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Fumigation potential of organic compounds against different stages of *Tribolium castaneum*

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ABSTRACT

Insects can cause severe damage to food grain and other commodities under storage condition. Several Coleoptera and Lepidoptera have been reported to feed on stored commodities and may cause approximately 10% loss. The primary means of controlling insect pests in stored grain is by fumigation with phosphine, but genetic resistance towards this fumigant has emerged across the globe. The present study investigated the fumigation potential of 16 compounds against stored grain pest *Tribolium castaneum* (Herbst) under laboratory conditions. Among all screened 16 compounds, 10 compounds, viz. 3-methyl-2-butenal, dipropyl disulphide, allylisothiocyanate, 1-bromohexane, 1-bromopentane, thioglycolic acid, *sec*-butyl bromide, triethyl orthoformate, ethyl bromide and thionyl chloride, showed high fumigant toxicity against larvae and adult of (7- day- old) *T. castaneum*. Allyl isothiocyanate was the most effective compound among the all tested, causing immediate adult and larval mortality after 12 h of exposure at the lower dosages of 0.50 to 0.75 $\mu L\ L^{-1}$. Results showed level of fumigant toxicity was highly correlated with the dosage (v/v) of chemical used for treatment. Larvae of tested insect were more tolerant than adults. The four compounds showing the best activity with larvae and adults were selected for study of ovicidal effects–allyl isothiocyanate, dipropyl disulphide, thionyl chloride and thioglycolic acid. All these four compounds showed complete mortality at 10 $\mu L\ L^{-1}$dosage when 24 h exposure was given. Maximum adulticide, larvicide and ovicidal activity were recorded with allyl isothiocyanate with 100% mortality at 1.0, 1.1 and 0.50 $\mu L\ L^{-1}$ respectively.

Key words: Allyl isothiocyanate*,* Fumigation, Organic compounds, *T. castaneum,* Wheat

There is a global increase in the demand of food grains for human and animal consumption in recent times. Population growth and rising incomes in developing countries place pressures on agricultural production to meet the increasing need for affordable food**.** Reports indicated that, the year 2050, the world population by will be over 9.1 billion. The biggest challenge before agricultural research, development and policy is how to feed them with safe food (Parfitt et al., 2010). Measurable qualitative and quantitative loss of food in the system after harvest has been termed as post-harvest loss or PHL. As reported by *FAO-World Bank* (2010) and Prusky (2011), 1.3 billion tonne of food have been shattered or lost each year. Several insect pests attack wheat (*Triticum aestivum* L.) grain and wheat grain products during storage.

Tribolium castaneum (Herbst) is a cosmopolitan pest that is known to infest 246 commodities worldwide (Hagstrum and Subramanyam, 2009), although flour and other milled products are preferred more (Good, 1936).Adult beetles and larvae of *T.castaneum* feed on stored produces like pulses, prepared cereal foods, dried fruits, bran, germ and flours (Atwal, 1976; Hamed and Khattak, 1985; Dars et al., 2001).They induce both qualitative and quantitative damage to grain. Insect feeding causes weight loss in grains which in turn inflicts quantitative damage (Golebiowska, 1969). Qualitative harm is because of changes in product like deterioration in aesthetic and nutritional qualities, high quantity of refuse in the grain heap, and loss of functional propertiessuch as baking qualities (Gallo et al., 1978; Vieira, 1983; Baier and Webster, 1992; Moino et al.,1998).

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Fumigation is the most widely used procedure to control stored-product pests to prevent economic and quality losses by providing various application methods and penetration capability into the treated commodity. However, it has been found to have destructive effects on human beings. Many of them have limitations in use due to various reasons. Methyl bromide (MB), according to Montreal Protocol, is scheduled to be phased out world-wide by 2015. Sulphuryl fluoride (SF) is known to have limited efficiency on egg stages of insects.

Currently, Phosphine (PH_3) is the most widely used fumigant worldwide ouring to its low cost and ease of application, though resistance observed in major pest species threatens the continued use of PH_3. In the case of phosphine, resistance is the inevitable result of the continuous use of the fumigants in leaky conditions, improper applications and exposures. The number of pest populations showing resistance to phosphine has been increasing world-wide since it was first shown by a global FAO survey on pesticide susceptibility in 1972, 1973 (Champ and Dyte, 1976). In view of these problems, scientists world-wide are looking towards alternatives for prophylactic and curative treatment of stored commodities and some encouraging results have been obtained in the past studies (Koul, 2004; Rajendran and Muralidharan 2004; Chen et al., 2011). In the present investigation, an attempt was made to search the fumigant toxicity of some compounds against *T. castaneum*, insect pests of stored grain.

MATERIALS AND METHODS

Sixteen organic compounds (3 Methyl-2-butenal, *trans*-2-hexenal, Dipropyl disulphide, Allyl isothiocyanate, Allyl bromide, 1-Bromo-hexane, 1-Bromo-pentane, 2-Bromo-pentane, 9-Ethyl iodide, Thioglycolic acid, *sec*-butyl bromide, Triethyl orthoformate, Sulphuryl chloride, Ethyl bromide, Thionyl chloride, Methyl iodide) were chosen on the basis of their physico-chemical properties and their probable potential for insecticidal activity.

Laboratory studies were carried out to screen these organic compounds against stored grain insect pest. The details of the rearing of population, bioassay technique, and the methodology adopted for recording observations are:

Rearing of test insect **-** Tribolium castaneum (Herbst)

The test insects were reared in the laboratory conditions as per the method reported by Bhatia and Pradhan (1968). The culture was maintained by rearing the insects at 30 ± 2°C and 70% r. h. on sterilized wheat flour mixed with 5% (weight/weight) brewer's yeast. About 300 to 400 adults were released in a rearing glass jar (15 cm × 10 cm) containing 100 g wheat flour media for oviposition. The adults were removed after every four to six days and released in a fresh jar. The insects in the jars were allowed to grow undisturbed till the adults started emerging. Successfully emerged adults were removed from the rearing glass jar for bioassay tests or for breeding the next generation in a similar way.

Bioassay technique

Bioassay tests were conducted with the candidate materials. Adult beetles (one to three weeks old) or two weeks old larvae were confined within plastic cups (10 beetles/larvae per cup) inside gas-tight desiccators that were used in exposure chambers. Organic compounds $\mu L\ L^{-1}$were poured on the filter paper which was hanged from adhering the top surface of air tight desiccators. To calculate the volume of desiccators, each desiccator was fully assembled and then completely filled with water. The weight of this water in gram closely equals the volume in millilitres. On the basis of the volume of each desiccator, the correct volume of organic compound was released into each desiccator to give the required dosage. Dosage volumes of the organic compounds were calculated using the source concentration, required dosage and volume of desiccators.

All test compounds (3 Methyl-2-butenal, *trans*-2-hexenal, Dipropyl disulphide, Allyl isothiocyanate, Allyl bromide, 1-Bromo-hexane, 1-Bromo-pentane, 2-Bromo-pentane, 9-Ethyl iodide, Thioglycolic acid, *sec*-butyl bromide, Triethyl orthoformate, Sulfuryl chloride, Ethyl bromide, Thionyl chloride, Methyl iodide) were screened at concentration 1, 5, 10 and 15 $\mu L\ L^{-1}$. Observations were recorded at 24 h after treatment. Total numbers of surviving (live) and dead insects were counted in each treatment.

On the basis of their efficacy, ten best compounds were selected for further experimental studies. These 10 compounds were tested against adult, larvae and egg stage of test insect.

Statistical analysis

The data on mortality were subjected to Abbott's correction formula for the correction of mortality. LC_{50} values were determined using probit analysis based computer programme STPR718.

RESULTS AND DISCUSSION

Fumigant toxicity of candidate fumigant materials against *T. castaneum* is presented in Table 1. All

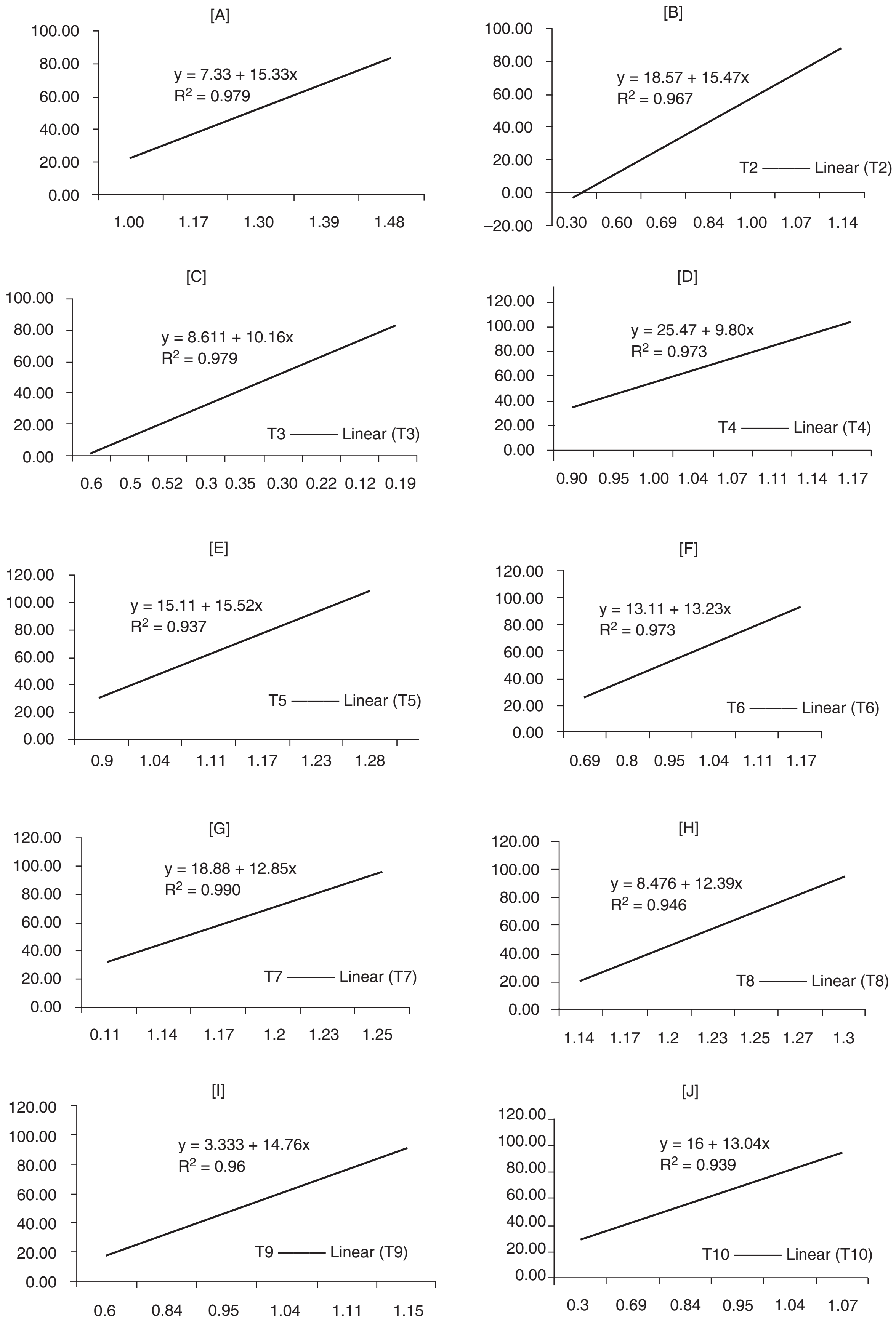

Fig. 1. Dosage response of compounds A, 3-Methyl-2-butenal; B, Dipropyl disulphide; C, Allylisothiocyanate; D, 1-bromo hexane; E, 1- bromo pentane; F, Thioglycolic acid; G, *s*-butyl formate; H, Triethyl orthoformate; I, Ethyl bromide; J, Thionyl chloride; against *T. castaneum* at 24 h exposure

Table 1 Dosage mortality response of organic compounds against *Tribolium castaneum* at 24 h exposure

Organic compounds	LC values (in µL L^{-1}air)												Chi-square value	Regression equation
	LC_{30}	Fiducial limit		RT at LC_{30}	LC_{50}	Fiducial limit		RT at LC_{50}	LC_{90}	Fiducial limit		RT at LC_{90}		
		Lower	Upper			Lower	Upper			Lower	Upper			
3-Methyl-2-butenal	12.61	9.42	14.84	1.15	17.62	15.00	20.29	1.00	40.18	31.62	65.45	1.00	0.37	y = 7.33 + 15.33x
Dipropyl disulphide	5.82	4.85	6.63	2.49	7.84	6.91	8.89	2.25	16.35	12.94	20.65	2.46	11.85	y = 18.57 + 15.47x
Allylisothiocynate	0.40	0.36	0.44	36.23	0.51	0.48	0.56	34.55	0.94	0.79	1.13	42.74	1.57	y = 8.61 + 10.16x
1-bromo hexane	7.98	6.65	8.74	1.82	9.43	8.56	10.07	1.87	14.21	12.76	17.80	2.83	5.09	y = 25.47 + 9.801x
1-bromo pentane	10.44	6.36	11.57	1.39	12.06	10.14	13.35	1.46	17.21	14.78	40.87	2.33	5.78	y = 15.11 + 15.52x
Thioglycolic acid	5.12	1.35	6.78	2.83	8.57	6.25	12.16	2.06	30.38	17.28	806.23	1.32	23.03	y = 13.11 + 13.23x
s-butyl bromide	13.28	11.63	14.06	1.09	14.42	13.37	15.01	1.22	17.68	16.82	19.75	2.27	2.10	y = 18.88 + 12.85x
Triethyl ortho formate	14.49	12.75	15.34	1.00	16.46	15.59	17.78	1.07	22.53	19.88	32.87	1.78	10.39	y = 8.47 + 12.39x
Ethyl bromide	7.43	5.98	8.36	1.95	9.20	8.12	10.10	1.92	15.57	13.53	20.50	2.58	6.45	y = 3.33 + 14.76x
Thionyl chloride	4.21	0.12	5.76	3.44	6.73	3.12	9.41	2.62	21.43	12.53	26410.42	1.87	23.79	y = 16.00 + 13.04x

RT, Relative toxicity

compounds were effective at some dosage against *Tribolium castaneum*. Allylisothiocynate was found most effective against adults, having LC_{50} value 0.51 µL L^{-1} with at 24 h exposure, followed by thionyl chloride, dipropyl disulphide, and thioglycolic acid (LC_{50} value, 6.73, 7.84 and 8.57 µL L^{-1}). Fumigants 1-bromo hexane and ethyl bromide were equally effective having LC_{50} of 9.43 and 9.20 µL L^{-1}. Compound 3-methyl-2-butenal found least effective (LC_{50}, 17.62 µL L^{-1}), similar to effectiveness of triethyl orthoformate (LC_{50} 16.46 µL L^{-1}).

Tested organic compounds against adult of *T. castaneum* were also tested against the larval stage of tested insect. Results depicted in Fig. 2 indicates that Allyl isothiocyanate found most effective at 0.75 µL L^{-1} , causing 86.70% mortality against the larval stage as well, followed by Dipropyl disulphide at 10 µL L^{-1} causing 86.67%, Thionyl chloride at 10 µL L^{-1} causing 80.00% and Thiglycolic acid at 15 µL L^{-1} , caused 100% mortality.

The four most fumigants against adult and larval stage of *T. castaneum,* were: Allyl isothiocyanate, thionyl chloride, dipropyl disulphide, and thioglycolic acid, were selected to check the efficacy against the egg stage. Fig 3 shows all four compounds were successful in stopping the hatching of eggs.

Koul (2004) reported dipropyl disulphide and diallyl disulphide (di-2-propenyl disulphide) were toxic when applied topically or as a fumigant to *Tribolium castaneum* adults and 8-, 12-, and 16-d-old larvae, and *Sitophilus oryzae* adults. Di-n-propyl disulphide significantly decreased the growth rate and dietary utilization with moderate inhibition of food consumption in both insects. Studies clearly demonstrated that di-*n*-propyl disulphide could be a potent toxicant, fumigant, and feeding deterrent for stored grain pests, if a suitable formulation and application procedure are developed. Similarly, Wu et al. (2009) reported that allyl isothiocyanate fumigation showed strong toxicity to the four species of stored-product pests. Adult mortality of 100% of all four pest species, recorded after 72 h exposure to allyl isothiocyanate fumes at an atmospheric concentration of 3 µg mL^{-1}, showed no significant difference from that of insects exposed to phosphine at 5 µg mL^{-1}, the recommended dosage for phosphine. Numerous studies on chemicals are being considered as alternative fumigants to methyl bromide, in according the present study is done to check the efficacy of some organic compounds which may lead in the future as a stored grain fumigants.

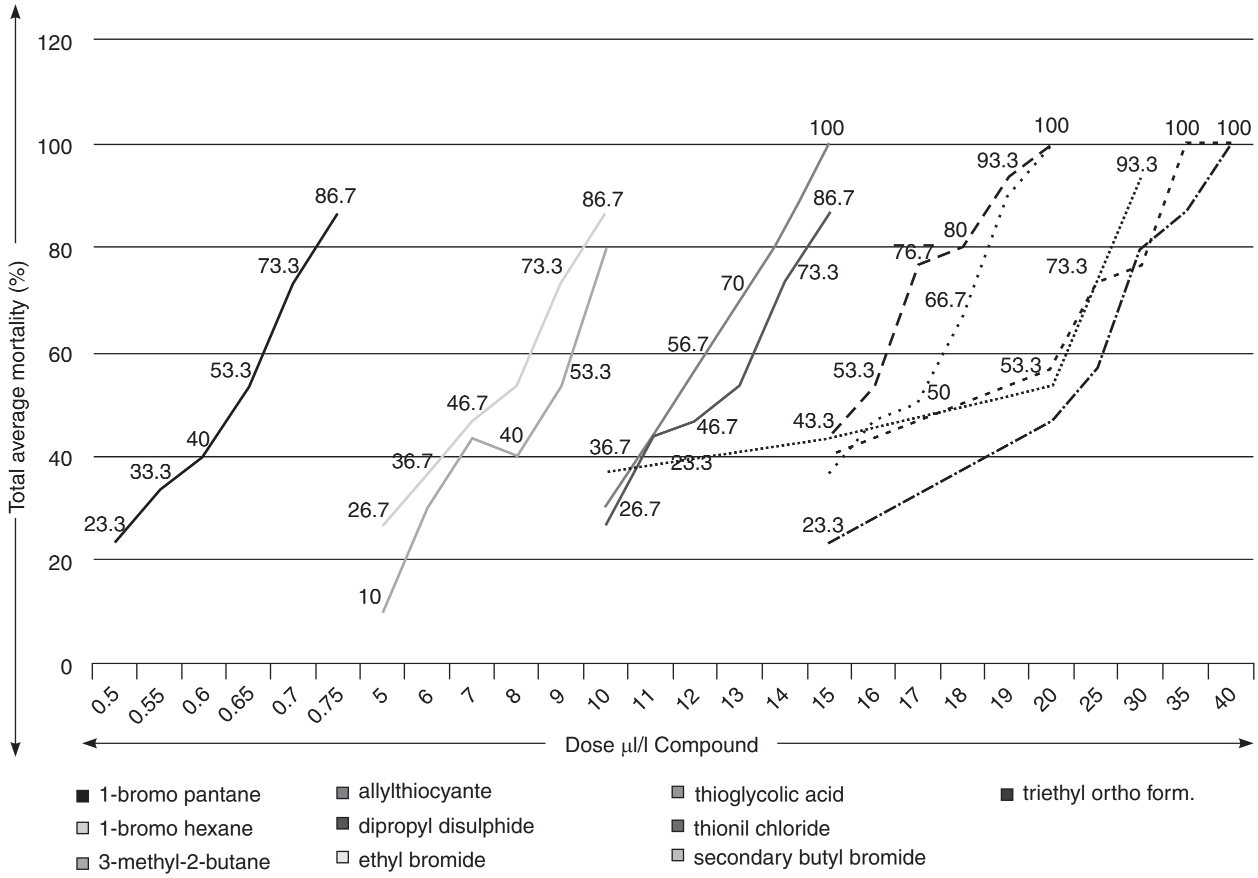

Fig. 2. Fumigant toxicity of organic compounds against larvae of *Tribolium castaneum*

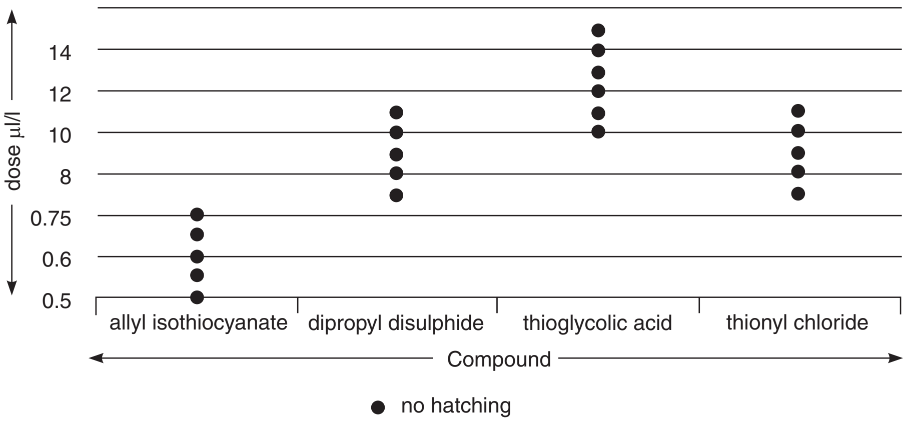

Fig. 3. Fumigant toxicity of organic compounds against eggs of *Tribolium castaneum*

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