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# Fumigant toxicity and joint action of garlic essential oil and its main compound, allyl disulphide with certain modified atmospheres against confused flour beetle, *Tribolium confusum*

ALI A IŞIKBER*, ÖZGÜR SAĞLAM[2], M KUBILAY ER[1], HASAN TUNAZ[1], SALMAN KERVANDA[1]

[1]*Agriculture Faculty, Plant-Protection Department, Kahramanmaraş Sütçü İmam University, Avşar Campus, 46100, Kahramanmaraş, Turkey*

ABSTRACT

In this study, fumigant toxicity of garlic, *Allium sativum*, essential oil and its main compound (allyl disulphide) in combination with high concentration (92%) of carbon dioxide ($CO_2$) and nitrogen ($N_2$) to all life stages of *Tribolium confusum* Jacquelin du Val was determined. Preliminary bioassay tests indicated that 10 µl/L concentration of garlic essential oil and allyl disulphide alone resulted in 100% mortality of only eggs and pupae of *T. confusum* without any necessity of $CO_2$ and $N_2$ combinations. However, these treatments did not cause 100% mortality of larvae and adults of *T. confusum***.** According to preliminary bioassay tests, combinations of garlic essential oil and allyl disulphide with 92% $CO_2$ and $N_2$ resulted in significant increase in the mortalities larvae and adults of *T. confusum*. Moreover, there were differences in susceptibility of life stages of *T. confusum* to combinations of garlic essential oil and allyl disulphide with $CO_2$ and $N_2$. While the egg was the most susceptible stage to the treatments, the larva and adult were the most resistance stages. Lethal concentration tests indicated that garlic essential oil and allyl disulphide in combination with 92% $CO_2$ and $N_2$ had 1.1- to 4.4-fold reduction in $LC_{90}$ values for all life stages of *T. confusum***.** Generally the combinations of garlic essential oil and its main compound (allyl disulphide) with 92% $CO_2$ were more toxic to all life stages of *T. confusum* than those in combinations with 92% $N_2$ as evident by significant decrease in their $LC_{50}$ and $LC_{90}$ values. It appears that high concentration of $CO_2$ and $N_2$ have a synergistic effect on all life stages of *T. confusum* when exposed together with garlic essential oil and allyl disulphide. Thus, that combinations of garlic essential oil and its main compound (allyl disulphide) with modified atmosphere can be potential as an alternative application to the most commonly used commercial fumigants.

**Key words:** Allyl disulphide, Fumigant, Garlic, Modified atmosphere, *Tribolium confusum*

Essential oils and their compounds are potential sources of alternative compounds to currently used fumigants. Various studies have demonstrated fumigant activity of various essential oils and their compounds against various stored-product insects (Shaaya et al., 1997; Huang et al., 2000; Tunç et al., 2000). A number of studies showed that garlic (*Allium sativum* L.) essential oil and its major components, (methyl allyl disulphide and diallyl trisulphide), have high toxicity against both *Sitophilus zeamais* (Motsch*)* and *Tribolium castaneum* (Herbst) (Ho et al., 1996; Huang et al., 2000), indicating their potential use in protecting grain and other stored products. Gözek (2007) reported that the adults and larvae of *Tribolium confusum* du Val. were the most tolerant stages and the eggs and pupae were the most susceptible stages to treatments of garlic essential oil, their active compounds (allyl sulphide, allyl disulphide and dipropyl disulphide) and mixture. In the same study, garlic essential oils,

[2]Namık Kemal University, Agriculture Faculty, Plant Protection Department, Tekirdağ, Turkey
*Corresponding author e-mail: *isikber@ksu.edu.tr*

their active compounds and mixture required less than 1 µl/L to kill 90% of the eggs whilst garlic essential oil and its active compounds required the dosages ranging from 6.4 to 23.3 µl/L 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; Williams, 1985). Laboratory tests with essential oils have shown a similar joint action with $CO_2$ atmospheres. Shaaya et al. (1999) 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 (larvae and pupae), *Rhyzopertha dominica* (Fabricius), *Sitophilus oryzae* (L.) and *Oryzaephilus surinamensis* (L.) (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.

Several studies revealed that garlic essential oil and its major components (allyl sulfide, allyl disulphide and diallyl trisulfide) had high fumigant toxicity against stored grain insects (Ho et al., 1996; Huang et al., 2000; Karcı, 2006; Gözek, 2007; and potential use for controlling stored grain insects. The use of garlic essential oil and its compounds with high concentrations of inert gases such as nitrogen ($N_2$) and carbon dioxide ($CO_2$) may contribute to increase their toxicity against stored product insects and penetration power in bulk commodity. In this context, the present study was conducted to determine fumigant toxicity of garlic essential oil and its main compound (allyl disulphide) in combination with high concentration (92%) of $CO_2$ and $N_2$ to all life stages of *T. confusum*.

## MATERIALS AND METHODS

### *Test insects*

Biological tests were carried out on all life stages (egg, larva, pupa and adult) of *T. confusum*. *Tribolium confusum* were obtained from cultures reared in 1 L glass jars at 25 ± 1°C and 65 ± 5% (r.h. on a diet of wheat (*Triticum* sp.) flour mixed with dry brewer's yeast (17:1, w:w). Eggs were daily separated from oviposition jars by sieving (60 mesh, 250 µm sieve). Eggs for exposure to treatments were transferred into the glass vials (2.5 cm diameter and 5 cm long). The 10 ml glass tube (18 cm height × 18 cm width × 12 cm diameter), each containing 50 eggs of 1–2 days old, was exposed to each treatment. Larvae 25–30 days after oviposition were removed from culture jars and exposed to the treatments. Two-day-old pupae were obtained by daily separation from culture jars and held in wheat flour for 24 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.

### *Fumigation chambers*

Fumigation chambers consisted of 3–l glass jars, 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.

### *Garlic essential oil and its main compound*

Essential oil from garlic and its main compound, (diallyl disulphide) were tested against all life stages of *T. confusum*. Garlic essential oil extracted by stem distillation method was provided commercially from ATL Canada Company. Diallyl disulphide (Sigma-Aldrich, 317691, 80%) was provided commercially by Sigma-Aldrich. After purchase, garlic essential oil and diallyl disulphide were collected in sealed glass containers and refrigerated in the dark at 4 ºC until their use.

### *Carbon dioxide and nitrogen gas*

Carbon dioxide ($CO_2$) and nitrogen ($N_2$) gas in a steel cylinder were supplied from Linde Gas (Ankara, Turkey) and was >99.9 % pure.

### *Bioassay and experimental procedures*

Garlic essential oil and its main compound, allyl disulphide were introduced as a liquid into the desiccators using 10 or 50 µl gas-tight syringes. Carbon dioxide 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 using hand-operated $O_2$/$CO_2$ analyzer (PBI Dansensor) and portable $O_2$/$N_2$ gas analyzer (BROTIE, China) respectively. Relative humidity during fumigations was also measured by placing small digital hygrometers (XIKAR Puro Temp$^{TM}$, USA) 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.

Bioassay tests on fumigation activity of garlic essential oil and allyl disulphide alone or in combination with either 92% $CO_2$ or 92% $N_2$ were carried out, to determine the effective concentrations of each treatment against all life stages of *T. confusum*. For garlic essential oil and allyl disulphide alone treatment, all life stages of *T. confusum* were exposed to a concentration of 10 µl/L of garlic essential oil and allyl disulphide for 24 h. Garlic essential oil and allyl disulphide were applied on filter paper (2 cm × 8 cm) attached to lower side of the lids of fumigation chamber by using 0–50 µl syringe. After all life stages of *T. confusum* 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 and allyl disulphide in a $CO_2$ and $N_2$ atmosphere, the insects were first placed in the fumigation chambers. Then, prior to the introduction of 10 µl/L of garlic essential oil and allyl disulphide concentration, the fumigation chambers were briefly evacuated to 60.8 mm Hg by using vacuum pump (KNF, Germany) with digital vacuum gage (Series DPG Digital pressure Gage, Dwyer Instruments, USA) and followed by flushing with $CO_2$ and $N_2$ until restoration of atmospheric pressure so as to obtain a uniform concentration of 92 ± 2% $CO_2$ and $N_2$. The 24-h exposure was used throughout all the experiment. Besides treatments, separate exposure to 92% $CO_2$ and $N_2$ alone was made and untreated control insects were exposed to atmospheric conditions. For all fumigations, r.h. and temperature were maintained at 65±5% at atmospheric pressure and 25±1°C respectively.

Additional bioassay tests were carried out to determine $LC_{50}$ and $LC_{90}$ values of garlic essential oil and allyl disulphide alone and in their combination with 92% $CO_2$ and $N_2$ for all life stages of *T. confusum*. Each stage of *T. confusum* was exposed to four to five different concentrations of garlic essential oil and allyl disulphide for 24 h. With garlic essential oil and allyl disulphide alone a range of 5 concentration levels from 0.25 to 40 µl/L and from 0.25 to 20 µl/L for all life stages of *T. confusum* was used respectively. With garlic essential oil and allyl disulphide in combination with 92% $CO_2$ ranges consisted of 5 concentrations from 0.25 to 15 µl/L and from 0.25 to 10 µl/L for all life stages of *T. confusum,* respectively. With garlic essential oil and allyl disulphide in combination with 92% $N_2$ ranges consisted of 5 concentrations from 0.25 to 35 µl/L and from 0.25 to 20 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. Three replicates were set up for each concentration and control. Fumigation procedures were the same as in above mentioned bioassay tests.

*Data processing and analysis*

After each treatment, larvae, pupae, and adults were transferred to 200-ml jars containing food medium and were held at 25 ± 1°C and 65 ± 5% r.h. until examined for mortality. 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 5% level (SAS Institute, 1985). Data obtained from each zero dose control and concentration-mortality responses were subjected to probit analysis by using maximum likelihood programme software (POLO-PC) (LeOra Software, 1987) to determine $LC_{50}$s (Lethal Concentration$_{50}$), $LC_{90}$s (Lethal concentration$_{90}$).

## RESULTS AND DISCUSSION

Bioassay tests indicated that all treatments except 92% $CO_2$ and $N_2$ alone and control resulted in 100% mortality for eggs and pupae of *T. confusum*. However, only garlic oil in combination with 92% $CO_2$ achieved almost 100% mortality of adults and larvae of *T. confusum,* which was significant higher mortality than those of garlic essential oil alone, garlic oil + 92% $N_2$, 92% $CO_2$ and $N_2$ alone and control treatment ($P <$ 0.0001; Table 1). Garlic essential oil alone and garlic, oil + 92% $N_2$ treatments resulted in low mortalities of *T. confusum* adults and larvae ranging from 6.7 to 38.3%. Exposure to 92% $CO_2$ and $N_2$ alone caused very low mortality of adults, pupae and larvae of *T. confusum*, but they resulted in relatively high mortality of *T. confusum* eggs (54 to 78%). Preliminary bioassay tests indicated that only garlic oil in combination with 92% $CO_2$ resulted in significant increase of mortality of *T. confusum* larvae and adults. On the other hand, garlic oil in combination with 92% $N_2$ resulted in very low increase of mortality of *T. confusum* larvae and adults.

Preliminary bioassay tests indicated that allyl disulphide in combination with 92% $CO_2$ achieved 100% mortality of all life stages of *T. confusum* while all treatments except 92% $CO_2$ and $N_2$ alone and the control resulted in 100% mortality for eggs and pupae of *T. confusum* (Table 2). Allyl disulphide alone and allyl disulphide + 92% $N_2$ treatments achieved high

Table 1 Percentage mortalities (%) of all life stages of *Tribolium confusum* exposed to 10 µl/L concentration of garlic essential oil alone, 10 µl/L of garlic essential oil in combination with 92% $CO_2$ and $N_2$, and 92% $CO_2$ and $N_2$ alone for 24 h exposure time at 25ºC and 65% r.h.

| Treatments | Mortality (%)±S.E | | | |
|---|---|---|---|---|
| | Egg | Larva | Pupa | Adult |
| Garlic oil | 100±0 A | 8.33±1.67 C | 100 ±0 A | 6.67±4.41 D |
| Garlic oil +92% $CO_2$ | 1000 A | 96.67±2.67 A | 100±0 A | 96.67 ±1.67 A |
| Garlic oil +92% $N_2$ | 1000 A | 38.33±1.67 B | 100±0 A | 35 ±2.89 B |
| 92% $CO_2$ | 78±1.16 B | 8.33±3.33 C | 28.33 ±1.67 B | 15±0 C |
| 92% $N_2$ | 54±1.15 C | 8.33±1.67 C | 15 ±2.89 C | 10±2.89 DC |
| Control | 10.67 ±0.67 D | 1.67±1.67 D | 8.33±3.33 D | 0±0 E |
| F and P value | $F_{5,12}$=3548.9 P<0.0001 | $F_{5,12}$=74.6 P<0.0001 | $F_{5,12}$=462.3 P<0.0001 | $F_{5,12}$=66.9 P<0.0001 |
| LSD value | 1.516 | 9.991 | 5.258 | 10.766 |

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

Table 2 Percentage mortalities of all life stages of *Tribolium confusum* exposed to 10 µl/L concentration of allyl disulphide alone, 10 µl/L of allyl disulphide in combination with 92% $CO_2$ and $N_2$, and 92% $CO_2$ and $N_2$ alone for 24-h exposure time at 25ºC and 65% r.h.

| Treatments | Mortality (%)±S.E | | | |
|---|---|---|---|---|
| | Egg | Larva | Pupa | Adult |
| Allyl disulphide | 100±0 A | 71.66±1.67 C | 100 ±0 A | 88.33±1.67B |
| Allyl disulphide +92% $CO_2$ | 100 ±0 A | 100±0 A | 100±0 A | 100±0 A |
| Allyl disulphide +92% $N_2$ | 100 ±0 A | 85±0 B | 100±0 A | 98.33±1.67 A |
| 92% $CO_2$ | 78±1.16 B | 8.33±3.33 D | 28.33 ±1.67 B | 15±0 C |
| 92% $N_2$ | 54±1.15 C | 8.33±1.67 D | 15 ±2.89 C | 10±2.89 C |
| Control | 10.67 ±0.67 D | 1.67±1.67 E | 8.33±3.33 C | 0±0 D |
| F and P value | $F_{5,12}$=1515.7 P<0.0001 | $F_{5,12}$=209.7 P<0.0001 | $F_{5,12}$=348.1 P<0.0001 | $F_{5,12}$=312.9 P<0.0001 |
| LSD value | 2.221 | 7.323 | 5.449 | 6.751 |

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

mortalities of *T. confusum* adults and larvae ranging from 71.6% to 98.3%, but these treatments did not resulted in the complete mortality of *T. confusum* adults and larvae. Exposure to 92% $CO_2$ and $N_2$ alone produced very low mortality of adults, pupae and larvae of *T. confusum*, but resulted in relatively high mortality of *T. confusum* eggs (54 to 78%). Preliminary bioassay tests indicated that both allyl disulphide in combination with 92% $CO_2$ and $N_2$ resulted in significant increase of mortality of *T. confusum* larvae and adults.

Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% $CO_2$ and $N_2$ for all life stages of *T. confusum* resulting from 24-h laboratory fumigations are given in Table 3. Garlic essential oil in combination with 92% $CO_2$ and $N_2$ reduced $LC_{50}$ and $LC_{90}$ values of all life stages of *T. confusum*. Garlic essential oil in combination with 92% $CO_2$ had 4.4, 3.8, 2.9 and 2.6-fold reduction in $LC_{90}$ values for eggs, larvae, pupae and adults of *T. confusum* respectively compared with garlic essential oil alone (Table 3). Garlic essential oil in combination with 92% $N_2$ had 3.3, 1.3, 1.4 and 1.2-fold reduction in $LC_{90}$ values for eggs, larvae, pupae and adults of *T. confusum* respectively compared with garlic essential oil alone. Generally the combinations of garlic essential oil with 92% $CO_2$ were more toxic to all life stages 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 disulphide alone and allyl disulphide in combination with 92% $CO_2$ and $N_2$ for all life stages of *T. confusum* resulting from

Table 3 Probit analysis data of garlic essential oil alone and garlic essential oil in combination with 92% $CO_2$ and $N_2$ for all life stages of *Tribolium confusum* resulting from 24-h laboratory fumigations at 25ºC and 65% r.h.

| Life stage | Treatments | N[a] | Slope[b] ± S.E. | $LC_{50}$ (µl/L) (Fiducial limit)[c] | $LC_{90}$ (µl/L) (Fiducial limit)[c] | $\chi^2$ [d] |
|---|---|---|---|---|---|---|
| Egg | Garlic oil | 600 | 2.67 ± 0.20 | 0.53 (0.46 – 0.59) | 1.59 (1.38 – 1.89) | 9.91 |
| | Garlic oil + 92% $CO_2$ | 600 | 6.25 ± 0.99 | 0.22 (0.19 – 0.24) | 0.36 (0.33 – 0.42) | 2.14 |
| | Garlic oil + 92% $N_2$ | 900 | 3.36 ± 0.47 | 0.20 (0.16 – 0.23) | 0.48 (0.42 – 0.58) | 3.71 |
| Larva | Garlic oil | 420 | 8.88 ± 1.08 | 19.44 (18.06 – 20.59) | 27.11 (25.44 – 29.58) | 4.74 |
| | Garlic oil + 92% $CO_2$ | 480 | 2.76 ± 0.24 | 2.45 (2.09 – 2.83) | 7.16 (6.03 – 8.91) | 8.33 |
| | Garlic oil + 92% $N_2$ | 360 | 5.48 ± 0.94 | 11.82 (9.90 – 13.15) | 20.26 (18.24 – 24.09) | 6.18 |
| Pupa | Garlic oil | 420 | 5.35 ± 0.83 | 2.42 (2.07 – 2.69) | 4.19 (2.69 – 3.69) | 2.88 |
| | Garlic oil + 92% $CO_2$ | 360 | 3.46 ± 0.39 | 0.61 (0.51 – 0.71) | 1.44 (1.20 – 1.83) | 12.75 |
| | Garlic oil + 92% $N_2$ | 360 | 5.62 ± 0.65 | 1.81 (1.62 – 1.99) | 3.07 (2.75 – 3.57) | 3.93 |
| Adult | Garlic oil | 360 | 7.43 ± 0.78 | 15.36 (14.36 – 16.36) | 22.84 (21.0 – 25.63) | 10.73 |
| | Garlic oil + 92% $CO_2$ | 420 | 3.21 ± 0.29 | 3.53 (3.04 – 4.03) | 8.85 (7.59 – 10.76) | 7.77 |
| | Garlic oil + 92% $N_2$ | 360 | 5.45 ± 0.52 | 10.96 (10.00 – 11.89) | 18.83 (17.05 – 21.42) | 9.71 |

[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

24-h laboratory fumigations are given Table 4. Allyl disulphide in combination with 92% $CO_2$ and $N_2$ reduced $LC_{50}$ and $LC_{90}$ values of all life stages of *T. confusum*. Allyl disulphide in combination with 92% $CO_2$ showed 1.7-, 2.9-, 1.9- and 3.6-fold reduction in $LC_{90}$ values for eggs, larvae, pupae and adults of *T. confusum* respectively compared with allyl disulphide alone (Table 4). Allyl disulphide in combination with 92% $N_2$ resulted in 1.1, 1.3, 1.2 and 1.4-fold reduction in $LC_{90}$ values for eggs, larvae, pupae and adults of *T. confusum,* respectively, compared with allyl disulphide alone. Generally, the combinations of allyl disulphide with 92% $CO_2$ were more toxic to all life stages of *T. confusum* than those in combinations with 92% $N_2$ as evident from significant decrements in their $LC_{90}$ values.

Thus, the use of 92% $CO_2$ and $N_2$ with garlic essential oil and allyl disulphide clearly resulted in significant reductions of $LC_{50}$ and $LC_{90}$ values for all life stages of *T. confusum*. This was particularly effective for the most tolerant larval and adults stage where combining garlic essential oil and allyl disulphide with 92% $CO_2$ decreased the $LC_{90}$ value from 27.1 to 7.2 µl/L and 22.8 to 8.9 µl/L; from 14.5 to 4.9 µl/L and 10.7 to 2.8 µl/L respectively. Similarly, garlic essential oil and allyl disulphide with 92% $N_2$ also decreased the $LC_{90}$ value of larval and adults stage from 27.1 to 20.6 µl/L and 22.8 to 18.8 µl/L; from 14.5 to 11.4 µl/L and 10.7 to 7.4 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 larval and adults stage of *T. confusum*. However, data without garlic essential oil and allyl disulphide indicated that there was only limited mortality of the larvae and adults on exposure to 92% $CO_2$ alone for 24 h. Therefore, the results indicate that $CO_2$ and $N_2$ have a synergistic effect on the test insect when exposed together with garlic essential oil and allyl disulphide. The addition of $CO_2$ has long been known to enhance the toxic effects of fumigant gases

Table 4 Probit analysis data of allyl disulphide alone and allyl disulphide in combination with 92% $CO_2$ and $N_2$ for all life stages of *Tribolium confusum* resulting from 24-h laboratory fumigations at 25°C and 65% r.h.

| Life stage | Treatments | N[a] | Slope[b] ± S.E. | $LC_{50}$ (µl/L) (Fiducial limit)[c] | $LC_{90}$ (µl/L) (Fiducial limit)[c] | $\chi^2$ [d] |
|---|---|---|---|---|---|---|
| Egg | Allyl disulphide | 750 | 4.36 ± 0.46 | 0.33 (0.29 – 0.36) | 0.65 (0.58 – 0.76) | 7.23 |
| | Allyl disulphide +92% $CO_2$ | 750 | 4.94 ± 0.73 | 0.22 (0.18 – 0.24) | 0.39 (0.36 – 0.45) | 4.19 |
| | Allyl disulphide +92% $N_2$ | 750 | 3.68 ± 0.46 | 0.26 (0.22 – 0.29) | 0.57 (0.51 – 0.68) | 4.44 |
| Larva | Allyl disulphide | 420 | 4.59 ± 0.53 | 7.61 (6.77 – 8.39) | 14.48 (12.83 – 17.16) | 5.15 |
| | Allyl disulphide +92% $CO_2$ | 420 | 2.16 ± 0.22 | 1.24 (0.97 – 1.52) | 4.86 (3.86 – 6.55) | 6.44 |
| | Allyl disulphide +92% $N_2$ | 420 | 4.43 ± 0.52 | 5.84 (5.07 – 6.53) | 11.38 (10.01 – 13.40) | 2.50 |
| Pupa | Allyl disulphide | 360 | 3.12 ± 0.49 | 1.33 (0.97 – 1.64) | 3.43 (2.83 – 4.53) | 5.71 |
| | Allyl disulphide +92% $CO_2$ | 420 | 2.86 ± 0.33 | 0.65 (0.53 – 0.78) | 1.83 (1.52 – 2.35) | 6.48 |
| | Allyl disulphide +92% $N_2$ | 420 | 3.39 ± 0.39 | 1.18 (0.97 – 1.36) | 2.82 (2.41 – 3.49) | 4.89 |
| Adult | Allyl disulphide | 420 | 6.08 ± 0.56 | 6.19 (5.71 – 6.66) | 10.07 (9.24 – 11.22) | 13.26 |
| | Allyl disulphide +92% $CO_2$ | 420 | 3.39 ± 0.31 | 1.16 (1.02 – 1.29) | 2.77 (2.39 – 3.35) | 4.76 |
| | Allyl disulphide +92% $N_2$ | 420 | 5.36 ± 0.48 | 4.24 (3.91 – 5.57) | 7.35 (6.65 – 8.38) | 5.72 |

[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

for some stored-product insects (Bond and Buckland, 1978; Navarro et al., 2004) and has been pursued as a method of reducing the amount of methyl bromide needed (Kawakami et al., 1996). Several modes of action have been proposed for the toxic action of elevated $CO_2$ levels (Friedlander, 1983). These include a reduction in various detoxification pathways from the mixed function oxidizes to the regeneration of acetyl-choline. The effects are through to occur $CO_2$ levels greater than 10%. At $CO_2$ levels as low as 1%, the insect increase spiracle opening, allowing the diffusion of fumigants into the tracheae to increase (Wigglesworth, 1972). Spiracles remain open in the $CO_2$ concentration range of 2–5%, depending on insect species (Wigglesworth, 1972), which facilities the entrance of the toxic gases into the insect body. In present study, $CO_2$ has a synergistic effect on the test insect when exposed together with garlic essential oil and allyl disulphide, which can be explained by mode of action of $CO_2$ as described above.

Other studies have shown that the admixture of $CO_2$ could increase the toxicity of fumigants, mainly MB and phosphine (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 µl/L 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 bostrychophila* Badonnel (Wing et al., 2001). However, the results obtained in our studies reveal that reductions in $LC_{50}$ and $LC_{90}$ caused by garlic essential oil and allyl disulphide in combination $CO_2$ and $N_2$ are much higher than those reported by the above authors.

The combinations of garlic essential oil and allyl disulphide with 92% $CO_2$ were more toxic to all life stages of *T. confusum* than those in combinations with 92% $N_2$, as evidenced by significant decrements in their $LC_{50}$ and $LC_{90}$ values. Nitrogen is not directly toxic gas to the insects, but is only lethal to the insects by producing a progressive hypoxia or anoxia only when used alone at a high purity level. 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 they are by lack of oxygen 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 and Jeyamkondan, 2002). The higher toxicity of combination of garlic essential oil and allyl disulphide with $CO_2$ can be attributed to these insecticidal properties of $CO_2$ as described above.

## CONCLUSION

The use of high concentration of $CO_2$ and $N_2$ appears to have a synergistic effect on all life stages of *T. confusum,* as evidenced by significant decrements in their $LC_{50}$ and $LC_{90}$ values. These results indicate that combination of garlic essential oil and allyl disulphide with $CO_2$ and $N_2$ can be potential as an alternative application to the most commonly used commercial fumigants, methyl bromide and phosphine.

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## REFERENCES

Bell CH, Spratt EC, Mitchell DJ (1980) The effects of nitrogen and carbon dioxide on eggs of *Ephestia cautella* (Walker) and *E. kuehniella* (Zeller) (Lepidoptera: Pyralidae). Bulletin of Entomological Research **70**: 293–298.

Bond EJ, and Buckland CT (1978) Control of insects (*Sitophilus granarius, Tribolium castaneum, Tenebroides mauritanicus*) with fumigants at low temperatures; toxicity of fumigants in a atmospheres of carbon dioxide. Journal of Economic Entomology **71**: 307–309.

Calderon M, Leesch JG (1983) Effect of reduced pressure and $CO_2$ on the toxicity of methyl bromide to two species of stored product insects. Journal of Economic Entomology **76**: 1,125–1,128.

Donahaye E, Navarro S (1989) Sensitivity of two dried fruit pests to methyl bromide alone, and in combination with carbon dioxide or under reduced pressure. Tropical Science **29**: 9–14.

Dumas T, Buckland CT, Monro HAU (1969) The respiration of insects at reduced pressures. II. The uptake of oxygen by *Tenebroides mauritanicus*. Entomologica Experimentalis et Applicita **12**: 389–402.

Friedlander A (1983) Biochemical reflection on a non-chemical control method. In: Mills et al. Proceedings of the 3rd Working Conference on Stored Product Entomology, 23–28 October, 1983, Kansas State University, Manhattan, Kansas, USA, pp. 471–486.

Gözek N (2007) Fumigant toxicity of garlic and onion essential oils and their active components against life stages of confused flour beetle, *Tribolium confusum* du Val. Master thesis, University of Kahramanmaraş, Sütçü Imam Institute of Natural and Applied Sciences, Department of Plant Protection, 46 pp. (Only Abstract in English)

Ho SH, Koh L, Ma Y, Huang Y, Sim KY (1996) The oil of garlic, *Allium sativum* L. (Amaryllidaceae), as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Postharvest Biology and Technology **9**: 41**–**46.

Huang Y, Xıng CS, Hung HS (2000) Bioactivities of methyl allyl disulphide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera : Tenebrionidae). Journal of Economic Entomology **93**: 537–543.

Jayas DS, Jeyamkondan S (2002) PH–Postharvest technology: modified atmosphere storage of grains meats fruits and vegetables. Biosystems Engineering **82**: 235–251.

Jones RM (1938) Toxicity of fumigant $CO_2$ mixture to the red flour beetle. Journal of Economic Entomology **31**: 298–309.

Karcı A (2006) Fumigant toxicity of some plant derived essential oils against all life stages of confused flour beetle, *Tribolium Confusum* Duval (Col.: Tenebrionidae). Master Thesis. University of Kahramanmaraş Sütçü Imam Institute of Natural and Applied Sciences, Department of Plant Protection, 45 pp. ( Abstract in English)

Kawakami F, Soma Y, Tsutsumi T, Sato T, Yuge T, Yamamoto M, Komatsu H, Inoue T (1996) Disinfestation of pests on cut flowers with gas mixtures of methyl bromide, phosphine and carbon dioxide. Research Bulletin and Plant Protection Service, Japan, **32**: 39–46.

LeOra Software (1987) POLO-PC: A Users' Guide to Probit or Logit Analysis. LeOra Software, Berkeley, CA.

Navarro S, Isikber AA, Finkelman S, Rindner M, Azrieli A, Dias R (2004) Effectiveness of short exposures of propylene oxide alone and in combination with low pressure or carbon dioxide against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Products Research **40**: 197–205.

SAS Institute Inc. (1989) SAS / STAT $^R$ User's Guide, Version 6, 4th ed, SAS Institute Inc., Cary, NC.

Shaaya E, Kostjukovski M, Eilberg J, Sukprakarn C (1997) Plant oils as fumigants and contact insecticides for the control of stored-product insects. Journal of Stored Products Research **33**: 7–15.

Shaaya E, Kostyukovsky M, Atsmi S, Chen B (1999) Alternatives to methyl bromide for the control of insects attacking stored products and cut flowers. (In) Zuxun J, Quan L, Yongsheng L, Xianchang T, Lianghua G (Eds), Proceedings of the 7$^{th}$ International Working Conference on Stored-Product Protection, 14–19 October 1998, Beijing, China, Sichuan Publishing House of Science and Technology, Chengdu, China, pp 526–530.

Tunç İ, Berger BM, Erler F, Dağlı F (2000) Ovicidal activity of essential oils from five plants against two stored-product insects. Journal of Stored Products Research **36**: 161–168.

Wang JJ, Tsai JH, Ding W, Zhao ZM, Li LS (2001) Toxic effects of six plant oils alone and in combination with controlled atmosphere on *Liposcelis bostrychophila* (Osocoptera: Liposcelididae). Journal of Economic Entomology **94**: 1,296–1,301.

Wigglesworth VB (1972) The Principles of Insect Physiology, Chapman & Hall, London.

Williams P (1985) Toxicity of methyl bromide in carbon dioxide enriched atmospheres to beetles attacking stored grain. General Applied Entomology **17**: 17–24.

Wong-Corral FJ, Castañé C, Riudavets J (2013) Lethal effects of $CO_2$-modified atmospheres for the control of three Bruchidae species. Journal of Stored Products Research **55**: 62–67.