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Enhanced fumigant toxicity of allyl acetate to stored-product beetles in the presence of carbon dioxide

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Abstract

Toxicity of allyl acetate (5–25 mg/l doses) to mixed-age cultures of stored-product beetles including *Cryptolestes ferrugineus*, *Lasioderma serricorne*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* in the presence of carbon dioxide (CO_2) (10% and 20%) with a 48-h exposure period was studied in the laboratory at 27 ± 2 °C. Depending on dosage and the insect species, the fumigant toxicity of allyl acetate was enhanced by CO_2. At most of the allyl acetate + CO_2 combinations, increased mortality was observed in *C. ferrugineus*, *O. surinamensis* and *S. oryzae*. In *L. serricorne* and *T. castaneum*, which are tolerant to allyl acetate, higher mortality due to CO_2 was achieved at selected dose combinations only (e.g. 15 mg/l allyl acetate + 20% CO_2). Significant increase in mortality of *R. dominica* (the most susceptible species to allyl acetate) exposed to allyl acetate in the presence of CO_2 was not evident except at the lower dose of 5 mg/l allyl acetate + CO_2 that caused 31.7% mortality. The mortality data show that CO_2 could be used as an adjuvant for allyl acetate.

Keywords: Allyl acetate; Allyl acetate + CO_2 mixtures; Fumigation toxicity; Stored-product beetles

1. Introduction

The currently used fumigants, phosphine and methyl bromide, have constraints such as development of insect resistance or environmental hazards due to ozone depleting potential. As alternatives to phosphine and methyl bromide, older fumigants including carbon disulphide (Yonglin and Allen, 1999) as well as ethyl formate (Desmarchelier et al., 1998) and newer compounds like alkylphosphines (Chaudhry et al., 2000), carbonyl sulphide (Desmarchelier, 1994), cyanogens (O'Brien et al., 1999) and isothiocyanates (Shaaya et al., 2003) have been investigated for stored-products protection. Sulfuryl fluoride (ProFume®) a structural fumigant, has now been cleared by the Environmental Protection Agency for use on food commodities also. ProFume® has been registered for insect pest control in food processing facilities in the USA and in some countries in Europe. Recently, Rajendran and Muralidharan (2005) reported the fumigation toxicity of allyl acetate, a liquid fumigant, against stored-grain beetle pests. The laboratory tests against mixed-age cultures of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), the lesser grain borer, *Rhyzopertha dominica* (F.), the rice weevil, *Sitophilus oryzae* (L.) and the rust-red flour beetle, *Tribolium castaneum* (Herbst) revealed that doses of 50–150 mg/l with 24–120 h exposures were necessary to achieve 100% mortality of all life stages. It was also observed that with allyl acetate, a dose of 100 mg/l over a 48-h exposure period was necessary to give 100% mortality of all stages of the above species. This dosage is relatively high when compared with that of currently used fumigants such as phosphine (1–3 mg/l) and methyl bromide (16–80 mg/l). It is therefore necessary to investigate ways of reducing the effective dosage of allyl acetate using suitable adjuvants. Sub-lethal concentrations of carbon dioxide (CO_2) have been shown to enhance the toxicity of fumigants such as methyl bromide (AliNiazee and Lindgren, 1969), phosphine (Kashi and Bond, 1975), propylene oxide (Navarro et al., 2004) and others (Jones, 1938).

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In this context, laboratory investigations were carried out to determine whether CO_2 could enhance the toxicity of allyl acetate against mixed-age cultures of stored-product insect pests including *C. ferrugineus*, *O. surinamensis*, *R. dominica*, *S. oryzae*, *T. castaneum* and the cigarette beetle *Lasioderma serricorne* (F.).

2. Materials and methods

2.1. Insects

Tribolium castaneum and *L. serricorne* were reared on whole-wheat flour fortified with 5% dried yeast, *C. ferrugineus* and *O. surinamensis* on a 5:5:1 mixture of broken wheat, rolled oats and yeast, whereas *S. oryzae* as well as *R. dominica* were reared on whole wheat. *Lasioderma serricorne* and *R. dominica* cultures were maintained at $30\pm1\,°C$ and 70% r.h. whilst cultures of other insects were maintained at $25\pm1\,°C$ and $65\pm10\%$ r.h. From these cultures, adults of *L. serricorne* (1–3 day old) and other species (1–2 weeks old) were taken for preparation of mixed-age cultures. About 300 adults of *L. serricorne*, *O. surinamensis* and *C. ferrugineus* were released into 1 kg of their respective culture media in 2-l glass jars. Similarly, 300 adults of *S. oryzae* and *R. dominica* were allowed to breed separately in 1 kg of wheat in 2-l glass jars. Details of preparation of mixed-age cultures of the insects have been described elsewhere (Rajendran and Muralidharan, 2005).

2.2. Tests with allyl acetate and CO_2

Mixed-age cultures of different insect species were exposed to allyl acetate (analytical grade from Merck India Ltd.) alone (5, 10, 15 and 25 mg/l), CO_2 alone (10% and 20%) and allyl acetate + CO_2 mixtures (5 mg/l + 10% and 20% CO_2, 10 mg/l + 10% and 20% CO_2, etc.) for 48 h at $27\pm2\,°C$. The allyl acetate and CO_2 doses were chosen based on the preliminary experiments so that the relative toxicity of allyl acetate, CO_2 and their mixtures to the insects tested could be determined. Rearing media containing mixed-age cultures of the insects were weighed in 50 g aliquots into cloth bags (20 cm × 14 cm size) and the bags were placed individually in 0.85-l desiccators that served as the fumigation chambers. In each desiccator, a Whatman No. 1 filter circle (9 cm size) was placed to serve as an evaporating surface for injected allyl acetate. For each species, there were five replicates for each dose of allyl acetate or CO_2 or their mixtures, with an equal number (five) of untreated control replicates.

For treatment with allyl acetate alone, the required quantity of the compound was injected into the fumigation chambers containing test insects using a microsyringe. To test with CO_2 alone and allyl acetate + CO_2 mixtures, a vacuum was created inside the desiccators to facilitate injection of CO_2. CO_2 was drawn from a cylinder source (purity 98.5%) with a gas-tight 500 ml Hamilton syringe for dosing the desiccators. Immediately after injecting CO_2, the desiccators were brought to normal atmospheric pressure. The required quantity of allyl acetate was then injected for the allyl acetate + CO_2 mixture treatments. Concentrations of allyl acetate and CO_2 inside the desiccators were not determined. After 48 h, the fumigation was terminated and the test insect bags were taken out. The contents of the bags were transferred to individual bottles (12 cm × 5 cm size) and kept at the rearing temperature and humidity conditions for 8 weeks. The insects which emerged from wheat (*S. oryzae* and *R. dominica*) or survived as adults (*T. castaneum*, *C. ferrugineus*, *L. serricorne* and *O. surinamensis*) in their respective media were checked at weekly intervals for 8 weeks. Similarly, counts were made in untreated control batches every week.

2.3. Data analysis

Mortality in allyl acetate alone, CO_2 alone and allyl acetate + CO_2 mixture treatments was determined by taking the survival/emergence in the controls as 100%. Mortality data of the different insects, after arcsine transformation, were subjected to ANOVA and Duncan's multiple range test to determine significant differences between treatments.

3. Results

CO_2 enhanced the toxicity of allyl acetate to the insects depending on the doses of the allyl acetate + CO_2 mixtures (Table 1). The susceptibility of the insects to allyl acetate alone was in the following decreasing order: *R. dominica* > *S. oryzae* > *C. ferrugineus* > *O. surinamensis* > *L. serricorne* > *T. castaneum*. Increased mortality of *C. ferrugineus* was observed at all allyl acetate + CO_2 combinations, *S. oryzae* at 5 mg/l of allyl acetate + 20% CO_2 and other higher dose combinations, *O. surinamensis* at 10 mg/l allyl acetate + 10% CO_2 and other higher dose combinations. Significant increase in mortality of *T. castaneum* was noted with 5 or 10 mg/l of allyl acetate in combination with 20% CO_2 and with higher allyl acetate doses + 10% or 20% CO_2.

Lasioderma serricorne was susceptible to CO_2 alone and an average mortality of 58.5% and 71.9% was achieved at 10% and 20% levels, respectively. In this species, significant increase in mortality was noticed only at 15 or 25 mg/l + 20% CO_2. *Rhyzopertha dominica* was the most susceptible to allyl acetate fumigant and about 80.7% and 99.0% average mortality was achieved with allyl acetate doses of 10 and 25 mg/l, respectively. In this species, 10% or 20% CO_2 with allyl acetate at 5 mg/l produced significantly increased mortality. Higher doses of allyl acetate in mixture with CO_2 caused $\geqslant 97\%$ mortality. One hundred percent mortality was not achieved with the insects tested at any of the allyl acetate + CO_2 combinations.

Table 1
Mortality (%) of mixed-age cultures of stored-product insects exposed for 48 h to allyl acetate alone, CO_2 alone, and allyl acetate + CO_2 mixtures at 27±2 °C

Allyl acetate (mg/l)	CO_2 (%)	Mean mortality (% ± SD)[a]					
		Tribolium castaneum	*Lasioderma serricorne*	*Oryzaephilus surinamensis*	*Cryptolestes ferrugineus*	*Sitophilus oryzae*	*Rhyzopertha dominica*
5	0	2.5 (±3.2)[ab]	12.9 (±12.6)[b]	6.3 (±13.0)[ab]	1.3 (±2.6)[a]	1.6 (±3.7)[a]	31.7 (±8.2)[b]
10	0	1.7 (±2.9)[ab]	3.4 (±5.7)[a]	7.6 (±15.8)[ab]	11.8 (±5.6)[bc]	17.6 (±1.7)[b]	80.2 (±3.0)[c]
15	0	0[a]	4.0 (±6.9)[a]	20.3 (±22.3)[b]	39.1 (±12.5)[d]	41.2 (±19.2)[c]	97.2 (±1.5)[d]
25	0	5.5 (±5.7)[abc]	21.9 (±9.4)[b]	50.0 (±8.9)[c]	60.5 (±18.3)[ef]	71.1 (±14.6)[de]	99.0 (±0.2)[de]
0	10	7.8 (±10.6)[bc]	58.5 (±15.1)[de]	8.5 (±7.8)[ab]	6.3 (±9.3)[b]	8.8 (±10.7)[ab]	3.3 (±4.6)[a]
0	20	3.6 (±3.1)[ab]	71.9 (±8.4)[def]	12.0 (±9.4)[ab]	3.0 (±4.3)[ab]	0.5 (±1.2)[a]	2.0 (±4.6)[a]
5	10	8.0 (±9.2)[bc]	43.9 (±8.8)[c]	1.7 (±3.9)[a]	15.7 (±8.7)[c]	0.4 (±1.0)[a]	86.9 (±1.9)[c]
	20	23.9 (±10.7)[d]	67.1 (±3.6)[de]	22.8 (±28.8)[b]	44.9 (±6.0)[de]	30.1 (±9.9)[c]	92.6 (±2.7)[c]
10	10	0.9 (±2.0)[ab]	68.9 (±10.5)[de]	58.0 (±3.3)[c]	69.3 (±6.8)[f]	38.9 (±9.2)[c]	97.8 (±1.6)[d]
	20	35.4 (±4.6)[d]	82.9 (±6.3)[efg]	78.8 (±13.9)[cd]	67.8 (±12.7)[f]	67.3 (±8.7)[d]	98.2 (±1.6)[de]
15	10	18.0 (±13.9)[c]	65.2 (±10.8)[de]	86.5 (±4.9)[d]	93.8 (±2.1)[g]	66.5 (±9.4)[d]	99.8 (±0.2)[e]
	20	58.5 (±8.9)[e]	87.2 (±7.6)[g]	85.6 (±7.7)[d]	95.1 (±1.8)[g]	84.4 (±12.7)[e]	99.0 (±2.1)[de]
25	10	54.1 (±6.5)[e]	71.3 (±6.6)[edf]	95.2 (±3.0)[d]	99.1 (±0.5)[gh]	82.8 (±9.8)[e]	99.8 (±0.2)[de]
	20	80.8 (±5.7)[f]	85.4 (±7.3)[fg]	94.9 (±5.7)[d]	99.7 (±0.7)[h]	97.9 (±1.9)[f]	99.9 (±0.2)[e]

[a]There were five replicates for each dose (50 g of infested wheat grain or flour per replicate). In untreated controls, the mean numbers of survivors were 217.8 ± 12.5 (*T. castaneum*), 63.4 ± 5.3 (*L. serricorne*), 62.4 ± 12.0 (*O. surinamensis*), 583.4 ± 108.7 (*C. ferrugineus*), 233.2 ± 10.1 (*S. oryzae*) and 252.8 ± 20.9 (*R. dominica*). Different letters within a column indicate that means are significantly different ($P<0.05$).

In untreated controls, the average numbers of survivors were lowest in *O. surinamensis* (62.4) as well as in *L. serricorne* (63.4) and the highest numbers were observed in *C. ferrugineus* (583.4).

4. Discussion

Several workers have examined the influence of CO_2 on the toxicity of fumigants against stored-product insect pests. Hazelhoff (1928) reported that CO_2 accelerates the penetration of fumigants, which are respiratory poisons in insects, by keeping the respiratory spiracles open. CO_2 is a well-known respiratory stimulant in insects (Case, 1957). Jones (1938) showed that CO_2 enhances the toxicity of several fumigants against *T. castaneum*. *Sitophilus* spp. (and diapausing larvae of the Khapra beetle, *Trogoderma granarium* Everts) are known to be tolerant to CO_2 treatment (Annis, 1987). In contrast, *S. oryzae* was highly susceptible to allyl acetate fumigant. In the presence of CO_2, the susceptibility of the pest was increased further (Table 1). CO_2 increased the toxicity to other stored-product insects also.

CO_2 in allyl acetate + CO_2 mixtures will have a triple action, i.e., (a) potentiating insect toxicity, (b) reducing the flammability risk of allyl acetate and (c) improving the penetration as well as distribution of the fumigant. Allyl acetate is highly flammable (1.1–7.5% in air, Class 1C). Hence, use of CO_2 has the advantage of reducing the flammability risk. CO_2 is safer for food commodities and even at high levels (>60%) it does not affect the quality of the commodities treated. The vapour pressure of allyl acetate is relatively low (2.8 kPa at 20 °C) (CHRIS, 1999) and therefore, allyl acetate alone cannot penetrate deep into bulk grain. CO_2 with a higher vapour pressure (5900 kPa at 21 °C) has the advantage of improving penetration and the effectiveness of allyl acetate.

Allyl acetate like other esters acts on insects as a fat solvent narcotic (Busvine, 1971) whereas CO_2 at and above 35% levels causes insect death by desiccation, acidification at the cellular level, and creating a lack of triglycerides for energy metabolism (Adler, 1994; Donahaye, 1991). Allyl acetate has been shown to be useful for the disinfestation of stored food grains in small quantities (<1 tonne) (Rajendran and Gunasekaran, 2004). For treatment of stored grains with CO_2 alone, the recommended dosages include 40% CO_2 for 17 days, 60% for 11 days or 80% CO_2 for 8.5 days at >21 °C (Annis, 1987). For fumigation of whole cereals with allyl acetate alone, an exposure period of 2–3 days is adequate (Rajendran and Gunasekaran, 2004). With the allyl acetate + CO_2 mixture, it has to be determined whether the exposure period and/or the allyl acetate dose could be reduced.

The results (Table 1) show that CO_2 has the effect of increasing mortality where the mortalities for allyl acetate alone are low. It is possible that CO_2 synergises the absorption of low doses of allyl acetate by opening the spiracles, but this becomes less important when the doses of allyl acetate become sufficiently high to ensure that the insect receives a lethal dose without assistance (as in the case of *R. dominica*). With *O. surinamensis* and *C. ferrugineus*, it was noted that some of the allyl acetate + CO_2 mixture doses failed to produce significant increases in mortality as CO_2 level increased. For example, allyl acetate 15 mg/l in the presence of either 10% or 20%

CO_2 produced nearly the same level of mortality of *O. surinamensis*. Similarly, mortalities in *C. ferrugineus* exposed to allyl acetate + CO_2 doses of 10 mg/l + 10% CO_2 and 10 mg/l + 20% CO_2 were nearly the same (69.3% and 67.8%, respectively). It may be that certain optimum combinations of allyl acetate with CO_2 are required for significant increases in mortality of these insects.

The present study shows that, depending on the doses, there is an increase in the toxicity of allyl acetate in the presence of CO_2, particularly in *S. oryzae* an internal infestor and in other species developing outside the grain, i.e., *O. surinamensis*, *C. ferrugineus* and *T. castaneum*. In view of the multiple advantages of CO_2, this could be used as an adjuvant for allyl acetate, a potential fumigant for stored food grains.

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