Alternative fumigants to methyl bromide for the control of pest infestation in grain and dry food products

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Abstract

The primary aim of the current study is to evaluate the potential use of the known isothyiocyanates (ITC) as compared to a new ITC isolated from *Eruca sativa* (salad rocket) as fumigants for the control of stored products insects. The biological activity of methyl iodide (CH_3I), carbon disulphide (CS_2), benzaldehyde (C_7H_60) and essential oils were also evaluated. The toxicity of the various fumigants was assessed against adults and larvae of a number of major stored-product insects. ITCs are potential candidates because only very low concentrations are needed for the control of stored-product insects. It should be mentioned that *Eruca sativa* is used worldwide as a food supplement. Methyl thio-butyl ITC the main bioactive component in this plant has high toxicity against insects, but lower mammalian toxicity as compared to other active ITCs. Comparative studies with CH_3I, CS_2 and C_7H_60 showed that the first was the most active compound against stored-product insects followed by the second and the third. CH_3I was found less sorptive and to be less penetrative in wheat than CS_2. The activity of some essential oils was also evaluated. In this context, we should keep in mind that a general consensus is very difficult to achieve in order to introduce broad-spectrum fumigants like methyl bromide or phosphine. Because of this, alternative fumigants could be developed against particular species of insects or to be used for specific food product commodity.

Keywords: Fumigants; Isothiocyanates; Methyl iodide; Carbon disulfide; Benzaldehyde.

1. Introduction

Insect damage in stored grain and other durable commodities may amount to 10-40% in developing countries, where modern storage technologies have not been introduced (Raja et al., 2001). Fumigation is still one of the most effective methods for the protection of stored grain and dry food from insect infestation. At present, mainly two fumigants are still in use: methyl bromide and phosphine. The first one is being mostly phased out in developed countries due to its ozone depletion effect (WHO, 1995; Shaaya and Kostyukovsky, 2006). Phosphine is mainly used today, but there are repeated reports that a number of storage pests have developed resistance to this fumigant (Rajendran and Karanth, 2000). Therefore, there is an urgent need for new strategies to focus on a search for alternative fumigants for the control of stored-product insects. In this paper we present a comprehensive study to evaluate the potential use of isothiocyanates (ITCs), methyl iodide (CH_3I), carbon disulfide (CS_2), benzaldehyde (C_7H_60) and essential oils (EOs) for the control of stored-product insects.

ITCs were chosen for this study because of the pesticidal properties of the chemicals (Fenwick et al., 1983) and the potential use of methyl ITC as fumigant for wheat (Ducom, 1994).

CH_3I is known as a potent insecticide. CS_2, according to Winburn (1952), was one of the most effective grain fumigants as viewed from efficiency and low cost. C_7H_60, occurs in kernels of bitter almonds, has low toxicity to mammals and it is used in topical antiseptics. The bioactivity of essential oils, the major volatile in aromatic plants, and their constituents has been well studied against a large number of stored-product insects. (Regnault-Roger and Hamraoui, 1995; Raja et al., 2001). In our laboratory, by screening a large number of EOs we could isolate two very active fumigants obtained from Lamiaceae plants. The main component of one of the oils is pulegone. The other is not yet identified.

2. Materials and methods

The tested stored-product insects were laboratory stains of *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *Trogoderma granarium* Everts and *Plodia interpunctella* (Hübner).

The ITCs were obtained by putting 100 g ground seeds into round bottom flask containing buffer solution (1% ascorbic acid). The flask is held in a water bath at 70°C for two hours to facilitate the hydrolysis of sinigrin to ITC by the enzyme myrosinase which is found inside the seeds. The second step is steam distillation with use of the Dean-Stark apparatus (Leoni et al., 1997). The yellow upper layer is then separated and extracted with petroleum ether. Finally, the petroleum ether is evaporated under a stream of air. The unknown ITC obtained from the seeds of *Eruca sativa* was identified as methylthio butyl isothiocyanate by gas chromatography (GC), nuclear magnetic resonance (NMR) and infra-red (IR) spectroscopy. CS_2, CH_3I and C_7H_60 were purchased from Sigma Chemical Company, St. Louis, MO, USA. The essential oils from the aromatic plants were obtained from freshly harvested leaves and stems by steam distillation.

Two types of bioassays were performed to evaluate the activity of the fumigants. The first screening of the compounds was space fumigation in glass chambers of 3.4-L capacity (Shaaya et al., 1991). The highly active compounds were then assayed in simulation glass columns 10 cm in diameter × 120 cm in height, filled to 70% by volume with wheat (11% moisture content). The insects were introduced in cages, each holding 20 insects of the same species together with food. Groups of four cages were suspended by a steel wire at different heights from the bottom of the column. Insect were exposed to fumigants for 3 to 72 h, removed from fumigation, held for 7 d and the mortality assessed.

3. Results

3.1. Toxicity of isothiocyanats (ITCs)

Mustard family (Brassicaceae) seeds contain ITCs, volatile essential oils that are known to possess insecticidal activity. By screening a number of various species of Brassicaceae seeds it was possible to isolate from seed oils of *Sinapis arvensis*, *Eruca sativa* and *Diplotaxis* spp. an unknown ITC at concentrations of 98%, 92% and 33%, respectively. Later, this compound was identified as methylthio butyl ITC. In space fumigation, the biological activity of this compound was compared with four common ITCs, namely, allyl, methyl, butyl and ethyl. Allyl and methyl ITCs were found to be the most potent against adults and larvae of four and three stored-product insects respectively. A concentration of 1 $\mu L \cdot L^{-1}$ air and exposure time of 4 h was enough to kill all the tested adult insects, except for *T. castaneum,* which was found much more susceptible to these two ITCs (Table 1).

Table 1 The fumigant toxicity 7 d after treatment with four active isothiocyanates compared with methylthio-butyl ITC isolated from the plant *Eruca sativa* (space fumigation).

			Mortality (%)						
			Larvae			Adults			
Compound	**Conc. (µL/L)**	**Exposure time (h)**	***Tribolium castaneum***	***Trogoderma granarium***	***Plodia interpunctella***	***Sitophilus oryzae***	***Rhyzopertha dominica***	***Oryzaephilus surinamensis***	***Tribolium castaneum***
Allyl ITC	1.0	3.0	-	-	-	100	100	100	100
	1.5	3.0	23	84	100	-	-	-	-
Methyl ITC	1.0	2.5	-	-	-	100	100	100	100
	1.5	3.5	100	81	100	-	-	-	-
Methylthio-butyl ITC	1.0	3.0	-	-	-	100	89	100	0
	1.0	3.0	100	100	87	-	-	-	-
Ethyl ITC	1.0	3.0	-	-	-	100	100	100	0
	1.5	3.0	20	6	100	-	-	-	-
Butyl ITC	1.5	3.0	-	-	-	65	43	68	25
	1.5	3.0	5.5	23	7	-	-	-	-

Third instar larvae were used; Mortality in control less than 5%

3.2. Efficacy of CH_3I, CS_2 and C_7H_60 as fumigants

In space fumigation, CH_3I was very effective against all insect stages tested. Exposure to a concentration of 3 to 5 $\mu L \cdot L^{-1}$ for 3 h was lethal and caused 100% mortality of all stages of the test insects, except for *T. granarium* larvae (Table 2). Adults of *T. castaneum* were found the most tolerant followed by *R. dominica, S. oryzae* and *O. surinamensis*. In the case of larvae, *T. granarium* was most tolerant, followed by *T. castaneum* and *P. interpunctella* (Table 2).

Table 2 Mortality 7 d after treatment with CH_3I, CS_2 and benzaldehyde against stored-product insects in space fumigation.

			Mortality (%)						
			Adults				Larvae		
Compound	**Exposure Time (h)**	**Conc. (µL/L)**	***Sitophilus oryzae***	***Rhyzopertha dominica***	***Oryzaephilus surinamensis***	***Tribolium castaneum***	***Tribolium castaneum***	***Trogoderma granarium***	***Plodia interpunctella***
CH_3I	3	3	94	85	100	65	40	-	100
		4	100	100	-	95	77	58	-
		5	-	-	-	100	100	70	-
CS_2	24	5	72	53	23	0	-	-	-
		7	100	100	74	10	-	-	100
		9	-	-	100	100	100	60	-
Benzal-dehyde	24	1.5	79	16	39	0	-	-	-
		3	100	100	100	65	0	0	-

CS_2 = Sp. gravity 1.26; Third instar larvae were used; mortality in control less than 5%

CS_2 was less effective than CH_3I and needed a concentration of 7-9 $\mu L \cdot L^{-1}$ air for one day to achieve total mortality of all the test insects for *T. granarium* larvae. In the case of CS_2, adults of *T. castaneum* were found to be the most tolerant, followed by *O. surinamensis, R. dominica* and *S. oryzae*. The larvae of *T. granarium* were more tolerant than *T. castaneum* (Table 2).

C_7H_60 was less active than CH_3I and CS_2 in space fumigation bioassays. A dosage of 3 $\mu L \cdot L^{-1}$ air and exposure time of 1 d caused 100% adult mortality of all the tested adult insects except for *T. castaneum* which caused only 65% kill (Table 2).

In glass columns filled to 70% wheat CS_2 penetrated better than CH_3I, but needed a higher concentration to achieve total mortality (Table 3). It should be mentioned that for methyl bromide fumigation the recommended concentration is 30 to 50 g/m^3.

Table 3 Toxicity of CH_3I and CS_2 to adult stored-product insects at different depths in 120 cm-high columns filled with 70% wheat by gravity.

				Mortality (%)			
Compound	**Conc. (µL/L)**	**Exposure Time (h)**	**Depth in grain (cm)**	***Rhyzopertha dominica***	***Oryzaephilus surinamensis***	***Sitophilus oryzae***	***Tribolium castaneum***
CH_3I	5	24	20	100	100	100	100
			120	10	10	30	0
		72	20	100	100	100	100
			120	95	75	80	0
CS_2	20	48	20	100	100	100	100
			120	100	0	30	10
		72	20	100	100	100	100
			120	100	100	100	100

Mortality in control less than 5%

3.3. Studies with essential oils (EOs).

The essential oils (EOs) of aromatic plants families are volatiles that can be easily extracted by hot water vapors. The main components of the EOs are monoterpenes and to a lesser extent, sesquiterpenes (Brielmann et al., 2006).

In order to isolate bioactive EOs, we screened a large number of EOs extracted from aromatic plants and isolated their main constituents by methods cited in Shaaya et al., 1991, 1994, 1997. Using space fumigation methodology two EOs obtained from *Lamiaceae* plants, were found to be the most potent fumigants as compared with all other essential oils obtained from a large number of aromatic plant species tested against stored-product insects. The main component of one of the EOs was pulegone, and of the other is not yet totally identified, and it is called SEM76. In space fumigation, these two volatiles caused total mortality of all adults tested at very low concentration of 0.5 $\mu L \cdot L^{-1}$ air and exposure time of 24 h. For comparison we tested also limonene which represents most of the other EOs tested (Table 4).

In glass columns filled to 70% volume with wheat showed that SEM76 at a concentration of 70 $\mu L \cdot L^{-1}$ air (equivalent to 70 $g \cdot m^{-3)}$ and 7 d exposure time caused 100% kill of adults of *S. oryzae* and *O. surinamensis* but not of *T. castaneum and R. dominica* (Table 4). Supplementation of 15% CO_2 (200 $g \cdot m^{-3}$) caused reduction in the effective volatile concentration. The concentration of 50 $\mu L \cdot L^{-1}$ air was enough to cause 96-100% kill of all adult insects tested (Table 4).

Table 4 Fumigant toxicity of SEM 76 and Pulegone in space fumigation and columns with and without CO_2.

Fumigation	Compound	Conc. (µL/L)	Mortality (%)			
			Rhyzopertha dominica	*Oryzaephilus surinamensis*	*Sitophilus oryzae*	*Tribolium castaneum*
Space	SEM 76	0.5	100	100	100	87
		1	100	100	100	100
	Pulegone	0.5	100	100	100	100
	Limonene	0.5	27	27	24	0
Columns	SEM 76	70	100	100	70	66
		70+15% CO_2	100	100	100	100
		50+15% CO_2	100	100	100	96

4. Discussion

Our findings showed that ITCs are potential candidates because only very low concentrations are needed for the control of stored-product insects. *Eruca sativa* (salad rocket) is used worldwide as a food supplement and methylthio butyl ITC, the main bioactive component in this plant, has lower mammalian toxicity as compared to the other active ITCs tested. The lower toxicity makes this fumigant a promising candidate for the disinfestations of grain and dry food products.

Comparative studies with C_7H_60, CH_3I and CS_2 showed that CH_3I was the most toxic compound to stored-product insects, followed by CS_2 and C_7H_60. CH_3I was found less penetrative in wheat then CS_2. CH_3I is toxic to humans and its use in food as a fumigant therefore is limited. CS_2 is flammable and can be used mainly as a supplement to increase the activity of other fumigants. In fact, a mixture of trichloroethylene, carbon disulphide and carbon tetrachloride (Calandrex^R) in a ratio 64:26:10, respectively, was developed by us and was found to be effective against stored-product insects (Polacek et al., 1960). C_7H_60 has low toxicity to mammals, but it is less effective against stored-product insects than all other fumigants tested. These three compounds may play a role mainly as supplements to increase the activity of other fumigants.

Our findings, as well as those of other researchers, suggest that certain plant essential oils and their active constituents, mainly terpenoids, have potentially high bioactivity against a range of insects and mites. They are also highly selective to insects, since they are probably targeted to the insect-selective octopaminergic receptor, a non-mammalian target (Kostyukovsky et al., 2002). The worldwide availability of plant essential oils and their terpenoids, and their use in cosmetics and as flavoring agents in food and beverages, is a good indication of their relative safety to warm-blood animals and humans. The ultimate goal is the introduction of these phytochemicals with low toxicity, which comply with health and environmental standards, as alternatives to methyl bromide and phosphine for the preservation of grain and dry food.

We should keep in mind that is very difficult to achieve the introduction of broad-spectrum fumigants like methyl bromide or phosphine. In this context, alternative fumigants could be developed against particular species of insects or to be used for a specific food product commodity.

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