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Original article (Orijinal araştırma)

Fumigant toxicity of monoterpenoid compounds against the confused flour beetle, *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae)[1,2]

Monoterpenoid bileşiklerin, Kırma biti *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae) üzerine fumigant toksisitesi

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Summary

This study was carried out to determine the fumigant toxicity of the monoterpenoid compounds; α-pinene, *p*-cymene, eugenol, cuminaldehyde, linalyl acetate, linalool, α-terpinene, γ-terpinene, limonene, β-pinene, allyl isothiocyanate and diallyl disulphide against all life stages of *Tribolium confusum* Jacquelin du Val. at 25°C and 65% RH. Preliminary biological tests were performed to test the fumigant activity of monoterpenoid compounds against all life stages of *T. confusum* exposed to a dose of 100 μLL^{-1} for 24-h. Preliminary biological tests indicated that allyl isothiocyanate and diallyl disulphide had high fumigant activity on all life stages of the test insect with mortality rates ranging from 92 to 100%, while cuminaldehyde was highly toxic to only its egg stage. Other monoterpenoid compounds, apart from cuminaldehyde, allyl isothiocyanate and diallyl disulphide, showed low fumigant toxicity to all life stages of the test insect with mortality rates ranging from 0 to 27.3%. Allyl isothiocyanate and diallyl disulfide were the only compounds to kill all life stages of *T. confusum*. Lethal concentration tests showed that allyl isothiocyanate was more toxic to *T. confusum* larvae, pupa and adults with 5.99, 2.69 and 3.50 μLL^{-1} LC_{90} values, respectively, than diallyl disulphide by 98.06, 42.26 and 47.57 μLL^{-1} LC_{90} values, respectively. The complete mortality of all life stages of *T. confusum* was achieved at a Ct product (Concentration x time) of 254 mg h L^{-1} of allyl isothiocyanate. Based on the toxicity data, allyl isothiocyanate has potential as a fumigant for controlling stored-grain insects.

Key words: Monoterpenoids, fumigant toxicity, *Tribolium confusum*, phytochemicals

Özet

Bu çalışma, önemli bir depo zararlısı olan Kırma Biti, *Tribolium confusum* du Val.' un tüm gelişme dönemlerine karşı, α-pinene, *p*-cymene, eugenol, cuminaldehyde, linalyl acetate, linalool, α-terpinene, gamma terpinene, limonene, β-pinene, allyl isothiocyanate ve diallyl disülfide monoterpenoid bileşiklerinin fumigant etkisinin belirlenmesine yönelik olarak, 25°C sıcaklık ve %65 nem koşullarında biyolojik testler yürütülmüştür. Ön biyolojik testlerde, *T. confusum'* un tüm dönemleri monoterpenoid bileşiklerin 100 μLL^{-1} konsantrasyonuna, 24 saat süreyle maruz bırakılmıştır. Ön biyolojik testler sonucunda allyl isothiocyanate ve diallyl disulfide bileşikleri *T. confusum'* un tüm dönemlere karşı yüksek fumigant etki göstermiş ve ölüm oranları %92 ile %100 arasında değişmiştir. Cuminaldehyde'in ise sadece yumurta dönemine yüksek toksik etki gösterdiği belirlenmiştir. Cuminaldehyde, allyl isothiocyanate ve diallyl disulphide bileşikleri dışındaki diğer monoterpenoid bileşikler %0 ila 27.3 arasında değişen ölüm oranlarına sahip olarak düşük bir fumigant etki göstermiştir. *Tribolium confusum'* un tüm yaşam dönemlerini sadece allyl isothiocyanate ve diallyl disulfide bileşikleri öldürücü etki göstermiştir. Lethal konsantrasyon testleri sonucunda *T. confusum*' un larva, pupa ve ergin dönemleri için allyl isothiocyanate ile dially disülfide' e ait LC_{90} değerleri sırasıyla 5.99, 2.69 and 3.50 μLL^{-1} ve 98.06, 42.26 and 47.57 μLL^{-1} olarak belirlenmiştir. Bu sonuçlar allyl isothiocyanate' ın *T.confusum*' un tüm yaşam dönemlerine, diallyl disulfide'den daha toksik olduğunu göstermiştir. Allyl isothiocyanate bileşiğinin, *T. confusum'* un tüm yaşam dönemlerini öldürmek için gerekli olacak Ct product değeri 254 mg h L^{-1} olarak belirlenmiştir. Sonuç olarak, toksisite verileri, allyl isothiocyanate bileşiğinin depolanmış tahıl zararlılarının kontrolünde potansiyel bir fumigant olabileceğini göstermiştir.

Anahtar sözcükler: Monoterpenoidler, fumigant toksisite, *Tribolium confusum*, fitokimyasallar

[1] This study is part of the PhD Thesis at Namık Kemal University, Plant Protection Department, Institute of Natural and Applied Science.
[2] This study was presented orally and a summary was included in proceedings of the International Conference of the IOBC/WPRS Working Group on "Integrated Protection of Stored Products", 4-7 July 2011, Volos, Greece
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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. Phosphine and methyl bromide are the most common fumigants used for stored-product protection throughout the world. Insect resistance to phosphine is a global issue now and control failures have been reported in field conditions in some countries (Taylor 1989; Collins et al. 2002). Methyl bromide, a broad-spectrum fumigant, has been declared an ozone-depleting substance (EPA 2001) and therefore, is being phased out. Hence, there is a global interest in alternative strategies including development of chemical substitutes, exploitation of controlled atmosphere and integration of physical methods (MBTOC 2002). Recently, the use of sulfuryl fluoride, a structural fumigant for termite and woodborer control, has been expanded to food commodities and food handling establishments (flour mills) in the USA, Canada and Europe (Prabhakaran 2006). New fumigants, such as carbonyl sulphide (Desmarchelier 1994), ethane dinitrile (Ryan et al. 2006) and the old fumigant ethyl formate (ethyl formate alone and mixtures of ethyl formate with CO_2) (Damcevski et al. 2003) have also been investigated as alternatives for food and non-food commodities. Furthermore, interest has been shown in plant products, i.e., essential oils and their components, for fumigants action since it is believed that natural compounds from plant sources may have the advantage over conventional fumigants in terms of low mammalian toxicity, except some of the monoterpenoid compounds, such as β-asarone, estragole, safrole, (+) – fenchone, which are known to be carcinogenic (Kim and Ahn 2001), rapidly degrade and have limited local availability (Isman, 2006).

Secondary plant compounds are a large and diverse group of molecules that have a key role in the interactions between herbivores and plants in both natural and agricultural ecosystems. There are over 24,000 known molecular structures that include classes of compounds known to have anti-nutritional and toxic effects on mammals (Harborne 1993), including the alkaloids, non-protein amino acids, cyanogenic glycosides, terpenoids, saponins and flavonoids. Most of these active ingredients are secondary metabolites secreted in plants as chemical defense against the insect pests. Volatile terpenoids are fat soluble, oxygenated hydrocarbons based on repeated isoprene units. Monoterpenes are based on two isoprene units (10-carbon-base; C_{10}) and are responsible for the characteristic odors of many plants. Monoterpenoid compounds have been considered as potential pest control agents because they are acutely toxic to insects and haverepellent (Watanabe et al. 1993) and antifeedant-properties (Hough-Goldstein 1990). Previous laboratory evaluations have established the biological activity of monoterpenoids on various insect pests as ovicides, fumigants, and contact toxicants (Karr and Coats 1988; Rice and Coats 1994; Tsao et al. 1995). Regnault-Roger and Hamraoui (1995) reported that oxygenated monoterpenoids (e.g. carvacrol, linalool, and terpineol) are more toxic than non-oxygenated compounds (*p*-cymene, cinnamaldehyde, anethole) against *Acanthoscelides obtectus* (Say) adults. Diallyl trisulphide, a constituent of garlic essential oil, was considered effective as a contact toxicant, fumigant and feeding deterrent (Huang et al. 2000). Acute toxicity of pulegone to the variegated cutworm (*Peridroma saucia* (Hubner)) was demonstrated by Harwood et al. (1990). Erler (2005) tested fumigant toxicities of six monoterpenoid compounds namely, carvacrol, thymol, menthol, gamma-terpinene, terpinen-4-ol and 1,8-cineole, against *T. confusum* adults and *Ephestia kuehniella* Zeller eggs, and among these monoterpenoid compounds, cavracrol was found to be the most active.

Most of the earlier studies indicated that 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 with different chemical groups against the immature stages of stored-products insects (Mansour 2012). 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, against all life stages (adult, egg, larva and pupa) of the confused flour beetle, *T. confusum,* and to discuss the possible use of these monoterpenoids as bio-fumigants against stored-grain insects.

Materials and methods

Test insects

Bioassays were carried out on all life stages (egg, larva, pupa and adult) of *T. confusum* in the Laboratory of Toxicology at Namık Kemal University in 2011. *Tribolium confusum* were reared on a diet of wheat flour mixed with dry brewer's yeast (17:1 w/w) in 1 l glass jars at 25±1°C and 65±5% relative humidity (RH) using standard culture techniques (Donahaye 1990). Eggs (1-2 days old), old larvae (4-7th instar), pupae (1-2 days old) and adults (7-10 days old) were used in bioassay tests. Eggs were separated daily from oviposition jars by sieving (70 mesh (210 µm) sieve; Retsch, Germany). Eggs were transferred into the 10 ml glass tubes for exposure to treatments. 10 ml glass tubes (2 cm diameter and 4.5 cm long), each containing 50 eggs 1–2 days old, were exposed to each treatment. Old larvae were removed from culture jars and exposed to the treatments 25-30 days after oviposition. Two day-old pupae were obtained by daily separation from culture jars and held in wheat flour for 48-h before the exposure. Newly emerged adults were held in pre-exposure jars containing wheat flour, and were exposed to treatment 7-10 days after emergence. Each glass tube, except of the egg stage, included 4 g wheat flour food.

Tested monoterpenoid compouds

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 used in fumigant toxicity tests. After purchase, the monoterpenoid compounds were transferred to sealed glass containers with frangible septum which allows easy penetration by a syringe needle to permit withdrawal of the compounds. Containers were kept at refrigerator temperature (4°C) in the dark until use.

Bioassay and experimental procedures

Each stage of *T. confusum* was collected from the cultures and placed separately 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. Bioassays were carried out in 3-L glass jars closed with metal screw-on lids, which served as fumigation chambers. For all treatments, RH and temperature were maintained at 65±5% at atmospheric pressure and 25±1°C, respectively. An aqueous saturated magnesium nitrite solution ($Mg(NO_3)_2$) was placed in small glass petri dishes of 7 cm diameter to provide 60 ± 5% RH in the glass jar (Greenspan 1977). Single-dose biological tests were carried out to determine the effective concentrations of each compound against the all biological stages of *T. confusum*. Each stage was exposed to a concentration of 100 µLL^{-1} air of each compound for 24 h. After each stage, *T. confusum* in glass vials were placed separately into fumigation chambers, monoterpenoid compounds were applied on filter paper (2 x 5 cm) attached to the under-side of the lids of fumigation chambers by using a 50 µL micropipette. The fumigation chambers were immediately closed with screw-on lids, which were made air-tight. Each treatment was replicated three times. For each treatment, untreated control insects were exposed to atmospheric conditions.

Cuminaldehyde, diallyl disulphide and allyl isothiocyanate, which showed high fumigant activity on life stages of *T. confusum* in single-dose biological tests, were included in lethal concentration tests to determine their LC_{50} and LC_{90} values. In lethal concentration tests with diallyl disulphide, 6 concentrations ranging from 0.25 to 10 µLL^{-1}, 5 to 75 µLL^{-1} and 1 to 75 µLL^{-1} for the eggs, pupae, and adults, respectively, and 9 concentrations from 1 to 120 µLL^{-1} for the larvae; allyl isothiocyanate, 6, 7, 8 and 5 concentrations ranging from 0.1 to 5 µLL^{-1}, 0.25 to 5 µLL^{-1},1 to 5 µLL^{-1} and 0.5 to 7.5 µLL^{-1} for the eggs, pupae, adults and larvae, respectively; and cuminaldehyde, 5 concentrations ranging from 0.25 to 10.5 µLL^{-1} for only the egg stage, were used. Concentrations were selected for each insect stage on the basis of single-dose biological tests. Three replicates were set up for each concentration and control. The same fumigation procedures described in single-dose biological tests were followed.

Data processing and analysis

After each treatment, larvae, pupae, and adults were transferred to clean 10 mL jars containing food medium and were held at 25±1°C and 65±5% RH until examined for mortality. The eggs on 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 had 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 using the Duncan's test at the 5% significance level (SPSS 2009). Data obtained from each zero dose control and concentration-mortality responses were subjected to probit analysis by using the maximum likelihood program software (POLO-PC) (LeOra Software 1987) to determine LC_{50}s (Lethal Concentration$_{50}$), LC_{90}s (Lethal Concentration$_{90}$) and their respective 95% confidence intervals. Differences in toxicity were considered significant when 95% confidence intervals did not overlap. The slopes and intercepts of concentration-mortality regressions for each tested insect were compared with the POLO-PC maximum-likelihood procedures (LeOra Software 1987). The concentrations × time (Ct) products (mg h L^{-1}) to obtain 90% mortality of all life stages of *T. confusum* were calculated using the LC_{90} values derived from probit analyses and exposure period (24 h).

Results

Percentage mortalities of all life stages of *T. confusum,* exposed to a concentration of 100 μLL^{-1} air of the tested monoterpenoid compounds for 24 h are given in Table 1. Single-dose biological tests indicated that both vapors of the monoterpenoid compounds ($F_{12,140}$ = 66.86, P<0.0001) and the insect stage ($F_{3,140}$=17.22, P<0.0001) had a significant effect on mortality of the life stages of *T. confusum* when exposed to a concentration of 100 μLL^{-1} for 24 h.

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

Compounds	Mortality (%) ± S.E.				F and P value
	Egg	Larva	Pupa	Adult	
α-pinene	17.3 ± 4.6 BCD a*	5.4 ± 1.3 CDE b	21.7 ± 3.9 BC a	1.3 ± 1.3 B b	$F_{3,8}$= 12.6, P <0.002
p- cymene	12 ± 1.1 CD a	6.8 ± 1.3 CDE bc	9.5 ± 1.5 DEF ab	4 ± 0 B c	$F_{3,8}$= 9.9, P <0.005
Cuminaldehyde	100 ± 0 A a	4.2 ± 2.4 E b	6.7 ± 2.6 EF b	1.3 ± 1.3 B b	$F_{3,8}$= 137.9, P <0.0001
Linalool	27.3 ± 1.7 B a	5.7 ± 1.3 CDE b	4.1 ± 2.3 F b	0 ± 0 B c	$F_{3,8}$= 24.9, P <0.0001
β-pinene	14.7 ± 2. 8 CD a	5.3 ± 2.6 DE bc	9.8 ± 1.1 DEF a	1.3 ± 1.3 B b	$F_{3,8}$= 5.2, P <0.027
Eugenol	16.7± 3.5 BC a	18.7 ± 3.5 C a	24 ± 2.3 B a	0 ± 0 B b	$F_{3,8}$= 17.6, P <0.001
α-terpine	15.3 ± 3.7 CD a	14.1 ± 1.6 CD a	17.3 ± 5.8 BCD a	5.3 ± 3.5 B a	$F_{3,8}$= 2.3, P=0.152
Linaly acetate	16 ± 4.1 CD ab	5.5 ± 1.4 CDE bc	21.8 ± 3.9 BC a	2.7 ± 2.6 B c	$F_{3,8}$= 7.5, P< 0.01
Diallyl disulphide	100 ± 0 A a	92 ± 4 B b	100 ± 0 A a	100 ± 0 A a	$F_{3,8}$= 4.0, P= 0.05
Allyl isothiocyanate	100 ± 0 A a	100 ± 0 A a	100 ± 0 A a	100 ± 0 A a	------
γ- terpinen	8.7 ± 1.3 DE bc	17.3 ± 4.8 C a	12 ± 2.3 CDE a	2.7 ± 2.6 B b	$F_{3,8}$= 4.9, P< 0.031
Limonene	9.3 ± 3.5 DE a	17.3 ± 4.8 CD a	9.3 ± 1.3 DEF a	8 ± 4 B a	$F_{3,8}$= 1.0, P=0.433
Control	2.7 ± 0.66 E a	1.4 ± 1.4 E a	0 ± 0 G a	2.7 ± 1.3 B a	$F_{3,8}$= 2.1, P= 0.16
F ve 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 uppercase letter do not differ significantly (Duncan test at 5% level).

According to the results obtained from single-dose biological tests, cuminaldehyde, allyl isothiocyanate and diallyl disulphide resulted in 100% mortality of *T. confusum* eggs, whilst other tested monoterpenoid compounds achieved low mortalities ranging from 8.7% to 27.3%. For larval, pupal and adult stages of *T. confusum*, allyl isothiocyanate and diallyl disulphide caused significantly higher mortalities than other monoterpenoid compounds. Generally, α-pinene, *p*-cymene, eugenol, cuminaldehyde (except egg stage), linalyl acetate, linalool, α-terpinene, γ-terpinene, limonene and β-pinene, had low fumigant toxicity against all life stages of *T. confusum* with mortalities ranging from 0 to 27.3% (Table 1). In contrast, allyl isothiocyanate and diallyl disulphide had high fumigant activity on all life stages of *T. confusum* and they caused significantly higher mortalities of all life stages of *T. confusum* than other monoterpenoid compounds, except for cuminaldehyde to the egg stage;cuminaldehyde had high fumigant toxicity against only *T. confusum* eggs. Therefore, allyl isothiocyanate and diallyl disulphide, which showed high fumigant activity in single-dose biological tests for all biological stages and cuminaldehyde for only the egg stage, , were used in lethal concentration tests.

The results of probit analysis (LC_{50} and LC_{90} values (µLL^{-1})) for all biological stages of *T. confusum* exposed to various concentrations of cuminaldehyde (only egg stage), allyl isothiocyanate and diallyl disulphide for 24-h are given in Table 2. Probit mortality regression data indicated a remarkable difference in toxicity between the tested monoterpenoid compounds (Table 2) against all life stages of *T. confusum*. Lethal concentration tests indicated that cuminaldehyde and diallyl disulphide against *T. confusum* eggs had values of 2.08 and 0.74 µLL^{-1} for LC_{90}, respectively; however, LC_{50} and LC_{90} values for allyl isothiocyanate were not able to be estimated since 100% mortality of eggs was obtained even at the lowest concentration (0.1 µLL^{-1}). The experiment demonstrated that allyl isothiocyanate is the most toxic compound to *T. confusum* eggs, followed by diallyl disulphide and cuminaldehyde, respectively. LC_{90} values for allyl isothiocyanate against larva, pupa and adult stages of *T. confusum* were significantly lower than those for diallyl disulphide, since the 95% confidence intervals (CLs) of allyl isothiocyanate did not overlap those for diallyl disulphide. Therefore, allyl isothiocyanate was more toxic to *T. confusum* larvae, pupae and adults with LC_{90} values of 5.99, 2.69 and 3.50 µLL^{-1} , respectively, than diallyl disulphide, with LC_{90} values of 98.06, 42.26 and 47.57 µLL^{-1} ,respectively (Table 2). Considering all results obtained from lethal concentration tests, allyl isothiocyanate was the most toxic compound to all life stages of *T. confusum*.

Table 2. Probit analysis data of monoterpenoid compounds for *Tribolium confusum* eggs, larvae, pupae and adults following 24-h laboratory fumigation

Life Stage	Monoterpenoid compounds	N[a]	Slope[b] ± S.E.	LC_{50} (µLL^{-1}) (Fiducial limit)[c]	LC_{90} (µLL^{-1}) (Fiducial limit)[c]	X^2[d]
Egg	Cuminaldehyde	1350	2.78 ± 0.16	0.72 (0.64-0.80)	2.08 (1.82-2.44)	30.70
	Allyl isothiocyanate	750	---	---	---	---
	Diallyl disulphide	900	4.04 ± 0.33	0.35 (0.31-0.40)	0.74 (0.65-0.89)	21.62
Larva	Allyl isothiocyanate	372	6.45 ± 0.83	3.79 (3.37-4.17)	5.99 (5.34-7.16)	14.63
	Diallyl disulphide	675	2.13 ± 0.27	24.64 (17.70-30.95)	98.06 (79.84-130.28)	22.92
Pupa	Allyl isothiocyanate	525	2.76 ± 0.25	0.92 (0.70-1.14)	2.69 (2.19-3.53)	30.95
	Diallyl disulphide	525	2.76 ± 0.26	14.56 (11.72-17.49)	42.26 (34.29-55.72)	21.34
Adult	Allyl isothiocyanate	600	6.38 ± 0.44	2.20 (2.03-2.37)	3.50 (3.19-3.93)	36.99
	Diallyl disulphide	525	2.54 ± 0.17	14.9 (11.66-19.04)	47.57 (34.81-74.66)	49.65

[a]:Number treated, excluding controls, [b]: Slopes are non-parallel and unequal where noted, [c]: Value in parentheses refers to the 95% confidence range, [d]: Chi-square.

Toxicity data for allyl isothiocyanate and diallyl disulphide indicate a remarkable difference in susceptibility between biological stages of *T. confusum.* Lethal Concentration$_{90}$ (LC_{90}) values for the larva were significantly different from those for the egg, pupa and adult, since 95% confidence intervals (CLs) of the larva did not overlap those of egg, pupa and adult. LC_{90} values for the egg were also significantly different than those of the larva, pupa and adult, since 95% CLs did not overlap. The larvae were the most resistant stage to allyl isothiocyanate and diallyl disulphide, with LC_{90} values of 5.99 and 98.06 μLL^{-1}, respectively, whilst the eggs were the most susceptible stage, with LC_{90} value of 0.72 μLL^{-1} (Table 2); an LC_{90} value for allyl isothiocyanate for the egg stage was not able to be estimated since 100% mortality of eggs was obtained at the lowest concentration, 0.1 μLL^{-1}.

Discussion

Many plant secondary metabolites, such as alkaloids, monoterpenoids and phenylpropanoids, show ovicidal, repellent, antifeedant, sterilization and toxic effects on stored-product insects (Nawrot and Harmatha 1994; Isman 2006). The toxicity may be by contact, ingestion or through fumigant activity. Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions. Therefore, the fumigant toxicity of monoterpenoids against stored-product insects has been studied (Regnault-Roger and Hamraoui 1995; Huang et al. 2000; Lee et al. 2003; Erler 2005, Mansour et al.). Lopez et al. (2008) noted that some monoterpenoids can be used as possible alternatives to synthetic insecticides against stored-product pests such as *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Plodia interpunctella* (Hubner) or *Cryptolestes pusillus* (Schönherr).

In the present study, the single-dose biological tests indicated that the tested monoterpenoids with different chemical groups had significantly different fumigant activity on all life stages of *T. confusum*. Vapour of monoterpenoids of allyl isothiocyanate and diallyl disulphide had a strong fumigant activity on all life stages *T. confusum*, while other tested monoterpenoids (except cuminaldehyde for only the egg stage) had very low fumigant action. These findings can be compared with several studies on some monoterpenoid compounds. Similiar to our results, Lee et al. (2003) reported a low toxicity of limonene and linalool against *Tribolium castaneum* (Herbst) adults. The high fumigant activity of allyl isothiocyanate against adults of *T. confusum* was similar to other studies with the maize weevil *Sitophilus zeamais* (Motsch.), *R. dominica*, the book louse *Liposcelis entomophila* (Enderline), *T. confusum,* and one other study with *T. castaneum* (Worfel et al. 1997; Demirel et al. 2009; Wu et al. 2009). In parallel with our results, Işıkber et al. (2009) reported that cumin essential oil, of which cuminaldehyde is the major monoterpenoid compound, had high fumigant toxicity against only *T. confusum* eggs.

There were remarkable differences in lethal concentration values of cuminaldehyde, allyl isothiocyanate and diallyl disulphide against all biological stages of *T. confusum*. It appears that allyl isothiocyanate was the most toxic compound to *T. confusum* eggs, followed by diallyl disulphide and cuminaldehyde, respectively. Allyl isothiocyanate was more toxic to *T. confusum* larvae, pupae and adults than diallyl disulphide. Considering all results obtained from lethal concentration tests, allyl isothiocyanate was the most toxic compound to all life stages of *T. confusum*. Tsao et al. (1995) reported that for 24-h allyl isothiocyanate fumigation against *R. dominica* adults, the LC_{50} value was 1.57 µg cm^{-3}, which showed high fumigant toxicity.

Stored-product insects and their stages varied in their susceptibility to monoterpenoid compounds (Stamopoulos 2007). In the present study, there was a difference between susceptibility of biological stages of *T. confusum*, to allyl isothiocyanate and diallyl disulphide. Toxicity data indicated that the larva was the most resistant stage to allyl isothiocyanate and diallyl disulphide tolerant (higher LC_{90} values), whilst the eggs were the most susceptible stage. The order of tolerance of the stages for allyl isothiocyanate and diallyl disulphide at LC_{90} was: larva > adult > pupa > egg. Mansour et al. (2012) reported that *T. confusum* larvae were more tolerant than adults to allyl isothiocyanate. Işıkber and Gözek (2009) noted that larvae of *T. confusum* were more tolerant to diallyl disulphide than eggs, adults and pupae of *T. confusum*, which parallels the findings in the present study.

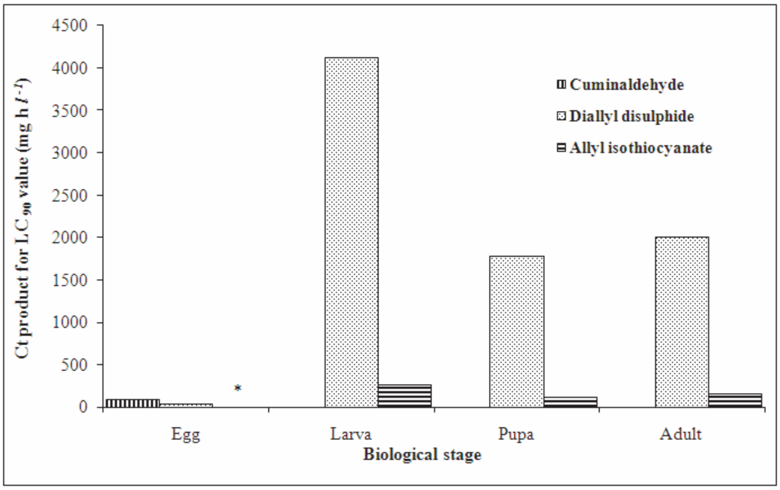

Figure 1. Concentration x time products (Ct product, mg h L^{-1}) required for LC_{90} values of cuminaldehyde, diallyl disulphide and allyl isothiocyanate for egg, larva, pupa and adult stages of *T. confusum* (*:LC_{90} values of allyl isothiocyanate for the egg stage was not able to be estimated since 100% mortality of eggs was obtained even at the lowest concentration 0.1 µLL^{-1}).

Calculations of Ct products reveal that there was also high variation in Ct products of different life stages of *T. confusum* required for LC_{90} values of allyl isothiocyanate and diallyl disulphide (Figure 1). A Ct product of 114.3 and 1778 mg h L^{-1} was required for allyl isothiocyanate and diallyl disulphide, respectively, to obtain 90% kill of pupae of *T. confusum* (Figure 1), whereas a Ct product of 148.7 and 2001 mg h $L^{-1}$of allyl isothiocyanate and diallyl disulphide, respectively, was required for 90% kill for adults of *T. confusum* (Figure 1). When these findings are compared with those of several studies on the two most commonly used fumigants, methyl bromide (MB) and phosphine for control of *T. castaneum*, whereas MB required Ct products of 62, 59 and 168 mg h L^{-1} to obtain 90% of kill of adults, larvae and pupae, respectively, (Lindgren and Vincent 1965; Rajendran 1990), phosphine required Ct products of 12, 47 and 56 mg h L^{-1} to achieve 90% kill of adults, larvae, and pupae, respectively (Bang and Telford 1966; Williams 1985). Tests on adults of *T. confusum* of carbonyl sulfide produced 90% mortality with a Ct product of 368.9 mg h L^{-1} (Zettler et al. 1997). Other fumigants, ethylene dichloride and carbon tetrachloride, produced 90% mortality of adults of *T. castaneum* with Ct products of 462 and 600 mg h L^{-1}, respectively (Busvine 1938; Bang and Telford 1966). It appears therefore that allyl isothiocyanate is less toxic to *T. castaneum* adults than phosphine and MB but is more toxic than carbonyl sulfide, ethylene dichloride and carbon tetrachloride. On the other hand, diallyl disulphide is less toxic to *T. castaneum* adults than phosphine and methyl bromide, carbonyl sulfide, ethylene dichloride and carbon tetrachloride.

Allyl isothiocyanate is highly volatile and its vapor density is 3.4 times higher than the air. Toxicity tests in the present study indicated that allyl isothiocyanate caused the complete mortality of all life stages of *T. confusum* at relatively low concentrations and short exposure period. Similarly, some authors reported that allyl isothiocyanate vapors are highly toxic to stored grain pests such as *Lasioderma serricorne* (Fabricius), *P. interpunctella* (Mansour et al. 2012), *T. confusum* (Worfel et al. 1997; Mansour et al. 2012) and *R. dominica* (Tsao et al. 1995) and *T. castaneum* (Santos et al. 2011).

In conclusion, the present study showed that among the tested monoterpenoids, allyl isothiocyanate has the potential to be an alternative to conventional fumigants. Clearly, further research is needed to obtain toxicity data on other stored-product insects, on its absorption by different commodities, and on its power of penetration into bulk commodities.

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