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Original article (Orijinal araştırma)

Fumigant toxicity of mustard essential oil and its main compound alone and combinations with modified atmosphere treatments against *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae)[1]

Hardal uçucu yağı ve ana bileşiğinin tek başına ve değiştirilmiş atmosfer uygulamaları ile kombinasyonun *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae)'a karşı fümigant etkisi

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Abstract

This study was carried out in 2017 in Entomology Laboratory of Kahramanmaraş Sütçü İmam University to determine fumigant toxicity of mustard essential oil and its main compound (allyl isothiocyanate) alone and in combination with high concentration (92%) of CO_2 or N_2 to all life stages of *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) was determined. Preliminary bioassay tests indicated that 10 µl/l of mustard essential oil and allyl isothiocyanate alone resulted in 100% mortality for all life stages of *T. confusum* without any necessity of CO_2 and N_2 combinations. Lethal concentration tests indicated that combinations of mustard essential oil or allyl isothiocyanate with 92% CO_2 produced 1.8 to 7.3 times reductions in LC_{90} values for larvae, pupae and adults of *T. confusum*. Generally, the combinations of mustard essential oil or allyl isothiocyanate with 92% CO_2 were more toxic to larvae, pupae and adults of *T. confusum* than those in combinations with 92% N_2 as evidenced by significant decrements in their LC_{50} and LC_{90} values. It appears that high concentration of CO_2 or N_2 might have a synergistic effect on larvae, pupae and adults of *T. confusum* when exposed together with mustard essential oil or allyl isothiocyanate. In conclusion, this study indicates that combinations of mustard essential oil or its main compound, allyl isothiocyanate with modified atmospheres can be a potential alternative to the most commonly used commercial fumigants, methyl bromide and phosphine.

Keywords: Allyl isothiocyanate, essential oil, modified atmosphere, mustard, *Tribolium confusum*

Öz

Bu çalışma 2017 yılında Kahramanmaraş Sütçü İmam Üniversitesi'nin Entomoloji Laboratuvarı'nda hardal uçucu yağı ve bunun ana bileşiği olan allyl isothiocyanate'ın yüksek CO_2 veya N_2 ile kombinasyonunun *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae)'un tüm biyolojik dönemlerine karşı fümigant etkinlikleri belirlemek amacıyla yürütülmüştür. Ön biyolojik etkinlik test sonuçları, hardal uçucu yağı veya allyl isothiocyanate'ın tek başına 10 µl/l konsantrasyonda *T. confusum* 'un tüm biyolojik dönemlerinde %100 ölümüne neden olduğunu göstermiştir. Hardal uçucu yağı veya allyl isothiocyanate'ın %92 oranındaki CO_2 veya N_2 ile birlikte uygulanması *T. confusum*'un larva, pupa ve erginlerine ait LC_{90} değerlerinde 1.8-7.3 arasında değişen oranlarda azalmalara neden olmuştur. Genel olarak hardal uçucu yağı veya allyl isothiocyanate'in %92 CO_2 ile kombinasyonun *T. confusum*'un larva, pupa ve erginlerine karşı bunların %92 N_2 ile kombinasyonuna kıyasla daha toksik olduğu belirlenmiştir. Biyolojik etkinlik testleri sonunda hardal uçucu yağı ve bunun ana bileşiği olan allyl isothiocyanate'ın yüksek konsantrasyonda CO_2 veya N_2 gazıyla birlikte uygulanmasının *T. confusum*'un larva, pupa ve erginlerine ait toksisite değerlerinde önemli azalmalara neden olduğu ve dolayısıyla CO_2 veya N_2 kullanımının sinerjik etki gösterebileceği görülmüştür. Sonuç olarak, hardal uçucu yağı ve ana bileşiği olan allyl isothiocyanate'ın değiştirilmiş atmosfer ile kombinasyon halinde uygulanmasının depolanmış ürün zararlılarının mücadelesinde konvansiyonel fümigantlara potansiyel alternatif olabileceği değerlendirilmiştir.

Anahtar sözcükler: Allyl isothiocyanate, uçucu yağ, değiştirilmiş atmosfer, hardal, *Tribolium confusum*

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Introduction

Plant essential oils in general have been recognized as an important natural resource and their major components, often various monoterpenoids, are among the best-known substances to have attracted attention in recent years as potential pest control agents due to their insecticidal, repellent and/or antifeedant properties (Tunç et al., 2000; Papachristos & Stamopoulos, 2002a,b; Lee et al., 2003; Trypathy, 2004; Ketoh et al., 2005; Isman, 2006). Most of these substances are volatile and can act as fumigants, thus offering the prospect of use against stored-product insects. Several studies have shown fumigant action of various essential oils and their compounds against some stored-product insects (Shaaya et al., 1997; Huang et al., 2000; Tunç et al., 2000). A number of studies showed that mustard essential oil and its major component, allyl isothiocyanate, have high toxicity against both *Sitophilus zeamais* (Motschulsky, 1855), *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Fabricius, 1792) (Worfel et al., 1997; Tsao et al., 2002; Paes et al., 2011), indicating their potential use in protecting grain and other stored products. Paes et al. (2011) reported that the vapor of mustard essential oil resulted in high mortality of all life stages of *S. zeamais*. However, there were significant differences in toxicity of mustard essential oil to its life stages. The egg was the most tolerant developmental stage, followed by pupae and larvae, respectively. This study indicates that mustard essential oil is a possible candidate for development of an environmentally-friendly fumigant for control of grain insect pests. However, the successful implementation of essential oils as fumigants is hampered by the relatively high concentrations needed for effective protection of stored grain against insect pests, the great difference in the sensitivity of various insect species, poor penetration into grain bulk current market prices and the effects on stored products being fumigated (Korunic & Rozman, 2008; Rajendran & Sriranjini, 2008; Rozman et al., 2008).

The use of CO_2 together with various fumigants has also been studied. Carbon dioxide, a respiratory stimulant, is known as an adjuvant for fumigants, such as methyl bromide. There are some advantages of using CO_2 in the mixture; higher toxicity of the fumigant, better gas distribution, limitation of harmful residue levels on the treated commodity, and also elimination of flammable hazard of some fumigants. Several investigations of fumigant and CO_2 mixtures have been done in the past (Cotton & Young, 1929; Jones, 1938), and these were followed by investigations which indicated that methyl bromide and CO_2 mixture caused an increase in the susceptibilities of some stored-product insects (Calderon & Leesch, 1983; Williams, 1985). Laboratory tests with essential oils have shown a similar joint action with CO_2 atmospheres. Shaaya et al. (1999) reported increased toxicity of essential oil, SEM76 (extracted from a species in the family Lamiaceae), in the presence CO_2 to *Tribolium castaneum* (Herbst, 1797) (adults, pupae and larvae), *Plodia interpunctella* (Hübner, [1813]) (pupae and larvae), *R. dominica*), *Sitophilus oryzae* (Linnaeus, 1763) and *Oryzaephilus surinamensis* (Linnaeus, 1763) (adults). However, toxicity studies on essential oils and CO_2 mixtures against stored-product insects to exhibit additive, synergistic or antagonistic effects are limited.

Several studies revealed that mustard essential oil and its major component, allyl isothiocyanate, had high fumigant toxicity against stored grain insects (Worfel et al., 1997; Tsao et al., 2002; Paes et al., 2011) and potential use for controlling stored grain insects. However, the problems regarding to decrease in the efficacy of mustard essential oil and its major component due to requiring their high toxicity values and exposure times to obtain the complete mortality of stored-product insects and a possible weak penetration power in bulk commodity limited to be potential alternative to current fumigants (especially phosphine) for controlling stored-product insects. The use of mustard essential oil and its compound, allyl isothiocyanate, with high concentrations of inert gases, such as N_2 and CO_2, may contribute to increase their toxicity against stored-product insects and penetration into bulk commodity. In this context, the present study was conducted to determine fumigant toxicity of mustard essential oil and its main compound (allyl isothiocyanate) in combination with high concentration (92%) of CO_2 and N_2 to all life stages of *T. confusum*.

Material and Methods

Test insects

Biological tests were conducted on all life stages (egg, larva, pupa and adult) of *T. confusum*. *Tribolium confusum* were obtained from standard cultures reared in 1-L glass jars at 25±1ºC and 65±5% RH on a standard diet with wheat flour mixture with dry brewer's yeast (17:1 by weight). Eggs were obtained by daily separation from oviposition jars by sieving (60 mesh, 250 µm sieve; Retsch, Haan, Germany). Eggs for exposure to treatments were transferred into the glass vials (5 cm long × 2.5 cm diameter). The 10 mL glass tube (18 cm high × 18 cm wide × 12 cm diameter), each containing 50 eggs 1 to 2-d old, was exposed to each treatment. Larvae were removed from insect culture jars 25-30 d after oviposition and exposed to the treatments. Two-d-old pupae for exposure to treatments were separated from insect culture jars and kept in wheat flour for 24-h before the treatment. Newly emerged adults were kept in pre-exposure vials containing wheat flour, and were exposed to treatment 7-10 d after emergence of the adults.

Fumigation chambers

Fumigation chambers consisted of 3-L glass jar, each capped with a ground-glass stopper equipped with entry and exit tubing. Two pieces of rubber tubing (5 cm long × 6.2 mm ID) were attached to the tubing and sealed with pinch-clamps.

Mustard essential oil and its main compound

Essential oil from mustard (*Sinapis nigra* L.) and its main compound, allyl isothiocyanate were tested against all life stages of *T. confusum*. Mustard essential oil extracted by stem distillation method was provided commercially from ATL Canada Company. Allyl isothiocyanate (Merck, 800260, 95% purity) was provided commercially by Sigma-Aldrich. After purchase, mustard essential oil and allyl isothiocyanate were collected in sealed glass containers and refrigerated in the dark at 4ºC until their use.

Carbon dioxide and nitrogen gas

Compressed CO_2 and N_2 were supplied by Linde Gas (Ankara, Turkey) and were >99.9% pure.

Bioassay and experimental procedures

Mustard oil and its main compound, allyl isothiocyanate were introduced as a liquid into the fumigation chamber using 10 or 50 µl gastight syringes (Hamilton Company, Switzerland). CO_2 and N_2 were transferred from the supply cylinder through a pipe equipped with a regulator valve. Concentrations of CO_2 and N_2 inside the glass jars were checked by hand-operated O_2/CO_2 analyzer (PBI Dansensor) and portable O_2/N_2 gas analyzer (Brotie, Beijing, China) respectively. Relative humidity during fumigations was also measured by placing small hygrometers within the fumigation chamber. Prior to each test, 20 larvae, pupae, adults and 50 eggs of *T. confusum* were confined, separately, inside 2.5 cm diameter by 5 cm long glass vials.

Firstly, preliminary bioassay tests on fumigation activity of mustard essential oil and allyl isothiocyanate alone; mustard essential oil and allyl isothiocyanate combination with 92% CO_2 or 92% N_2 were conducted to determine the effective concentrations of each treatment against all life stages of *T. confusum*. For mustard essential oil and allyl isothiocyanate alone treatment, all life stages of *T. confusum* were exposed to a concentration of 10 µl/l of mustard essential oil and allyl isothiocyanate for 24-h. Mustard essential oil and allyl isothiocyanate were applied on filter paper (2 x 8 cm) attached to lower side of the lids of fumigation chamber by using 50 µl syringe. After all life stages of *T. confusum* held in the glass vials, insects were transferred separately into fumigation chamber, fumigation chambers were closed by screwed lids, which were made airtight. Each treatment and control were replicated three times. For the treatments with mustard essential oil and allyl isothiocyanate in a CO_2 and N_2 atmosphere, test insects were first placed in the fumigation chambers. Then, prior to the introduction of 10 µl/l of mustard essential oil or allyl isothiocyanate concentration, the fumigation chambers were briefly evacuated to 60.8 or 295 mm Hg followed by flushing with CO_2 or N_2 respectively until restoration of atmospheric pressure so as to achieve a uniform concentration of 92±3% CO_2 or 92±3% N_2. The 24-h exposure was used throughout all the experiments. In addition to these treatments, separate exposure to 92% CO_2 or 92% N_2 alone was made and untreated control insects were exposed to atmospheric conditions. For all fumigations, relative humidity and temperature were maintained at 65±5% at atmospheric pressure and 25±1°C, respectively. The relative humidity level was maintained in the fumigation chamber by using saturated solutions of sodium nitrite (Greenspan, 1977).

Concentration-mortality tests were conducted to determine LC_{50} and LC_{90} values of mustard essential oil and allyl isothiocyanate alone and in their combination with 92% CO_2 or 92% N_2 for all life stages of *T. confusum*. Each stage of *T. confusum* was exposed to four to five different concentrations of mustard essential oil or allyl isothiocyanate for 24-h. With mustard essential oil and allyl isothiocyanate alone a range of five concentrations from 0.25 to 5 µl/l and from 0.25 to 2 µl/l for all life stages of *T. confusum* was used, respectively. With mustard essential oil and allyl isothiocyanate in combination with 92% CO_2 ranges consisted of five concentrations from 0.25 to 1.5 µl/l and from 0.25 to 2 µl/l for all life stages of *T. confusum,* respectively. With mustard essential oil and allyl isothiocyanate in combination with 92% N_2 ranges consisted of five concentrations from 0.25 to 5 µl/l and from 0.25 to 2.6 µl/l for all life stages of *T. confusum,* respectively. Concentrations were selected for all life stages of *T. confusum* on basis of preliminary bioassay tests. Each concentration and control treatment were replicated three times. Fumigation procedures were the same as described above.

Data processing and analysis

After each treatment, larvae, pupae, and adults were transferred to clean 200-ml jars containing wheat flour and were kept at 25±1ºC and 65±5% RH until mortality checking. The eggs were held in their Perspex slides under the same conditions and examined for egg hatch after 7 d. Mortality data obtained from preliminary tests were normalized using arcsine transformation and then were analyzed using one-way ANOVA. The means were separated using the LSD test at 5% significance level (SAS Institute, 1985). Data obtained from each zero dose control and concentration-response mortality were subjected to probit analysis PoloPlus (LeOra Software, Petaluma, CA) (LeOra Software, 2005) to determine LC_{50} (Lethal Concentration$_{50}$) and LC_{90} (Lethal Concentration$_{90}$) values for each treatment and life stage of *T. confusum* and their 95% confidence intervals.

Results and Discussion

Preliminary bioassay tests indicated that all treatments (mustard essential oil and allyl isothiocyanate alone, mustard essential oil and allyl isothiocyanate+92% N_2 or 92% CO_2) except 92% CO_2 and N_2 alone and control resulted in 100% mortality for all life stages of *T. confusum* (Table 1). However, exposure to 92% CO_2 and 92% N_2 alone produced very low mortality of adults, pupae and larvae of *T. confusum*, but they resulted in relatively high mortality of *T. confusum* eggs (54-78%). Preliminary bioassay tests indicated that 10 µl/l concentration of mustard essential oil and allyl isothiocyanate alone resulted in 100% mortality for all life stages of *T. confusum* without any necessity of CO_2 and N_2 combinations (Table 1).

Table 1. Percentage mortalities (%) of all life stages of *T. confusum* exposed to 10 µl/l concentration of mustard essential oil and allyl isothiocyanate alone, 10 µl/l of mustard essential oil in combination with 92% CO_2 or 92% N_2, and 92% CO_2 and 92% N_2 alone for 24-h exposure time.

Treatments	Mean mortality(%)±Standard error			
	Egg	Larva	Pupa	Adult
Mustard oil	100.00±0.00 A*	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A
Mustard oil+92% CO_2	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A
Mustard oil+92% N_2	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A
Allyl isothiocyanate	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A
Allyl isothiocyanate+92% CO_2	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A
Allyl isothiocyanate+92% N_2	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A	100.00±0.00 A
92% CO_2	78.00±1.16 B	8.33±3.33 B	28.33±1.67 B	15.00±0.00 B
92% N_2	54.00±1.15 C	8.33±1.67 B	15.00±2.89 C	10.00±2.89 C
Control	10.67±0.67 D	1.67±1.67 C	8.33±3.33 C	0.00±0.00 D
F and P value	$F_{8,18}$ = 3548.9 $P < 0.0001$	$F_{8,18}$ = 253.3 $P < 0.0001$	$F_{8,18}$ = 242.7 $P < 0.0001$	$F_{8,18}$ = 1331.6 $P < 0.0001$
LSD value	2.338	8.708	7.114	3.591

* Means within a column with the same letter are not significantly different (LSD at 5% level). One-way ANOVA was applied to the data.

Probit analysis data of mustard essential oil alone and mustard essential oil in combination with 92% CO_2 or 92% N_2 for larval to adult stages of *T. confusum* resulting from 24-h laboratory fumigations are given Table 2. Since the lowest concentration of mustard essential oil alone (0.25 µl/l of air) resulted 100% mortality of *T. confusum* eggs, the lethal concentration values were not able to be estimated. Mustard essential oil in combination with 92% CO_2 or 92% N_2 reduced LC_{50} and LC_{90} values for adults, larvae and pupae of *T. confusum*. Mustard essential oil in combination with 92% CO_2 had 7.3, 1.8 and 4.6 times reduction in LC_{90} values for larvae, pupae and adults of *T. confusum,* respectively, compared with mustard essential oil alone (Table 2). Mustard essential oil in combination with 92% N_2 had 1.2, 1.1 and 3.1 times reduction in LC_{90} values for larvae, pupae and adults of *T. confusum,* respectively, compared with mustard essential oil alone. Generally, the combinations of mustard essential oil with 92% CO_2 were more toxic to larvae, pupae and adults of *T. confusum* than those in combinations with 92% N_2 as evidenced by significant decrements in their LC_{90} values.

Toxicity data of allyl isothiocyanate alone and allyl isothiocyanate in combination with 92% CO_2 or 92% N_2 for all life stages of *T. confusum* resulting from 24-h laboratory fumigations are given Table 3. Allyl isothiocyanate in combination with 92% CO_2 and N_2 reduced LC_{50} and LC_{90} values of all life stages of *T. confusum*. Allyl isothiocyanate in combination with 92% CO_2 had 4.1, 1.9 and 4.1 times reduction in LC_{90} values for larvae, pupae and adults of *T. confusum,* respectively, compared with allyl isothiocyanate alone (Table 3). Allyl isothiocyanate in combination with 92% N_2 had 2.3, 1.2 and 2.1 time reduction in LC_{90} values for larvae, pupae and adults of *T. confusum,* respectively, compared with allyl isothiocyanate alone. Generally, the combinations of allyl isothiocyanate with 92% CO_2 were more toxic to larvae, pupae and adults of *T. confusum* than those in combinations with 92% N_2 as evidenced by significant decrements in their LC_{90} values.

Table 2. Probit analysis data of mustard essential oil alone and mustard essential oil in combination with 92% CO_2 or 92% N_2 for larval to adult stages of *Tribolium confusum* resulting from 24-h laboratory fumigations

Life stage[f]	Treatments	N[a]	Slope[b]±S.E.	LC_{50} (µl/l) (Fiducial limit)[c]	LC_{90} (µl/l) (Fiducial limit)[c]	χ^{2} [d] H[e]
Larva	Mustard oil alone	420	4.59±0.52	1.76 (1.54-1.97)	3.35 (2.94-3.98)	6.19 0.39
	Mustard oil+ 92% CO_2	300	8.33±1.31	0.32 (0.29-0.34)	0.46 (0.42-0.52)	5.22 0.58
	Mustard oil+ 92% N_2	420	6.05±0.70	1.73 (1.57-1.87)	2.82 (2.58-3.19)	8.55 0.43
Pupa	Mustard oil alone	360	4.37±0.69	0.83 (0.67-0.95)	1.75 (1.55-2.06)	7.93 0.61
	Mustard oil+ 92% CO_2	300	3.41±0.52	0.41 (0.33-0.48)	0.98 (0.82-1.31)	5.81 0.58
	Mustard oil+ 92% N_2	420	4.76±0.73	0.92 (0.76- 1.07)	1.62 (1.39-2.03)	10.71 0.67
Adult	Mustard oil alone	360	6.02±0.55	2.25 (2.08-2.43)	3.67 (3.32-4.19)	9.87 0.76
	Mustard oil+ 92% CO_2	360	4.00±0.41	0.42 (0.37-0.47)	0.88 (0.77-1.05)	5.39 0.41
	Mustard oil+ 92% N_2	420	4.71±0.42	0.65 (0.59-0.71)	1.21 (1.09-1.41)	9.85 0.62

[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 value, [e]Heterogeneity value, [f]The lethal concentration values were not able to be estimated since the lowest concentration of mustard essential oil alone (0.25 µl/l of air) resulted 100% mortality of *Tribolium confusum* eggs.

The results of probit analyses indicate that the use of 92% CO_2 or 92% N_2 with mustard essential oil and allyl isothiocyanate clearly resulted in significant reductions of LC_{50} and LC_{90} values for larvae, pupae and adults of *T. confusum*. This was particularly effective for the most tolerant stages, larvae and adults, where combining mustard essential oil and allyl isothiocyanate with 92% CO_2 decreased the LC_{90} value from 3.35 to 0.46 µl/l and 2.65 to 0.65 µl/l; from 3.67 to 0.88 µl/l and 1.98 to 0.49 µl/l, respectively. Similarly, mustard essential oil and allyl isothiocyanate with 92% N_2 also decreased the LC_{90} value of larval and adults' stage from 3.35 to 2.82 µl/l and 2.65 to 1.16 µl/l; from 3.67 to 1.21 µl/l and 1.98 to 0.93 µl/l, respectively. It might be argued that low O_2 concentrations could influence the potentiating effect of CO_2 and N_2 for the most tolerant stages of *T. confusum* (larvae and adults). However, data without mustard essential oil and allyl isothiocyanate indicated that exposure of 92% CO_2 alone for 24 h resulted in only limited mortality of the larvae and adults. Therefore, the results suggest that CO_2 or N_2 might have a synergistic effect on target insects when exposed together with mustard essential oil and allyl isothiocyanate. The addition of CO_2 has long been known to increase the toxicity of fumigant gases for some storage insect pests (Bond & Buckland, 1978; Navarro et al., 2004) and has been recommended as a possible method of lowering the amount of methyl bromide required (Kawakami et al., 1996). Several modes of action have been proposed for the toxic action of elevated CO_2 levels (Friedlander, 1983), which include a reduction in various detoxification pathways from the mixed function oxidizes to the regeneration of acetylcholine. At CO_2 levels as low as 1% in air, the insects increase their spiracle opening, allowing the diffusion of fumigant gases into the tracheae to increase (Wigglesworth, 1972). Depending on insect species, insect spiracles remain open in the CO_2 concentration ranging from 2% to 5%, (Wigglesworth, 1972), which facilities the entrance of the toxic gases into the insect body. In the present study, CO_2 might have had a synergistic effect on target insects when exposed together with mustard essential oil and allyl isothiocyanate, which can be explained by mode of action of CO_2 as described above.

Other studies have indicated that the addition of CO_2 can increase the toxicity of methyl bromide (Dumas et al., 1969; Calderon & Leesch, 1983; Williams, 1985; Donahaye & Navarro, 1989). All these studies reported that the susceptibilities of target insects to fumigants mixture with CO_2 were increased by a factor of one to three. A similar joint action with CO_2 atmospheres has been recorded for some essential oils. The peel oils of *Citrus* spp. and *Eucalyptus citriodora* Hook at 10 and 20 µl/l concentrations in presence of two different modified atmospheres (15% CO_2+1% O_2+84% N_2 and 12% CO_2+5% O_2+83% N_2) were more toxic to the psocid, *Liposcelis bostrychophila* Badonnel, 1931 (Wang et al., 2001). However, toxicity results from present study show that reductions in LC_{50} and LC_{90} caused by mustard essential oil and allyl isothiocyanate in combination CO_2 and N_2 are higher than those reported by Wang et al. (2001).

Table 3. Probit analysis data of allyl isothiocyanate alone and allyl isothiocyanate in combination with 92% CO_2 or 92% N_2 for larval to adult stages of *Tribolium confusum* resulting from 24-h laboratory fumigations

Life stage[f]	Treatments	N[a]	Slope[b]±S.E.	LC_{50} (µl/l) (Fiducial limit)[c]	LC_{90} (µl/l) (Fiducial limit)[c]	χ^2 [d] H[d]
Larva	Allyl isothiocyanate alone	360	7.52±1.11	1.79 (1.61-1.93)	2.65 (2.45-2.99)	4.96 0.31
	Allyl isothiocyanate+92% CO_2	300	4.35±0.59	0.33 (0.27-0.38)	0.65 (0.56-0.79)	6.16 0.62
	Allyl isothiocyanate+92% N_2	360	4.09±0.59	0.57 (0.46- 0.65)	1.16 (1.02-1.41)	5.81 0.36
Pupa	Allyl isothiocyanate alone	360	4.14±0.56	0.53 (0.43-0.62)	1.08 (0.93-1.32)	4.09 0.32
	Allyl isothiocyanate+92% CO_2	300	3.74±0.59	0.26 (0.20-0.31)	0.58 (0.49-0.73)	7.06 0.71
	Allyl isothiocyanate+92% N_2	420	4.25±0.55	0.45 (0.37-0.51)	0.89 (0.79-1.07)	6.13 0.38
Adult	Allyl isothiocyanate alone	360	5.69±0.55	1.18 (1.08-1.79)	1.98 (1.79-2.25)	11.39 0.88
	Allyl isothiocyanate+92% CO_2	300	3.21±0.58	0.19 (0.13-0.25)	0.49 (0.41-0.62)	4.78 0.48
	Allyl isothiocyanate+92% N_2	360	3.90±0.40	0.44 (0.38-0.49)	0.93 (0.81-1.11)	11.50 0.88

[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, [e]Heterogeneity value, [f]The lethal concentration values were not able to be estimated since the lowest concentration of mustard essential oil alone (0.25 µl/l of air) resulted 100% mortality of *Tribolium confusum* eggs.

The combinations of mustard essential oil and allyl isothiocyanate with 92% CO_2 were more toxic to larvae, pupae and adults of *T. confusum* than those in combinations with 92% N_2 as evidenced by significant decrements in their LC_{50} and LC_{90} values. N_2 is not directly toxic to the insects, but is only lethal to the insects by producing a progressive hypoxia or anoxia (decreasing O_2 availability) only when used alone at a high concentration, producing low O_2 atmospheres. However, a higher CO_2 concentration, accompanied by a reduction of O_2, leads to hypercarbia, which directly affects the nervous, endocrine, respiratory and circulatory systems, as well as general metabolism (Wong-Corral et al., 2013). Insects are generally killed more rapidly by CO_2 than by lack of O_2 (high level of N_2 atmosphere) and therefore, CO_2 is more effective method than use of N_2 because CO_2 stimulates insect respiration by displacing O_2 (Bell et al., 1980; Jayas & Jeyamkondan, 2002). The higher toxicity of combination of mustard essential oil and allyl isothiocyanate with CO_2 can be attributed to these insecticidal properties of CO_2 as described above.

In conclusion, the use of high concentration of CO_2 or N_2 with mustard essential oil and allyl isothiocyanate might have a possible synergistic effect on larvae, pupae and adults of *T. confusum* as evidenced by significant reductions in their LC_{50} and LC_{90} values. These results indicate that combination of mustard essential oil and allyl isothiocyanate with CO_2 or N_2 can be potential alternative to the most commonly used commercial fumigants, methyl bromide and phosphine.

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