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Use of carbon dioxide (CO_2) and phosphine (PH_3) for management of major stored-product insects of paddy (*Oryza sativa*)

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ABSTRACT

Laboratory experiments were conducted in a fumigation chamber to study the effect of carbon dioxide (CO_2) and phosphine (PH_3) mixtures on different life stages of red flour beetle [*Tribolium castaneum* (Herbst)] and rice weevil [*Sitophilus oryzae* (L.)]. Mortality of different life stages of stored-product pests were recorded after exposure to different times to mixtures of 40% CO_2; 3 g/m^3, 2 g/m^3 or 1 g/m^3 phosphine gas; and the rest air. Mortality of both pests and their life stages increased with the increase in exposure time. An application of CO_2 in combination with phosphine reduced the dose and time required for fumigation of the stored paddy [*Oryza sativa* (L.)]. An application of 3 g/m^3 phosphine + 40% CO_2 and 2 g/m^3 phosphine + 40% CO_2 could achieve 100% mortality to all stages of *Tribolium castaneum* and *Sitophilus oryzae* in 5 days instead of 7 days, the normal practice in warehouses. Phosphine residues in the treated paddy were (0.1 mg/kg), which is below the recommended level, hence safe for consumption.

Key words: Carbon dioxide, Paddy, Phosphine, Residues, *Sitophilus oryzae, Tribolium castaneum*

India handled 253 million tonnes (Mt) of food grains in 2014–15 production season (FAO, 2014). These grains need to be preserved with the aid of the available fumigants (Pattanaik, 2012). Phosphine has been the choice fumigant for three decades for treating various commodities. Indiscriminate use of phosphine has led to the development of phosphine resistant strains as well as residue problems in food grains (Bhatia, 1990; Rajendran, 2001; Lorni et al., 2007). Although commercial fumigations have been generally successful, development of resistant strains is increasing (Tyler et al., 1983; Benhalima et al., 2004) due to the application of sub-lethal doses, leakages from the treated structures and lack of proper sealing techniques. In addition, environmental regulations will require the elimination of methyl bromide in many countries and will mitigate a scheduled phase-out in developing countries (EPA, 1998). The development of alternative treatments for pest control in food commodities has been increasing in demand from the food industry and new treatment methods should meet consumer demands for the reduced use or elimination of pesticides. Mixture of (CO_2) and (PH_3) is considered as a potential fumigant for the management of stored-product pests (Cotton and Young, 1929; Primental et al., 2009). Addition of CO_2 to PH_3 has several advantages such as reduction in the flammability level, reduction in the dose and time required for fumigation and increase in the susceptibility of stored product insects (Williams, 1985). Phosphine-carbon dioxide mixture is non-flammable and is highly toxic to insects. Carbon dioxide increases the respiration rate of insects and reduces the dose and time required for fumigation. The objective of the present study was to use PH_3 and CO_2 mixture in controlling different stages of red flour beetle [*Tribolium castaneum* (Herbst)] and rice weevil [*Sitophilus oryzae* (L.)] in paddy.

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MATERIALS AND METHODS

Laboratory experiments were conducted at the Indian Institute of Crop Processing Technology (IICPT), Thanjavur, using 40% CO_2 with 3, 2, or 1g/m^3

of phosphine, and phosphine alone at 3 g/m^3 was used as control. Mortality of various stages of *T. castaneum* and *S. oryzae* were recorded. Also, phosphine residues in the treated paddy after fumigation were measured using a gas chromatograph (GC-14B, Shimadzu Technologies, Columbia, USA).

Culturing of test insects

Red flour beetle and rice weevil were cultured and maintained in the Storage Engineering Laboratory at the IICPT, Thanjavur. Insects were cultured on whole maize (*Zea mays* L.) and wheat flour (*Triticum aestivum* L.) at 30°C and 70% r.h.. Different life stages of insects such as egg, pupa, larva and adult were maintained separately to carry out mortality studies. The study on mortality of egg stage was not determined.

Lab model fumigation set up for toxicity study

A laboratory-scale phosphine fumigation chamber was designed and fabricated at the IICPT (Fig. 1). It consisted of a circular outer acrylic cylindrical tube with the dimension of 37 cm × 27 cm (height × diameter) which was pasted to a flat acrylic sheet at the bottom. Inside the fumigation chamber, four compartments were made by partitioning it with wire mesh and were fitted in a stand. Fumigation cups of 4 cm × 6 cm (height × diameter) were made by cutting small acrylic tubes and were pasted with fine wire mesh at the bottom. The top cover of the fumigation chamber was a PVC end cap that made the set up airtight. Rubber septum was placed in the top cover for injecting phosphine. Provision of CO_2 gas inlet and outlet was kept for injecting and releasing CO_2 from the cylinder. Phosphine gas was generated from aluminium phosphide tablets using sulphuric acid as the medium (Valmas and Elbert, 2006). Required volume of phosphine gas was calculated and injected using Hamilton syringe through the rubber septum located in the top of the fumigation chamber. The 40% CO_2 in air continuously flowed through the chamber.

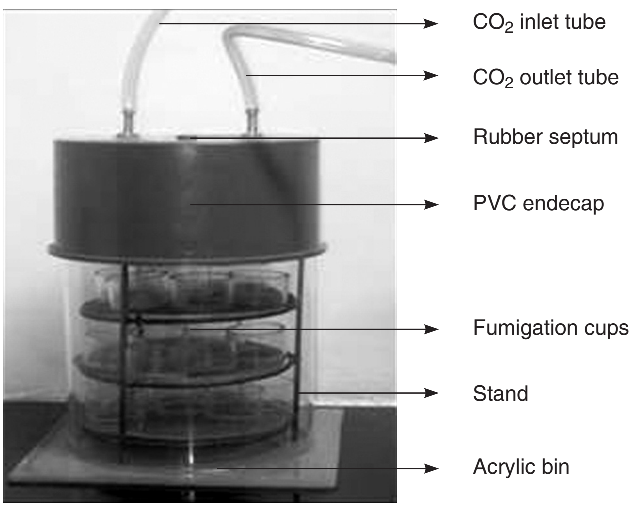

Fig. 1. A laboratory-scale fumigation chamber used for bioassay studies

Bioassay study

Fifteen grams of the food material was taken in fumigation cups and 10 insects of different life stages were released into the fumigation cups and the top of the fumigation cups was covered with muslin cloth and secured with rubber band. After the treatment, the insects were transferred with food material and were kept in environmental chamber at 25°C and 60% r.h.. The number of insects dead after fumigation at different time intervals was recorded and the percent mortality was calculated. Empty space test was conducted for different stages of insects to determine the effective combination of gases on the mortality of *T.castaneum* and *S. oryzae.* The mortality (%) was calculated for different combinations of PH_3 and CO_2 at 40% plus phosphine at 3, 2, 1 g/m^3 and were compared with phosphine alone at 3 g/m^3 as control.

Determination of PH_3 residues in the paddy

After fumigation treated paddy was kept in a tray and ventilated by natural movement of air around the tray for 5 days and were labeled and packed for residue analysis. PH_3 residue analysis of the paddy was conducted by a GC/FPD (Gas Chromatograph/ Flame Photometric Detector) method, using a gas chromatograph (GC-14B, Shimadzu Technologies, Columbia, USA). A representative sample of 15 g aerated paddy after fumigation from three replications were taken in a 500 mL flask, filled with 150 mL water, sealed with a stopper and injected with 5 mL HCL from an attaching syringes sampling adapter (stopper with silicone septum). The flasks were put in an ultrasonic wave-cleaner and shaken for 5 min and then allowed to stand for 30 min. The headspace analysis was done using Gas Liquid Chromatography (GLC, Shimadzu Technologies, Columbia, USA).

RESULTS AND DISCUSSION

The laboratory study indicated that there is a need to use critical levels of gases for the successful fumigation (Tables 1, 2). The insect mortality was higher with increase in PH_3 concentration level. The exposure period can be reduced by increasing the concentration of PH_3 in mixtures. Based on the test results of the preliminary trials with different concentrations of CO_2, 40% CO_2 was fixed in this study. The mortality results of increasing

Table 1 Percentage mortality of different life stages of *Tribolium castaneum* exposed to different levels phosphine in 40% carbon dioxide and rest air

Hours after fumigation	40 % CO_2 + 3 g/m^3 PH_3			40 % CO_2 + 2 g/m^3 PH_3			40 % CO_2 + 1 g/m^3 PH_3			Control 3 g/m^3 PH_3		
	Adult	Larva	Pupa	Adult	Larva	Pupa	Adult	Larva	Pupa	Adult	Larva	Pupa
6	83.00	60.00	43.33	80.00	66.66	35.00	63.33	56.00	30.00	76.66	60.00	28.00
12	85.33	63.33	43.33	82.00	60.00	35.00	65.00	56.00	30.00	77.00	60.33	28.00
24	88.00	65.55	52.66	86.00	63.00	53.00	66.00	58.00	43.00	80.00	63.00	33.33
36	92.00	68.66	58.00	88.00	65.00	56.00	70.00	60.00	47.00	82.00	65.66	38.00
48	95.00	79.00	69.00	93.00	76.00	67.00	76.00	64.00	58.00	88.88	74.00	41.00
60	98.00	87.00	96.00	95.00	83.00	90.00	88.00	68.00	61.00	90.00	75.00	46.00
72	100	95.00	85.33	100	100	91.00	91.00	73.00	75.00	96.66	80.00	58.66
96	100	100	95.00	100	100	93.00	100	85.00	77.00	100	87.00	69.00
120	100	100	100	100	100	100	100	100	89.00	100	100	78.33
144	100	100	100	100	100	100	100	100	100	100	100	100

Table 2 Percentage mortality of different life stages of *Sitophilus oryzae* exposed to different levels of phosphine in 40% carbon dioxide and rest air

Hours after fumigation	40% CO_2 + 3 g/m^3PH_3			40% CO_2 + 2 g/m^3 PH_3			40% CO_2 + 1 g/m^3 PH_3			Control 3 g/m^3PH_3		
	Adult	Larva	Pupa	Adult	Larva	Pupa	Adult	Larva	Pupa	Adult	Larva	Pupa
6	90.00	46.00	40.00	88.00	43.00	40.00	80.00	38.00	38.00	82.00	40.00	41.00
12	93.00	48.00	42.00	90.00	46.00	40.00	88.00	43.00	38.00	84.00	41.00	42.00
24	98.00	56.60	45.00	96.00	56.00	44.00	93.00	46.00	41.00	93.00	45.00	44.50
36	100	70.00	68.00	100	70.50	59.00	97.00	57.00	49.00	95.00	56.00	54.00
48	100	88.00	79.00	100	86.33	76.00	100	78.00	58.66	98.00	77.00	55.00
60	100	100	91.00	100	100	90.44	100	89.00	76.00	100	88.88	73.66
72	100	100	100	100	100	100	100	100	83.00	100	100	88.00
96	100	100	100	100	100	100	100	100	100	100	100	100
120	100	100	100	100	100	100	100	100	100	100	100	100
144	100	100	100	100	100	100	100	100	100	100	100	100

PH_3 in CO_2 on the life stages of *T. castaneum* is given in Table 1. The adult and larval mortality was higher in all the treatments when compared to pupa. Compared to other stages, pupal stage of *T. castaneum* was resistant and required longer exposure period. Among the different treatments, 40% CO_2 + 3 g/m^3 PH_3 and 40% CO_2 + 2 g/m^3 PH_3 were better treatments than 40% CO_2 + 1 g/m^3. Complete mortality (100%) of adults was achieved in 40% CO_2 + 3 g/m^3 and 40% CO_2 + 2 or 3 g/m^3 PH_3 with 72 h exposure period.But the larva and pupa required 96 and 120 h to achieve 100% mortality. Addition of CO_2 to PH_3 enhanced the toxicity of PH_3, resulting in quick death of insects. Ren et al. (1994) reported that when insects were exposed to greater than 20% v/v of intake of phosphine was doubled and phosphine toxicity increased with increasing concentration of CO_2 (Navarro et al., 1985).

Mortality of different stages of *S. oryzae* is given in Table 2. Rice weevil adults were killed more quickly than *T. castaneum* under the same conditions. Complete mortality of adults was obtained in the treatments, 40% CO_2 + 2 or 3 g/m^3 PH_3 with 36 h exposure period, whereas it took 72 h to kill 100% *Tribolium* adults. Larva and pupa required 60 and 72 h exposure period for the treatments with 40% CO_2 + 3 g/m^3 PH_3 and 40% CO_2 + 2 g/m^3 PH_3.

CONCLUSION

It can be concluded that PH_3 in combination with CO_2 is more effective than conventional PH_3 fumigation where no CO_2 is added. Residues of PH_3 in all the treatments including control were below detectable level. There is a need to establish ovicidal effects of PH_3-CO_2 combinations for different stored product insects.

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