

Chapter 16

The Potential of Biofumigants as Alternatives to Methyl Bromide for the Control of Pest Infestation in Grain and Dry Food Products

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Abstract Fumigation is still one of the most effective methods for the protection of stored grain and dry food from insect infestations. Phosphine and methyl bromide are the most widely used fumigants for the control of stored-product insects. Phosphine is mainly used today, but there are repeated reports that a number of storage pests have developed resistance to this fumigant. Methyl bromide has been identified as a contributor to ozone depletion by the United Nations World Meteorological Organization in 1995 and, thus, was phased out in most developed countries. Thus, there is an urgent need to develop alternatives with the potential to replace these fumigants.

The primary aims of the current study are to evaluate the potential use of essential oils obtained from aromatic plants as insect fumigants and to evaluate the toxicity of the known isothiocyanates (ITCs) as compared to a new ITC isolated from *Eruca sativa* (salad rocket) as fumigants for the control of stored-product insects. Also, the biological activity of carbon disulphide (CS₂), methyl iodide (CH₃I), and benzaldehyde (C₇H₆O) is evaluated.

The toxicity of the various fumigants was assessed against adults, larvae, and pupae of six major stored-product insects. Two essential oils isolated from *Lamiaceae* plants were found to be the most potent fumigants as compared with a large number of other essential oils. ITCs are also potential candidates, especially methylthio-butyl isothiocyanate, the main bioactive component in *E. sativa*, because of its low toxicity. Comparative studies with CH₃I, CS₂, and C₇H₆O showed that CH₃I was the most active compound against stored-product insects, followed by CS₂ and C₇H₆O. CH₃I was also found to be less sorptive and less penetrative in wheat than CS₂.

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16.1 Introduction

In developing countries, the post-harvest losses of cereals and other durable commodities caused by insect damage and other bio-agents range from 10 to 40% (Raja et al., 2001).

Fumigation with methyl bromide or phosphine is a quick and effective tool for the control of stored-product insect pests. In view of the scheduled phaseout of methyl bromide under the Montreal protocol, the role of phosphine in grain protection has increased and stands as the main alternative to methyl bromide. Lately, insect resistance to phosphine has become an important issue for effective grain treatment (Nakakita and Winks, 1981; Tyler et al., 1983; Rajendran and Karanth, 2000). A global survey of pesticide susceptibility demonstrated that 9.7% of the strains tested showed resistance to phosphine (Champ and Dyte, 1976). Another compound, 2,2 dichlorovinyl dimethyl phosphate, which is widely used as a fog fumigant for insect control in empty structures, is classified by the US Environmental Agency as a possible human carcinogen (Mueller, 1998). Therefore, there is an urgent need for new strategies. Thus, in recent years, research has focused on a search for alternative fumigants for the control of stored-product insects. In this chapter, we present a comprehensive laboratory and semi-field studies to evaluate the potential use of essential oils (EOs) obtained from aromatic plants and isothiocyanates (ITCs), methyl iodide (CH_3I), carbon disulfide (CS_2), and benzaldehyde ($\text{C}_7\text{H}_6\text{O}$) for the control of stored-product insects.

During previous centuries, traditional agriculture in developing countries has developed effective means for insect control using *botanicals*. Their efficiency and optimal use still need to be assessed in order to make these means of insect control cheap and simple for users. Lately, a new field has evolved which emphasizes the use of phytochemicals for insect pest management. The bioactivity of essential oils (EOs), the major volatile in aromatic plants, and their constituents, has been well documented against a large number of insect pests. An example is the EO obtained from the leaves of *Thugopsis dolabrata hondai* which was found to have high bioactivity against the cockroach (*Periplaneta fuliginosa*), the mite (*Dermatophagoids farinae*), and the termite (*Coptotermes formosanus*) (Asada et al., 1989; Lee, 2004). Some EOs were found to exhibit repellent activity against various insects (Kalemba et al., 1991; Hassalani and Lwande, 1989; Mwangi et al., 1992). Others were found to be potent growth inhibitors and anti-feedants (Jermy et al., 1981; Koul et al., 1990). These essential oils were also found to be effective as nematicidal (Oka et al., 2000), anti-bacterial (Matasyoh et al., 2007), virucidal (Schuhmacher et al., 2003), and repellents against ectoparasites (Mumcuoglu et al., 1996).

The efficacy of essential oils as fumigants for the control of pest infestations in grain and dry food products was also evaluated. EOs and their constituents are known to possess insecticidal (Wilson and Shaaya, 1999; Shaaya et al., 1997) and insect repellent activity (Jilani et al., 1988) and to cause a reduction in progeny (Regnault-Roger and Hamraoui, 1995). For example, the fumigant toxic activity, anti-feedant, and reproduction inhibition induced by a number of EOs and their monoterpenoids were evaluated against the bean weevil *Acanthoscelides obtectus*

(Say) and *Callosobruchus maculatus* (F.) (Klingauf et al., 1983; Regnault-Roger and Hamraoui, 1995; Raja et al., 2001). EOs extracted from *Pogostemon heyneanus*, *Ocimum basilicum* (basal), and *Eucalyptus* showed insecticidal activity against *Sitophilus oryzae*, *Stegobium paniceum*, *Tribolium castaneum*, and *Callosobruchus chinensis* (Deshpande et al., 1974; Deshpande and Tipnis, 1977).

In our laboratory, in order to isolate active EOs, we screened a large number of EOs extracted from aromatic plants and isolated their main constituents. We have already isolated many such compounds from the EOs of a large number of aromatic plants (Shaaya et al., 1991, 1994, 1997). Using space fumigation (see Shaaya et al., 1997), two EOs obtained from Lamiaceae plants were found to be the most potent fumigants of all oils tested. The main component of one of the oils is pulegone. The other is not yet identified and it is called SEM76 (Shaaya and Kostyukovsky, 2006).

In our study of the mode of action of EOs, we could show that the target for EO's neurotoxicity is the octopaminergic system in insects. We can thus postulate that EOs may affect octopaminergic target sites (Kostyukovsky et al., 2002; Shaaya et al., 2002).

ITCs were chosen for this study because of the pesticidal properties of these chemicals (Fenwick et al., 1983) and because of the potential use of methyl ITC as fumigant for wheat (Ducom, 1994). In our study on the rates of sorption of homologous series of ITCs on wheat, we could show that the rate of sorption decreases with increasing molecular weight (Shaaya and Desmarchelier, 1995). In the case of methyl ITC, a withholding period over 1 week would be required before residues decayed to levels near the limit of detection (Shaaya and Desmarchelier, 1995).

Comparative studies with CH_3I , CS_2 , and $\text{C}_7\text{H}_6\text{O}$ showed that CH_3I was the most potent compound against stored-product insects, followed by CS_2 and $\text{C}_7\text{H}_6\text{O}$. CS_2 , according to Winburn (1952), was one of the most effective grain fumigants as viewed from efficiency and low cost points of view. $\text{C}_7\text{H}_6\text{O}$ occurs in kernels of bitter almonds, has low toxicity to mammals, and has widespread use in topical antiseptics.

16.2 The Materials and Methods

The *materials and methods* employed in the current study are described as follows.

The tested stored-product insects were laboratory strains of *S. oryzae*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, *T. castaneum*, *Trogoderma granarium*, *Plodia interpunctella*, and *Ephestia cautella*.

The isothiocyanates (ITCs) are 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 (temperature = 70°C) for 2 h 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 *E. sativa* was identified as methyl thio-butyl isothiocyanate by gas chromatography (GC), nuclear magnetic resonance (NMR), and infra-red (IR) spectroscopy. CS₂, CH₃I, and C₇H₆O 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.

Three 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 (for details see Shaaya et al., 1991). The highly active compounds were then assayed in 600-mL glass chambers, filled to 70% by volume with wheat (11% moisture content). Pilot tests were carried out in simulation glass columns of 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. Percentage of insect mortality was then determined.

The *essential oils* (EOs) of aromatic plant 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* (Briellmann et al., 2006). The majority of EOs contain a limited number of main constituents, although minor compounds in the oil can also play an important role in the fragrance and biological activity.

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, the two EOs obtained from our experimental 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 (Table 16.1)

Table 16.1 List of aromatic plants whose essential oils were tested for bioactivity

Species	Family	Species	Family
<i>Apium graveolens</i>	Apiaceae	<i>Micromeria fruticosa</i>	Lamiaceae
<i>Artemisia arborescens</i>	Compositae	<i>O. basilicum</i>	Lamiaceae
<i>A. judaica</i>	Compositae	<i>O. gratissimum</i>	Lamiaceae
<i>Carum carvi</i>	Apiaceae	<i>Origanum vulgare</i>	Lamiaceae
<i>Citrus limonum</i>	Rutaceae	<i>Pelargonium graveoleus</i>	Geraniaceae
<i>Coriandrum sativum</i>	Apiaceae	<i>Petroselinum crispum</i>	Apiaceae
<i>Cuminum cyminum</i>	Apiaceae	<i>Pimpinella anisum</i>	Apiaceae
<i>Cymbopogon citrates</i>	Poaceae	<i>Rosmarinus officinalis</i>	Lamiaceae
<i>Foeniculum vulgare</i>	Apiaceae	<i>Ruta chalepensis</i>	Rutaceae
<i>Laurus nobilis</i>	Lauraceae	<i>Salvia dominica</i>	Lamiaceae
<i>Lavandula officinalis</i>	Lamiaceae	<i>Salvia fruticosa</i>	Lamiaceae
<i>Majorana siriaca</i>	Lamiaceae	<i>Salvia officinalis</i>	Lamiaceae
<i>Matricaria camomilla</i>	Asteraceae	<i>Salvia sclarea</i>	Lamiaceae
<i>Mentha piperita</i>	Lamiaceae	<i>Satureja thymbra</i>	Lamiaceae
<i>M. rotundifolia</i>	Lamiaceae	<i>Thymus vulgaris</i>	Lamiaceae

Table 16.2 Fumigant toxicity of SEM76 and pulegone on some stored-product insects (space fumigation)

Terpenoids	Concentration, $\mu\text{L}\cdot\text{L}^{-1}$	Stage	% Mortality (7 days after treatment)					
			<i>Sitophilus</i>	<i>Oryzaephilus</i>	<i>Rhyzopertha</i>	<i>Tribolium</i>	<i>Plodia</i>	<i>Trogoderma</i>
SEM76	0.5	Adult	100	100	100	87		
	1		100	100	100	100		
Pulegone	0.5		100	100	100	100		
Limonene	0.5		27	27	24	0		
SEM76	2	Larvae				60	90	55
	4					96	100	100
Pulegone	2					58	63	55
	4					100	100	100

Exposure time –24 h.

Third instar larvae and 3-day old pupae were used.

The main component of one of the oils 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 concentrations of $0.5 \mu\text{L}\cdot\text{L}^{-1}$ air and exposure time of 24 h. A higher concentration of $4 \mu\text{L}\cdot\text{L}^{-1}$ air was needed to kill larvae of *Tribolium*, *Trogoderma*, and *Plodia*. Limonene which is regarded as active monoterpene has much lower activity (Table 16.2).

Table 16.3 Fumigant toxicity of SEM76, with and without CO₂, against five stored-product insects on winter wheat, in columns 70% filling, in pilot tests

Stage	Concentration, $\mu\text{L}\cdot\text{L}^{-1}$	% Mortality (7 days after treatment)				
		<i>Sitophilus</i>	<i>Tribolium</i>	<i>Oryzaephilus</i>	<i>Rhyzopertha</i>	<i>Plodia</i>
Adults	70	100	66	100	70	–
	50 + 15% CO ₂	100	96	100	100	–
	70 + 15% CO ₂	100	100	100	100	–
Pupae	70 + 15% CO ₂	–	75	–	–	100
Larvae	70	–	60	–	–	87
	70 + 15% CO ₂	–	80	–	–	100

Exposure time – 7 days.

Pilot tests in simulation glass columns filled to 70% volume with wheat, under conditions similar to those present in large grain bins, showed that SEM76 at a concentration of $70 \mu\text{L}\cdot\text{L}^{-1}$ air (equivalent to $70 \text{ g}\cdot\text{m}^{-3}$) and 7 days exposure time caused 100% kill of adults of *Sitophilus* and *Oryzaephilus*, but not of *Rhyzopertha* and *Tribolium* (Table 16.3). Supplementation of 15% CO_2 ($200 \text{ g}\cdot\text{m}^{-3}$) caused reduction in the effective volatile concentration. A concentration of $50 \mu\text{L}\cdot\text{L}^{-1}$ air was enough to cause 96–100% kill of all adult insects tested. For pupae and larvae of *Tribolium* and *Plodia*, a higher concentration is needed (Table 16.3).

16.3 Efficacy of Isothiocyanates (ITCs) as Fumigants for the Control of Pest Infestations in Grain and Dry Food Products

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, namely, *Brassica nigra*, *B. carinata*, *B. tournefortii*, *Lepidium*

Table 16.4 The fumigant toxicity of four active isothiocyanates compared with methylthio-butyl ITC against adults of major stored grain insects. (Space fumigation)

Compound	Concentration, $\mu\text{L}\cdot\text{L}^{-1}$	Exposure time, h	% Mortality (7 days after treatment)			
			<i>Sitophilus</i>	<i>Rhyzopertha</i>	<i>Oryzaephilus</i>	<i>Tribolium</i>
Allyl ITC	1.0	1.5	–	100	100	0
	1.0	3.0	100	100	100	100
Methylthio-butyl ITC	1.0	3.0	100	89	100	0
	2.0	6.0	–	100	–	52
Methyl ITC	1.0	1.5	100	100	73	13
	1.0	2.5	100	100	100	100
Ethyl ITC	1.0	1.5	17	15	–	0
	1.0	3.0	100	100	–	0
	1.5	3.0	100	100	100	18
Butyl ITC	1.5	3.0	65	43	68	25
	3.0	3.0	100	100	100	100

Methylthio-butyl ITC was isolated from the plant *Eruca sativa*.

sativa, *Sisymbrium irio*, *Sinapis alba*, *S. arvensis*, *E. sativa*, and *Diplotaxis spp.*, only in the last three species was it possible to isolate from the seed oil 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 active against adults of four stored-product insects. A concentration of $1 \mu\text{L}\cdot\text{L}^{-1}$ air and exposure time of 3 h were enough to kill all the tested adult insects. The activity of methylthio-butyl ITC was comparable to that of allyl and methyl ITCs except for *Tribolium*, which was found to be much more susceptible to the two ITCs (Table 16.4).

In the case of *Plodia* larva also, a concentration of $1.5 \mu\text{L}\cdot\text{L}^{-1}$ air of the three active ITCs and exposure time of 3 h were enough to get 100% kill. For larvae of *Tribolium* and *Trogoderma*, a higher concentration of $2.5 \mu\text{L}\cdot\text{L}^{-1}$ air and exposure time of 3 h were needed. The pupae of these three insect species were the most resistant to the ITCs tested (Table 16.5).

Using high columns filled to 70% wheat to evaluate the toxicity of allyl ITC in grain, we could show that $20 \mu\text{L}\cdot\text{L}^{-1}$ air ($=20 \text{ g m}^{-3}$) and exposure time of 1 day were not effective in killing the insects at the bottom of the column when the fumigant was applied at the upper layer of the grain. Addition of CO_2 and circulation caused 100% kill at the different heights. Increasing the exposure time to 4 days and cycling was enough to obtain 100% kill (Table 16.6).

Table 16.5 The fumigant toxicity of four active isothiocyanates compared with methylthio-butyl ITC against larvae and pupae of major stored grain insects. (Space fumigation)

Compound	Concentration, $\mu\text{L}\cdot\text{L}^{-1}$	Exposure time, h	% Mortality (7 days after treatment)					
			Larvae			Pupae		
			<i>Tribolium</i>	<i>Trogoderma</i>	<i>Plodia</i>	<i>Tribolium</i>	<i>Trogoderma</i>	<i>Plodia</i>
Allyl ITC	1.5	3.0	23	84	100	52	65	–
	2.5	3.0	95	100	100	68	80	45
	5.0	3.0	100	100	100	100	98	100
Methyl ITC	2.5	1.5	65	77	100	50	7	97
	3.5	1.5	100	81	100	100	32	100
	5.0	1.5	–	100	–	–	–	–
Methylthio-butyl ITC	1.0	3.0	100	100	87	–	–	–
	2.0	3.0	100	100	90	–	–	–
Ethyl ITC	1.5	3.0	20	6	100	–	–	–
	1.5	4.5	49	23	100	–	–	–
	1.5	6.0	100	100	100	–	–	–
	2.5	3.0	–	–	–	2.5	3	27
Butyl ITC	1.5	3.0	5.5	23	7	–	–	–
	5.0	3.0	98	78	100	–	–	–

Methylthio-butyl ITC was isolated from the plant *Eruca sativa*.

Third instar larvae and 3-day old pupae were used

Table 16.6 Toxicity of allyl ITC against stored-product insects, using high columns filled with 70% wheat with and without CO₂

Insect	Insects' height, cm (top–bottom)	% Mortality (7 days after treatment)				
		Exposure time – 1 day			Exposure time – 4 days	
		20 $\mu\text{L}\cdot\text{L}^{-1}$ + 20% CO ₂	20 $\mu\text{L}\cdot\text{L}^{-1}$ + cycling	20 $\mu\text{L}\cdot\text{L}^{-1}$ + cycling + 20% CO ₂	20 $\mu\text{L}\cdot\text{L}^{-1}$	20 $\mu\text{L}\cdot\text{L}^{-1}$ + cycling
<i>Tribolium</i>	20	100	70	100	100	100
	120	0	17	90	0	100
<i>Sitophilus</i>	20	100	100	100	100	–
	120	35	100	100	94	–
<i>Rhyzopertha</i>	20	100	100	100	100	–
	120	5	85	100	78	–
<i>Oryzaephilus</i>	20	–	100	100	100	–
	120	–	100	100	100	–

16.4 Efficacy of CH₃I, CS₂, and C₇H₆O as Fumigants for the Control of Stored-Product Insects

In space fumigation, CH₃I was very effective against all insect stages tested. Exposure to a concentration of 3–5 $\mu\text{L}\cdot\text{L}^{-1}$ for 3 h was lethal and caused 100% mortality of all stages of the test insects, except for *Trogoderma* larvae (Table 16.7). Adults of *Tribolium* were found to be the most tolerant, followed by *Oryzaephilus*, *Rhyzopertha*, and *Sitophilus*. In the case of larvae and pupae, *Trogoderma* was the most tolerant, followed by *Tribolium* and *Plodia* (Table 16.7).

CS₂ was less effective than CH₃I and needed a concentration of 6–9 $\mu\text{L}\cdot\text{L}^{-1}$ air for 1 day to achieve total mortality of all the test insects except for *Trogoderma* larvae. In the case of CS₂, adults of *Tribolium* were found to be the most resistant, followed by *Sitophilus*, *Oryzaephilus*, and *Rhyzopertha*. The larvae of *Trogoderma* were more resistant than *Tribolium* (Table 16.8).

In experiments with 600-mL glass chambers filled to 70% volume with wheat, CH₃I also showed higher activity than CS₂. The concentration of CH₃I and exposure time needed to obtain a total mortality of the test insects were comparable to those in space fumigation tests (see Table 16.7). For CS₂, higher concentrations

Table 16.7 Toxicity of CH₃I against stored-product insects, in space fumigation and in 600-mL chambers filled with 70% wheat

Test	Concentration, $\mu\text{L}\cdot\text{L}^{-1}$	% Mortality (7 days after treatment)								
		<i>Rhyzopertha</i>	<i>Oryzaephilus</i>	<i>Sitophilus</i>	<i>Tribolium</i>		<i>Trogoderma</i>		<i>Plodia</i>	
		Adults				Larvae	Pupae	Larvae	Pupae	Larvae
Space fumigation – 3 h	2.0	41	10	55	7	–	–	–	–	–
	3	94	85	100	65	40	–	–	–	100
	4	100	100	–	95	77	–	58	–	–
	5	–	–	–	100	100	100	70	0	–
	6	–	–	–	–	–	–	100	–	–
600-mL chambers with grain – 3 h	2.5	88	63	100	63	50	–	15	–	–
	3.5	100	88	–	80	60	–	40	–	–
	5	–	100	–	100	100	–	100	–	–

Specific gravity of CH₃I –2.28

Third instar larvae and 3-day old pupae were used.

were needed (see Table 16.8). The large difference in the activity between the two compounds was probably due to higher sorption rate of CS₂ in wheat, as compared with that of CH₃I. In the pilot tests, in glass columns filled to 70% wheat, CH₃I again showed higher activity than CS₂, when circulation was applied. A concentration of 5 $\mu\text{L}\cdot\text{L}^{-1}$ air and exposure time of 3 h were enough to obtain 100% kill (Table 16.9) as compared with 20 $\mu\text{L}\cdot\text{L}^{-1}$ air CS₂ and 24 h exposure time (Table 16.10). In gravity applications, CS₂ penetrated better than CH₃I, but needed a higher concentration and exposure time to achieve total mortality (Tables 16.9 and 16.10). It should be mentioned that for methyl bromide fumigation the recommended concentration is 30–50 g·m⁻³.

16.5 Conclusions

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

Table 16.8 Toxicity of CS₂ against stored-product insects, in space fumigation and in 600-mL chambers filled with 70% wheat

Test	Concentration, $\mu\text{L}\cdot\text{L}^{-1}$	Exposure time, days	% Mortality (7 days after treatment)					
			<i>Rhyzopertha</i>	<i>Oryzaephilus</i>	<i>Sitophilus</i>	<i>Tribolium</i>	<i>Trogoderma</i>	
			Adults			Larvae		
Space fumigation	5	1	72	53	23	0	–	–
	6		100	90	30	0	–	–
	7		–	100	74	10	–	–
	8		–	–	93	70	–	–
	9		–	–	100	100	100	60
600-mL chambers with grain	10	1	100	0	0	0	–	–
		2	–	17	32	0	–	–
		4	–	24	100	56	–	–
		5	–	100	–	100	–	–
	20	1	100	62	100	40	–	–
		2	100	100	100	100	–	–

Specific gravity of CS₂– 1.26

Third instar larvae were used.

non-mammalian target. 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-blooded animals and humans. They are also classified as *generally recognized as safe (GRAS)*. 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.

C₇H₆O was less active than CH₃I and CS₂ in space fumigation bioassays. A concentration of 3 $\mu\text{L}\cdot\text{L}^{-1}$ air and exposure time of 1 day caused 100% adult mortality of *Sitophilus*, *Rhyzopertha*, and *Oryzaephilus*. In the case of *Tribolium*, 65% mortality for adults and no effect on eggs and pupae were recorded (Table 16.11). In the case of *Ephesia*, this concentration caused 100% mortality of the eggs, but had no effect on pupae (data not shown). In studies with 600-mL fumigation chambers, a concentration of 50 $\mu\text{L}\cdot\text{L}^{-1}$ air and exposure time of 7 days caused 100% mortality of the adults tested except for *Tribolium*. Increasing the concentration to 100 $\mu\text{L}\cdot\text{L}^{-1}$ air yielded very low mortality of larvae, pupae, and adults of *Tribolium* (Table 16.11).

Table 16.9 Penetration of CH₃I in 120-cm high columns filled with 70% wheat by gravity or circulation

Method used	Concentration, $\mu\text{L}\cdot\text{L}^{-1}$	Exposure Time, h	Insects' height, cm (top-bottom)	% Mortality (7 days after treatment)			
				<i>Rhyzopertha</i>	<i>Oryzaephilus</i>	<i>Sitophilus</i>	<i>Tribolium</i>
Gravity	5	24	Top	100	100	100	100
			20				
			120	10	10	30	0
			Bottom				
	5	72	Top	100	100	100	100
			20				
			120	95	75	80	0
			Bottom				
Circulation 3 × 45 min	5	3	Top to bottom	100% mortality of all insects			

ITCs are also potential candidates because only very low concentrations are needed for the control of stored-product insects. It should be mentioned that *E. sativa* (salad rocket) is used worldwide as a food supplement, and methyl thio-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 disinfestation of grain and dry food products.

Comparative studies with CH₃I, CS₂, and C₇H₆O showed that CH₃I was the most toxic compound to stored-product insects, followed by CS₂ and C₇H₆O. CH₃I was found to be less sorptive and less penetrative in wheat than CS₂. CH₃I is toxic to humans and its use in food as a fumigant is therefore limited. It should be mentioned that CS₂ is flammable and 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 of 64:26:10, respectively, was developed by us and was found to be effective against stored-product insects (Polachek et al., 1960). C₇H₆O has low toxicity to mammals, but it is less effective against stored-product insects than all other fumigants tested. CH₃I, CS₂, and C₇H₆O may play a role mainly as supplements to increase the activity of other fumigants.

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

Table 16.10 Penetration of CS₂ in 120-cm high columns filled with 70% wheat by gravity or circulation

Method used	Concentration, μL.L ⁻¹	Exposure time, h	Insects' height, cm (top–bottom)	% Mortality (7 days after treatment)			
				<i>Rhyzopertha</i>	<i>Oryzaephilus</i>	<i>Sitophilus</i>	<i>Tribolium</i>
Gravity	20	48	Top	100	100	100	100
			20	100	0	30	10
			120				
Circulation 3 × 45 min	20	72	Bottom				
			Top to bottom	100%	mortality of all insects		
			Top to bottom	100%	mortality of all insects		

Table 16.11 Fumigant toxicity of benzaldehyde against various developmental stages of stored-product insects using space fumigation and fumigation in 600-mL chambers filled with 70% wheat

Treatment	Concentration, $\mu\text{L}\cdot\text{L}^{-1}$	Exposure time, days	% Mortality (7 days after treatment)						
			<i>Sitophilus</i>	<i>Rhyzopertha</i>	<i>Oryzaephilus</i>	<i>Tribolium</i>			
			Adults				Pupa	Larva	Eggs
Space fumigation	1.5	1	39	79	16	0	–	–	–
	3	1	100	100	100	65	0	–	0
Fumigation in 600-mL chambers	50	7	97	95	100	0	–	–	–
	100	7	100	100	100	19	–	0	–

Adult mortality in control was less than 5. Third instar larvae and 3-day old pupae were used.

phosphine. Because of this, alternative fumigants could be developed against particular species of insects or used for a specific food product commodity.

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