Fumigant toxicity of garlic essential oil in combination with carbon dioxide (CO_2) against stored-product insects

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Abstract

Fumigant toxicity of garlic essential oil alone and in combination with carbon dioxide (92% CO_2) to adults and pupae of *Tribolium confusum* and *Ephestia kuehniella* at 24 h of exposure time was studied. Results indicated that adults of *E. kuehniella* were the most sensitive, since garlic essential oil alone resulted in their complete mortality at each concentration, whereas adults of *T. confusum* were the most tolerant with LC_{90} value of 30 µL L^{-1}. In contrast to the results for the adults, pupae of *T. confusum* were more sensitive with LC_{90} value of 11.1 µL L^{-1} than those of *E. kuehniella* with LC_{90} value of 27.4 µL L^{-1}. When 92% CO_2 was combined with garlic essential oil, the LC_{50} and LC_{90} values for adult of *T. confusum* and pupa of *T. confusum* and *E. kuehniella* were significantly reduced. Combination of garlic essential oil with 92% CO_2 produced decrements in LC_{90} value of *E. kuehniella* pupa from 27.4 to 0.95 µL L^{-1}, while it resulted in reduction in LC_{90} value of *T. confusum* pupa from 11.1 to 1.0 µL L^{-1}. Combination of garlic essential oil with 92% CO_2 also produced significant decrements in LC_{90} value for adults of *T. confusum* (4.9-fold) compared with those exposed to garlic essential oil alone. These results indicated that 92% CO_2 had a synergistic effect on toxicity of garlic essential oil to *T. confusum* and *E. kuehniella*. In conclusion, present study revealed that the combination of garlic essential oil with CO_2 enhanced its fumigant toxicity to stored product insects.

Keywords: Garlic essential oil, Carbon dioxide, *Tribolium confusum*, *Ephestia kuehniella*, Fumigant toxicity, Synergistic effect

1. Introduction

Control of insect-pest infestation in storage may cause special problems on stored products. In many storage systems, methyl bromide and phosphine are the most economical fumigants for management of stored-grain insect pests. EPA (2001) has proposed elimination of the production of methyl bromide by 2005 because of its ozone depletion potential. Additionally, some stored-product insects are found to have developed resistance to methyl bromide and phosphine (Subramanyam and Hagstrum, 1995; Champ and Dyte, 1977). These problems have highlighted the need for the development of natural products derived from plants as an alternative to conventional insecticides. Many types of spices and herbs are known to possess insecticidal activities (Tripathi et al., 1999), especially in the form of essential oils (Shaaya et al., 1991). They do not leave residues toxic to the environment and have medicinal properties for humans with lower toxicity to mammals (Duke, 1985).

Essential oils are potential sources of alternative compounds to currently used fumigants. Various studies have demonstrated fumigant activity of various essential oils against various stored product insects (Shaaya et al., 1991; Shaaya et al., 1997; Tunç et al., 2000; Lee et al., 2003). Toxicity of various essential oils and their volatile constitutes against all life stages of the flour beetle, *Tribolium confusum* Jacguelin du Val (Coleoptera: Tenebrionidae) indicated that two essential oils, garlic and onion had potent fumigant activities (Karcı, 2006). Gözek (2007) reported that the adults and larvae of *T. confusum* were the most tolerant stages and the eggs and pupae were the most susceptible stages to treatments of garlic essential oil, their active compounds and mixture. In the same study, garlic essential oils, their active compounds and mixture required less than 1 µL L^{-1} to kill 90% of the eggs whilst garlic essential oil and its active compounds required the dosages ranging from 6.4 and 23.3 µL L^{-1} to kill 90% of the larvae.

The use of CO_2 together with conventional fumigants has also been studied. Carbon dioxide, a respiratory stimulant, is a known adjuvant for fumigants including phosphine and methyl bromide. The advantages of using CO_2 in the mixture are to increase the toxicity of the fumigant, improve the distribution pattern, limit the levels of harmful residues in the treated commodity, and also eliminate the flammable hazard of some fumigants. Several general studies on fumigant/CO_2 mixtures have been made in the past (Jones, 1938), and these were followed by investigations which showed that the addition of CO_2 to methyl bromide (MB) resulted in an increase in the susceptibilities of some stored-product insects (Calderon and Leesch, 1983; Willliams, 1985). Laboratory tests with essential oils have shown a similar joint action with CO_2 atmospheres. Shaaya et al. (1997) demonstrated enhanced toxicity of essential oil, SEM76 (from a Lamiaceae plant), in the presence CO_2 to *T. castaneum* (larvae, pupae and adults), *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) (larvae and pupae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (adults). However, toxicity studies on mixture of essential oils with CO_2 against stored-product insects to demonstrate additive, synergistic or antagonistic effects are rare.

The present study was conducted to investigate fumigant toxicity of garlic essential oil alone and in combination with high concentration of carbon dioxide (92% CO_2) to adults and pupae of *T. confusum* and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae).

2. Materials and methods

2.1. Test insects

Tests were carried out on pupae and adults of *E. kuehniella* and *T. confusum.* Adults and pupae of *T. confusum* were obtained from cultures reared at 25 ± 1°C and 65 ± 5% relative humidity (r.h.) on a diet of wheat flour mixed with yeast (17:1, w/w) using standard culture techniques (Donahaye, 1990). Larvae of *E. kuehniella* were reared on a 10:2:1 mixture of wheat flour, wheat germ and dried brewer's yeast at the same environmental conditions as *T. confusum*. Pupae were obtained by daily separation from culture jars and were exposed to the treatments at the age of 2 d. Seven- to 10-d-old and newly-emerged (0-1 d) adults of *T. confusum* and *E. kuehniella*, respectively, were exposed to the treatments in empty exposure jars.

2.2. 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.

2.3. Garlic Essential oil

Essential oil from bulbs of garlic, *Allium sativum* L. (Alliaceae) was tested against all life stages of *T. confusum* and *E. kuehniella*. Garlic essential oil extracted by stem distillation method was provided commercially (Liberty Natural Products, Inc., Oregan City, OR, USA). After purchase, garlic essential oil collected in sealed glass containers was refrigerated in the dark at 4ºC until their use. Carbon dioxide was supplied from a cylinder and was 99% pure.

2.4. Bioassay and Experimental Procedures

Garlic essential oil was introduced as a liquid into the desiccators using 10- or 50-µL gas-tight syringes. Carbon dioxide was transferred from the supply cylinder through a pipe equipped with a regulator valve. Concentrations of CO_2 inside the glass jars were checked by using hand-operated O_2/CO_2 analyzer (PBI Dansensor, Ringsted, Denmark). Relative humidity during fumigations was also measured by placing small hygrometers within the fumigation chamber. Prior to each test, 20 larvae and adults of *T. confusum* and *E. kuehniella* were confined, separately, inside 2.5-cm diameter by 5-cm long glass vials.

Firstly, preliminary bioassay tests on fumigation activity of garlic essential oil alone and garlic essential oil combination with 92% CO_2 were carried out to determine the effective concentrations of each treatment against adults and pupae of *T. confusum* and *E. kuehniella*. For garlic essential oil alone treatment, adults and pupae of each tested species were exposed to a concentration of 10 µL L^{-1} of garlic essential oil for 24 h. Garlic essential oils were applied on filter paper (2 x 8 cm) attached to lower side of the lids of fumigation chamber by using a 50-µL syringe. After adults and pupae of *T. confusum* and *E. kuehniella* kept in the glass vials were transferred separately into fumigation chamber, fumigation chambers were closed by screwed lids, which were made air-tight. Each treatment and control was replicated three times. For the treatments with garlic essential oil in a CO_2 atmosphere, the insects were first placed in the fumigation chambers. Then, prior to the introduction of 10 µL L^{-1} of garlic essential oil concentration, the fumigation chambers were briefly evacuated to 60.8 mm Hg followed by flushing with CO_2 until restoration of atmospheric pressure so as to obtain a uniform concentration of 92±2% CO_2. The 24-h exposure was used throughout all the experiments. In addition to these treatments, separate exposure to 92% CO_2 alone was made and untreated control insects were exposed to atmospheric conditions. For all fumigations, conditions were maintained at 65±5% r.h. at atmospheric pressure and 25±1°C respectively.

Separate bioassay tests were carried out to determine LC_{50} and LC_{90} values of garlic essential oil alone and in combination with 92% CO_2 for adults of *T. confusum* and pupae of *T. confusum* and *E. kuehniella*. Each stage of tested species were exposed to different concentrations of garlic essential oil for 24 h. With garlic essential oil alone a range of 5 concentration levels from 10 to 40 µL L^{-1} for *T. confusum* adult, from 1 to 15 µL L^{-1} for *T. confusum* pupae and from 5 to 35 µL L^{-1} for *E. kuehniella* pupae was used. With garlic essential oil in combination with 92% CO_2 ranges consisted of 5 concentrations, from 0.5 to 8 µL L^{-1} for *T. confusum* adults, from 0.25 to 2.5 µL L^{-1} for *T. confusum* pupae and from 0.25 to 2 µL L^{-1} for *E. kuehniella* pupae. Concentrations were selected for the eggs of insect species on basis of preliminary bioassay tests. Three replicates were set up for each concentration and control. Fumigation procedures were the same as in above mentioned bioassay tests.

2.5. Data processing and analysis

After each treatment, adults and pupae of *T. confusum* and *E. kuehniella* were transferred to 250-mL jars containing standard diets and were held at 26±1°C and 65±5% r.h. until examined for mortality. Mortality counts for adults were made 1-2 d after exposure and pupal mortality was based on those pupae that failed to produce adults 9 d after exposure. Mortality data were subjected to arcsine transformation and then analyzed using one-way analysis of variance (ANOVA). The means were separated using the LSD (Least Significant Difference) method at the 1% level (SAS Institute, 1985). Data obtained from each zero dose control and concentration-mortality responses were subjected to probit analysis by using maximum likelihood program software (POLO-PC) (LeOra Software, 1989) to determine LC_{50}s (Lethal Concentration$_{50}$), LC_{90}s (Lethal Concentration$_{90}$) and their respective 95% confidence intervals.

3. Results

Percentage mortalities (%) of adults and pupae of *T. confusum* and *E. kuehniella* exposed to a concentration of 10 µL L^{-1} of garlic essential oil alone, 10 µL L^{-1} of garlic essential oil in combination with 92% CO_2 and 92% CO_2 alone for 24-h exposure time are given in Table 1. Preliminary bioassay tests indicated that all treatments except control resulted in almost 100% mortality for adults of *E. kuehniella*. However, only garlic essential oil in combination with 92% CO_2 achieved almost mortality of 100% against adult of *T. confusum,* which was significant higher mortality than those of garlic essential oil alone, 92% CO_2 alone and control treatment (P<0.0001). Similarly, garlic essential oil in combination with 92% CO_2 resulted in 100% mortality of pupae of *T. confusum* and *E. kuehniella*, which was significant higher mortality than those of garlic essential oil alone, 92% CO_2 alone and control treatment (P<0.0001). Preliminary bioassay tests indicated that garlic essential oil in combination with 92% CO_2 resulted in the increase of mortality of adults of *T. confusum* and pupae of *T. confusum* and *E. kuehniella*. Exposure to 92% CO_2 alone produced a very low mortality of *T. confusum* adults, but it resulted in relatively high mortality of *T. confusum* and *E. kuehniella* pupae (27% to 31%).

Table 1 Percentage mortalities (%) of adults and pupae of *T. confusum* and *E. kuehniella* exposed to a concentration of 10 µL/L of garlic essential oil alone, 10 µL/L of garlic essential oil in combination with 92% CO_2 and 92% CO_2 alone for 24-h exposure time.

	Mortality (%)±SE			
	T. confusum		***E. kuehniella***	
Treatments	**Adult**	**Pupa**	**Adult**	**Pupa**
Garlic oil alone	17.6±2.22 b	34.4±2.94 b	97.8±2.22 a	34.4±2.94 b
Garlic oil + 92% CO_2	98.3±1.67 a	100±0 a	100±0 a	100±0 a
92% CO_2 alone	23.3±3.85 b	31.1±5.88 b	100±0 a	26.7±3.85 b
Control	2.2±2.22 c	17.2±2.22 b	6.7±3.33 b	16.2±2.22 b

Means within a column with the same letter are not significantly different (LSD test at 1% level). One-way ANOVA was applied for data.

Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for pupae of *T. confusum* and *E. kuehniella* resulting from 24-h laboratory fumigations are given Tables 2 and 3, respectively. The tables show that garlic essential oil in combination with 92% CO_2 reduced LC_{50} and LC_{90} values of pupae of *T. confusum* and *E. kuehniella*. Garlic essential oil in combination with 92% CO_2 produced a significant decrease in LC_{90} values from 11.1 to 1.0 µL L^{-1} and from 27.4 to 0.95 µL L^{-1} for pupae of *T. confusum* and *E. kuehniella* respectively. Thus, garlic essential oil in combination with 92% CO_2 had 11.1- and 28.9-fold reduction in LC_{90} values for pupae of *T. confusum* and *E. kuehniella*, respectively, compared with garlic essential oil alone (Tables 2 and 3). The decrements in LC_{50} values were higher than those in LC_{90} values, the LC_{50} values obtained from garlic essential oil in combination with 92% CO_2 were still reduced by 14.3- and 58.3-fold for pupae of *T. confusum* and *E. kuehniella*, respectively, compared with garlic essential oil alone.

Table 2 Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for pupae of *Tribolium confusum* resulting from 24-h laboratory fumigations.

Treatment	**n**[a]	**Slope±SE**	**LC_{50} (µL/L) (Fiducial limit)**[b]	**LC_{90} (µL/L) (Fiducial limit)**[b]	**λ^{2c}**
Garlic oil alone	315	4.15±0.64	5.45 (4.34-6.52)	11.09 (9.11-14.81)	4.712
Garlic oil + 92% CO_2	315	3.02±0.51	0.38 (0.22-0.42)	1.00 (0.78-1.46)	5.191
Ratio			14.34	11.09	

[a]: Number treated, excluding controls. [b]: Numbers in brackets give the 95% confidence range. [c]: Chi-square (chi-square is significant, $P < 0.05$).

Table 3 Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for pupae of *Ephestia kuehniella* resulting from 24-h laboratory fumigations.

Treatment	**n**[a]	**Slope±SE**	**LC_{50} (µL/L) (Fiducial limit)**[b]	**LC_{90} (µL/L) (Fiducial limit)**[b]	**λ^{2c}**
Garlic oil alone	315	10.99±2.56	20.98 (18.22-22.73)	27.44 (25.23-32.33)	9.897
Garlic oil + 92% CO_2	315	3.03±0.52	0.36 (0.31-0.40)	0.95 (0.74-1.39)	4.184
Ratio			58.28	28.89	

[a]: Number treated, excluding controls. [b]: Numbers in brackets give the 95% confidence range. [c]: Chi-square (chi-square is significant, $P < 0.05$).

Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for adults of *T. confusum* resulting from 24-h laboratory fumigations are given Tables 4. Garlic essential oil in combination with 92% CO_2 reduced LC_{50} and LC_{90} value of adults of *T. confusum*. Garlic essential oil in combination with 92% CO_2 produced a significant decrease in LC_{90} values from 30.1 to 6.1 µL L^{-1} for adults of *T. confusum*. Thus, garlic essential oil in combination with 92% CO_2 had 4.9-fold reduction in LC_{90} values for adults of *T. confusum* compared with garlic essential oil alone (Table 4). The decrements in LC_{50} values were higher than those in LC_{90} values, the LC_{50} values obtained from garlic essential oil in combination with 92% CO_2 were still reduced by 9-fold for adults of *T. confusum* compared with garlic essential oil alone.

Table 4 Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% CO_2 for adults of *Tribolium confusum* resulting from 24-h laboratory fumigations.

Treatment	n[a]	Slope ± SE	LC_{50} ($\mu L\ L^1$) (Fiducial limit)[b]	LC_{90} ($\mu L\ L^1$) (Fiducial limit)[b]	λ^{2c}
Garlic oil alone	420	5.38±0.54	17.38 (15.74-19.88)	30.07 (27.37-33.99)	9.099
Garlic oil + 92% CO_2	420	2.55±0.27	1.93 (1.55-2.29)	6.12 (4.98-.807)	6.727
Ratio			9.01	4.91	

[a]: Number treated, excluding controls. [b] : Numbers in brackets give the 95% confidence range. [c] : Chi-square (chi-square is significant, $P < 0.05$).

4. Discussion

From this study, it can be seen that the use of 92% CO_2 with garlic essential oil clearly resulted in significant reductions of LC_{50} and LC_{90} values for adults and pupae of *T. confusum* and *E. kuehniella*. This was particularly effective for the most tolerant pupal stage where combining garlic essential oil with 92% CO_2 decreased the LC_{90} value from 27.4 to 0.95 $\mu L\ L^{-1}$. It might be argued that low O_2 concentrations could influence the potentiating effect of CO_2 for pupae of *T. confusum* and *E. kuehniella*. However, data without garlic essential oil indicated that there was only limited mortality of the adults and pupae on exposure to 92% CO_2 alone for 24 h. Therefore, the results suggest that CO_2 has a synergistic effect on the test insects when exposed together with garlic essential oil.

Other studies have shown that the admixture of CO_2 could increase the toxicity of fumigants, mainly MB and phosphine (Monro et al., 1966; Dumas et al., 1969; Calderon and Leesch, 1983; Williams, 1985; Donahaye and Navarro, 1989). In all these studies the susceptibilities of test insects to fumigants combined with CO_2 were found to increase by only a factor of one to three. Laboratory tests with essential oils have shown a similar joint action with CO_2 atmospheres. The peel oils of *Citrus* spp. and *Eucalyptus citriodora* Hook at 10 and 20 $\mu L\ L^{-1}$ doses were more toxic in presence of two different controlled atmospheres (15% CO_2 + 1% O_2 + 84% N_2 and 12% CO_2 + 5% O_2 + 83% N_2) to the psocid, *Liposcelis bostrychophilus* Badonnel (Psocoptera: Liposcelidae) (Wang et al., 2001). However, the results obtained from our studies reveal that reductions in LC_{50} and LC_{90} caused by garlic essential oil in combination CO_2 are much higher than those reported by the above authors.

In conclusion, the use of high concentration of CO_2 appears to have a synergistic effect on these species as evidenced by significant decrements in LC_{50} and LC_{90} values for the adults and pupae. These results indicate that combination of garlic essential oil with CO_2 can be potential as an alternative application to the most commonly used commercial fumigants, methyl bromide and phosphine.

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