1}) showed that cuminaldehyde had a low toxicity to the eggs of *T.confusum* and *E. kuehniella*, placed at bottom position of 2 kg of wheat, since it could have weak penetration into the commodity. Diallyl disulphide had a low toxicity to adults, pupae and adults of both tested species placed at bottom position in present of 2 kf of wheat (Fig. 1).

Table 2. Percent mortality of *Ephestia kuehniella* eggs, larvae, pupae and adults exposed to 100 µl l^{-1} concentration of tested monoterpenoid compounds for 24 h

Monoterpenoid compounds	Means of Mortality (%) ± S.E.				F and P value
	Egg	Larva	Pupa	Adult	
α-pinene	2±1.15 CD c*	4±0 BC bc	9.4±3.5 CD b	100±0 A a	$F_{3,8}$=259.5 P < 0.0001
p- cymene	3.3 ±1.33 CD b	6.7 ±2.66 BC b	8.2 ±2. 26 CD b	100 ± 0 A a	$F_{3,8}$=223.38 P < 0.0001
Cuminaldehyde	100±0 A a	10.7±1.33 BC b	13.9±3.46 CD b	100±0 A a	$F_{3,8}$=635.98 P < 0.0001
Linalool	14.7±3.52 B b	14.7±5.81 BC b	13.3±5.85 CD b	100±0 A a	$F_{3,8}$= 74.18 P < 0.0001
β-pinene	6.7 ± 1.33 BC c	16 ±2.30 BC b	17.4 ±3.34 C b	100 ± 0 A a	$F_{3,8}$=397.31 P < 0.0001
Eugenol	4±2.3 CD a	2.9±2.89 BC a	6.8±2.83 CD a	2.7±1.33 C a	$F_{3,8}$= 0.43 P = 0.73
α-terpine	2.7±0.66 CD c	7.7 ±2.33 BC b	7.7 ±2.05 CD b	100 ± 0 A a	$F_{3,8}$=451.27 P < 0.0001
Linaly acetate	2.7±1.33 CD b	6.7±1.33 BC ab	13.1±5.81 CD a	13.3±3.5 B a	$F_{3,8}$= 5.14 P = 0.02
D.disulphide	100±0 A a	22.7±1.33 B b	90.7±6 B a	100±0 A a	$F_{3,8}$= 26.90 P < 0.0001
A. isothiocyanate	100 ± 0 A a	100 ±0 A a	100 ± 0 A a	100 ± 0 A a	---**
γ- terpinen	6±2 C a	12±0 BC b	18±2.66 C b	100±0 A a	$F_{3,8}$=346.92 P < 0.0001
Limonene	4±1.15 C c	10.7±2.66 BC b	9.3±2.66 CD bc	100±0 A a	$F_{3,8}$=322.18 P < 0.0001
Control	1 ±0.68 D a	4 ± 2.30 C a	2.7 ± 1.33 D a	0 ± 0 D a	$F_{3,8}$= 1.63 P = 0.25
F and P value	$F_{12,26}$=187.126 P< 0.0001	$F_{12,26}$=29.27 P< 0.0001	$F_{12,26}$=44.85 P< 0.0001	$F_{12,26}$=697.79 P< 0.0001	

*Two-way ANOVA was applied to the data. Means within a row with the same lower-case letter and a column with the same upper-case letter do not differ significantly (Duncan test at 5% level).

Study determined that the toxicity of diallyl disulphide in descending order according to life stages in commodity was egg > larva > pupa > adult for *T. confusum.* Allyl isothiocyanate had high fumigant toxicity to all life stge of tested species placed in each position of 2 kg od wheat. All tested monoterpenoid compounds except 2 of them (allyl isothiocyanate and dially disulphide) showed low toxicity. Similar results were obtained for *E. kuehniella* eggs and pupae at concentration of 100 µl l^{-1}. Larvae and adults of *E.kuehniella* were tested at concentration of 80 µl l^{-1} and 55 µl l^{-1}. Allyl isothiocyanate for *E. kuehniella* larvae and 10 monoterpenoid compounds (α-pinene, *p*-cymene, cuminaldehyde, linalool, α-terpinene, gamma terpinene, limonene, β-pinene, allyl isothiocyanate and diallyl disulphide) for *E. kuehniella* adults showed high fumigant toxicity. Cuminaldehyde against to *E. kuehniella* egg indicated same result at single-dose tests in absent and present of commodity. It was determined that the toxicity of diallyl disulphide in descending order according to life stages in present of commodity adult > egg > pupa for *E. kuehniella* respectively (Fig. 2).

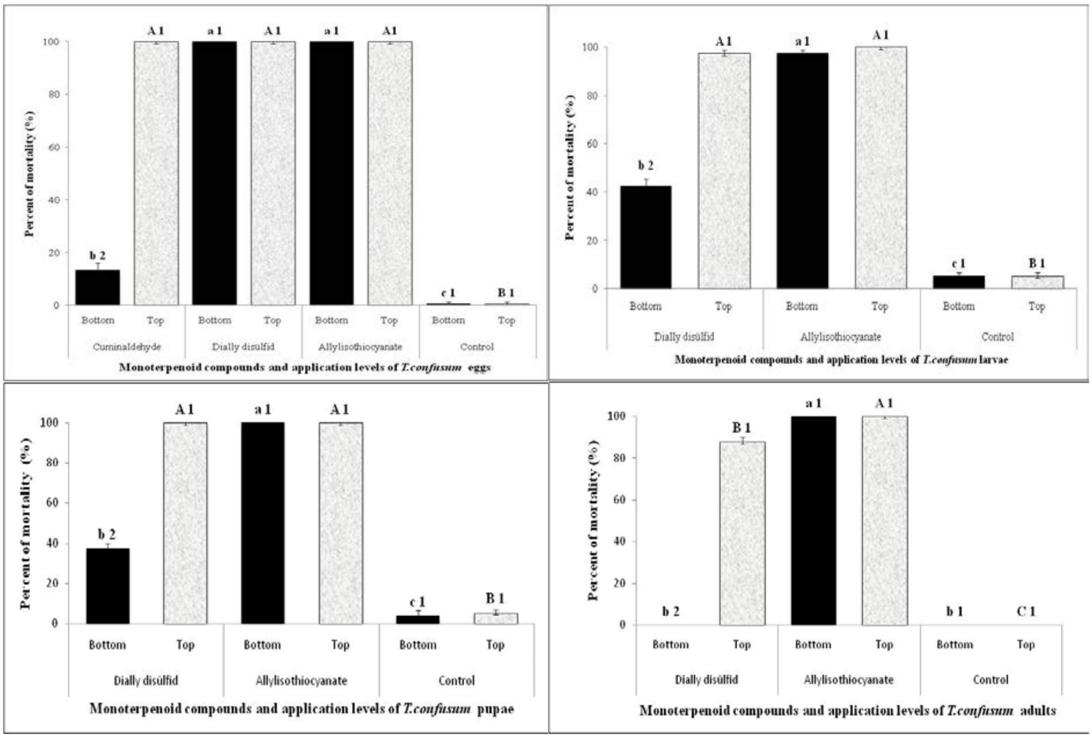

Fig. 1- Percent mortality of *Tribolium confusum* eggs, larvae, pupae and adults placed at different positions (bottom and top) in present of 2 kg of wheat, which were exposed to 100 µl l^{-1} concentration of tested monoterpenoid compounds. (Two-way ANOVA was applied to the data. Means within a row with lower-case letter, a column with upper-case letter and each numbers indicated that differences of percentage mortality. Same characters do not differ significantly (Duncan test at 5% level).

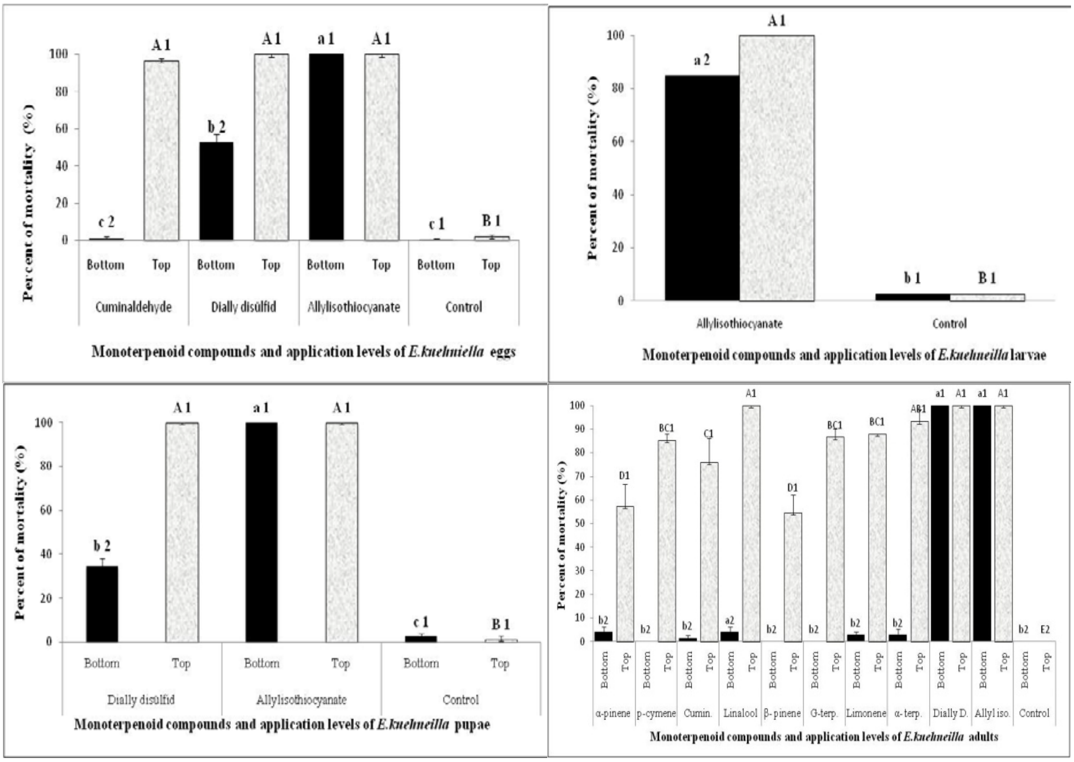

Fig. 2- Percent mortality of *Ephestia kuehniella* eggs, larvae, pupae and adults placed at different positions (bottom and top) in present of 2 kg of wheat, which were exposed to 100, 80, 100 and 55 µl l^{-1} concentration of tested monoterpenoid compounds respectively. (Two-way ANOVA was applied to the data. Means within a row with lower-case letter, a column with upper-case letter and each numbers indicated that differences of percent of mortality. Same characters do not differ significantly (Duncan test at 5% level).

Diallyl disulphide had a very low toxicity to larvae, pupa and eggs of *E. kuehniella* in present of commodity indicated poor penetration through the commodity. Allyl isothiocyanate was more toxic than diallyl disulphide to both *T. confusum* and *E. kuehniella* egg, larva, pupa and adult in present and absent of commodity.

In conclusion, it was determined that allyl isothiocyanate would be a potential compound in controlling stored-product insects, since it had high toxicity to all biological stages of both species in both absent and present of commodity.

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