Propylene oxide: a fumigant for quarantine purposes as a potential alternative to methyl bromide

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Abstract: In this study, PPO at low pressure (100 mm Hg) was tested in his study for rapid disinfestation of durable stored products as replacement for methyl bromide by evaluating its toxicity to confused flour beetle (*Tribolium castaneum* (Herbst)) in present of various commodities, its sorption and residue on various commodities. It required a dosage of 19.9, 24.1, 47.4 and 83.4 mg/l to kill 99% of the pupae of *T. castaneum* when fumigated in empty space and in presence of wheat, cacao beans and corn, respectively. Thus, much higher dose of PPO required for PPO fumigation in presence of corn and cacao beans to obtain the complete mortality of the pupa of *T. castaneum*. Sorption of PPO by corn, wheat and cacao bean after 4-h exposure time was relatively high, varying from 57% to 79% of initial concentration. The greatest sorption of PPO for 4 h exposure period was observed by corn (79%). The PPO residue in corn, cocoa bean and wheat were a maximum average of 157, 117 and 133 ppm respectively at 0-1 day after termination of aeration, which all were below the 300 ppm maximum tolerance. Based on its high and rapid toxicity to insects, its reasonable sorption by the commodities and its rapid desorption from the commodities, the combination of PPO with low pressure can be a potential as fumigant for replacing alternative methyl bromide for quarantine purposes.

Key words: Propylene oxide, quarantine fumigation, toxicity, stored product insects, sorption

Introduction

Propylene oxide (PPO) is a liquid fumigant under normal temperature pressure (NTP) with a low boiling point (35°C) and a noticeable ether odour. It is also not an ozone depleter and therefore is environmentally benign. It is a safe fumigant for use on food as a sterilant because they are quickly converted to non-toxic glycols in the stomach. Therefore, it has been used for food sterilization since 1958 and is an FDA approved fumigant to control microbial contamination on certain dry food product such as dry and shelled walnuts, cocoa powder and spices. It was also found to have potential as soil fumigants to control some soil born pathogens, nematodes and weeds. PPO has been recently demonstrated by preliminary tests to have insecticidal properties under vacuum conditions and to show potential as fumigant by killing all stages of the confused flour beetle, the Indian meal moth and the warehouse beetle. PPO is flammable from 3% to 37% volume in air and therefore, to avoid flammability it should be applied under low pressure or CO_2-enriched atmospheres. PPO effectiveness is enhanced at low pressure or in a combination with CO_2.

Some preliminary studies reported by Griffith (1999), Isikber et al. (2001) and Navarro et al. (2004) PPO has insecticidal properties under vacuum conditions as a fumigant by killing all stages of various stored product insects within a short exposure time. Zettler et al. (2002) also showed that the 8%:92% mixture of PPO and CO_2 was effective in controlling postharvest insect pests and a dose of 45 mg/l PPO at 38°C for 48 h produced complete

control of mixed life stages of various stored product insects. Methyl bromide (MB) is apparently the only fumigant available for quarantine treatment of commodities where rapid disinfestation techniques are essential. The loss of MB could have a significant negative impact on world agriculture, particularly because no available alternatives to either fumigant currently exist for rapid disinfestation of commodities. Thus, there is a critical need to develop new fumigants for quarantine purposes.

A non-ozon depletion fumigant, propylene oxide (PPO) has been considered for rapid disinfestation of durable stored products as replacement for methyl bromide (Griffith, 1999; Isikber et al., 2001; Zettler et al., 2002 and Navarro et al., 2004). In this study, PPO at low pressure (100 mm Hg) was tested for rapid disinfestation of durable stored products as replacement for methyl bromide by evaluating its toxicity to confused flour beetle (*Tribolium castaneum* (Herbst)) in present of various commodities, its sorption and residue on various commodities.

Materials and Methods

Test insects

Tests were carried out on only pupal stage of *T. castaneum*, which is known the most resistance stage to PPO. The pupae were obtained from laboratory cultures reared at 26 ± 1°C and 70 ± 5% relative humidity (r.h.) on standard cultures (Donahaye, 1990). Two days old pupae were obtained by daily separation from culture jars and held in wheat flour for 24-h before the exposure.

Commodities

Hard red winter wheat (*Triticum sp*.) at a moisture content (m.c.) of 11.2%±0.2, Grade no.2 yellow U.S.A. corn for feed (*Zea mays* L.) with m.c. of 11.8%±0.1 and Ivory Coast cocoa beans (*Theobroma cacao* L.) at m.c. of 6.3%±0.3 were used in the tests.

Fumigation chambers

Test chambers consisted of 2.64-L desiccators, each capped with a ground-glass stopper equipped with entry and exit tubing. A magnetic stirrer placed in the bottom well beneath a wire-mesh disc served to mix the air with the fumigant. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps. The desiccators were sealed with silicone vacuum grease.

The fumigant

The fumigant was 99 + % pure liquid PPO that was withdrawn from a sealed vial fitted with a rubber septum, using a gas-tight syringe. CO_2 was supplied from a cylinder and was 99 + % pure.

Dosing and fumigation procedures

Propylene oxide was introduced as a liquid into the desiccators using 50 or 250 μL gas-tight syringes. Pressure in each desiccator was measured using a 0 to 800 mm Hg Celesco, U.S.A. vacuum gauge (model SE-2000). The 100 mm Hg measure referred to herein is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Prior to each test, each desiccator was filled up with one kg of wheat, corn and cacao beans and then 50 pupae confined, separately, inside 3 cm diameter by 8 cm long wire-mesh cages were placed into the commodity. For fumigations at low pressure, prior to the introduction of the required PPO concentration, 100 mm Hg was obtained by evacuating air. PPO at 100 mm Hg was tested at

four to five dosages varying from 5 mg/liter to 82 mg/liter. Each test was replicated at least twice. The 4-h exposure was used throughout all the experiments. The gas mixtures in the desiccators were stirred for at least 20 min. For all fumigations, r.h. and temperature were maintained at 65 ± 5% at atmospheric pressure and 30 ± 1°C respectively**.**

Measurement of sorption and residue in the commodity

Each commodity weighing 1.0±0.01 kg placed into the fumigation chamber. Thereafter, the lids of each fumigation chamber were tightly closed using high vacuum silicone grease. The fumigation chambers before treatment were held for two or three hours for preconditioning of the commodities at 30°C. Sorption profiles of PPO were determined for each commodity at a dose of 82 mg/l applied over a 4 h period. The calculated volumes of PPO were introduced as a liquid into the desiccators containing the commodities by using 50 gas-tight syringes. Controls consisting of sealed, empty fumigation chambers were also dosed to determine the "chamber effect" on fumigation concentrations. All exposures were conducted at 30±2°C and 60%±10 relative humidity, ambient conditions. PPO was sampled from the free-space of each chamber to determine the decrease in fumigant concentration due to sorption.

The gas concentration of PPO was measured using a Shimadzu 17A GC fitted with an FID (Flame Ionization Detector) and an EC^{TM}–WAX capillary column (30 m length x 0.25 mm ID x 0.25 μm Film Thickness) run at 170°C isothermal. During the operation, gas flow rates were 30 mLmin^{-1}, 50 mLmin^{-1}, 500 mLmin^{-1} for helium, hydrogen and air, respectively. Temperatures were 170°C, 250°C and 260°C for column oven, injector port and detector, respectively. With these conditions, the retention time of PPO was ca. 2.65 min. The PPO residues in wheat, corn and cocoa beans were measured after 4-h fumigation at 30°C at the sole dose of 112 mg/l PPO. The levels of PPO residue on each commodity were determined at the end of the fumigation and following a 3-day aeration period. The levels of PPO residue in the commodities were determined by a commercial analytical laboratory service (Aminolab Ltd. Israel) following the analytical method that was a modification of the ASTA analytical method of the Official Methods of Analysis of the AOAC (Anonymous, 2000).

Data processing and analysis

After each treatment, the pupae were transferred to 200-mL jars containing food medium and were held at 26 ± 1°C and 70 ± 5% r.h. until examined for mortality. Mortality counts for pupae were made 9 d after exposure based on those pupae that failed to produce adults Zero dose control and dose-mortality responses were subjected to probit analysis by the POLO-PC computer program (LeOra Software 1987) to determine LD_{50}s, LD_{99}s and their respective 95% confidence intervals.

Results and Discussions

The LD_{50} and LD_{99} levels for PPO at 100 mm Hg against pupal stage of *T. castaneum* resulting from 4-h laboratory fumigations in empty space and presence of wheat, corn and cocoa beans are presented in Table 1. There was a significant difference in toxicity of PPO at 100 mm Hg against the pupae fumigated in empty space and presence of wheat, corn and cocoa beans. It required a dosage of 19.9, 24.1, 47.4 and 83.4 mg/l to kill 99% of the pupae of *T. castaneum* when fumigated in empty space and in presence of wheat, cacao beans and corn, respectively (Table 1.). The results indicated that a four-fold increase in LD_{99} value of PPO at low pressure took place when the pupae were fumigated in presence of corn as compared to those fumigated in empty space. Whereas, there was two and half-folds increase in LD_{99} value of PPO at low pressure fumigated in cacao beans as compared to those fumigated in empty

space. On the other hand, there was not much difference in LD_{99} values for PPO fumigation in presence of wheat as compared with empty space PPO fumigation. Present study indicated that much higher dose of PPO required for PPO fumigation in presence of corn and cacao beans to obtain the complete mortality of the pupa of *T. castaneum*. This could be due to a higher sorption of PPO by these corn and cacao beans. It is a well-recognized fact that a much higher dose of fumigants is required to kill an insect in a container filled with a commodity than in an empty one, owing to the sorption of the gas by the product. Thus, Punj (1969) reported that LD_{50} value of different fumigants against *T. castaneum* in presence of paddy and groundnut kernels varied from 2.7 to 7.5 times as in empty space.

Table 1. Probit analysis data for propylene oxide at low pressure of 100 mm Hg for the pupae of *T. castaneum P* resulting from 4-h laboratory fumigations at 30°C in empty space and in presence of wheat, cacao beans and corn.

Tested insect species	n[a]	Slope±SE	LD_{50} (Fiducial limit)[b] (mg/L)	LD_{99} (Fiducial limit)[b] (mg/L)	H[c]
Empty	200	19.8±3.94	16.2 (14.33 – 17.33)	21.2 (19.75 – 23.76)	0.79
Wheat	200	8.4±0.87	12.8 (11.72 – 13.81)	24.2 (21.75 – 27.99)	0.20
Corn	200	10.3±1.03	47.2 (44.01 – 50.08)	79.5 (72.99 – 89.55)	0.75
Cacao Beans	200	6.5±0.82	20.2 (18.57 – 21.70)	45.9 (39.36 - 58.96)	0.32

[a] Number treated, excluding controls.

[b] Numbers in brackets give the 95% confidence range.

[c] Heterogeneity factor, chi-square/degrees of freedom (chi-square is significant, $P < 0.01$)

Concentrations of PPO (mg/l) in fumigation chamber of 2.64 l during four hours of exposure after the application of PPO dose of 82 mg/l to 1 kg of wheat, corn and cacao beans at 30°C and 60±5% relative humidity are presented in Figure 1. In the corn and cacao beans cases, it is clear that there is an initial rapid decrease in concentrations of PPO during first 1 h exposure followed by a more gradual subsequent drop. This indicates that PPO is initially taken up faster by corn and cacao beans than by wheat. Sorption of PPO by corn, wheat and cacao bean after 4-h exposure time was relatively high, varying from 57% to 79% of initial concentration. The greatest sorption of PPO for 4 h exposure period was observed by corn (79%), whereas the wheat displayed the lowest sorption of PPO (57%), which indicates a reasonable sorption of PPO by the commodities tested at short exposure time (4-h). These findings may be compared with several studies on sorption of other fumigants by wheat, although sorption varies in extent according to the type of commodity fumigated, as well as with other factors such as the temperature during and following fumigation, moisture content, fumigation concentration, and dosage time (Banks, 1986). The results obtained by Berck (1965) indicated that at 27°C, sorption of ethylene dibromide, ethylene dichloride and carbon tetrachloride by wheat at 13.5% m.c. after 4 h exposure was less than 40%, 20% and 5% respectively. Cherif et al. (1985) reported that at 26°C, sorption of methyl bromide by wheat at 12 m.c. after 6 h exposure was less than 70 %. It appears therefore that PPO tends to be more highly sorbed by wheat than ethylene dibromide, ethylene dichloride and carbon tetrachloride, whilst it is sorbed slightly higher than methyl bromide.

The PPO residue in corn, cocoa bean and wheat were a maximum average of 157, 117 and 133 ppm respectively at 0-1 day after termination of aeration, which all was below the 300 ppm maximum tolerance determined by US FDA. A very low PPO residues ranging from 6 to 14 ppm was detected at 3 days after termination of aeration (Table 2). This data

indicate that the PPO rapidly desorbs from the commodity at conditions of NAP and 30-35 °C. Very low levels of PPO residue in the commodities indicate that PPO was physically bound to the commodity and the fumigation chamber, and did not chemically react with components of the commodity. Thus, it is clear that most of the sorption of PPO by the commodity was physical. These data also support those of Zettler et al. (2002) who showed that the PPO residues among almonds, pecans and walnuts immediately after 4-h fumigations were well below the 300 ppm tolerance and that residues could not be detected following three days aeration.

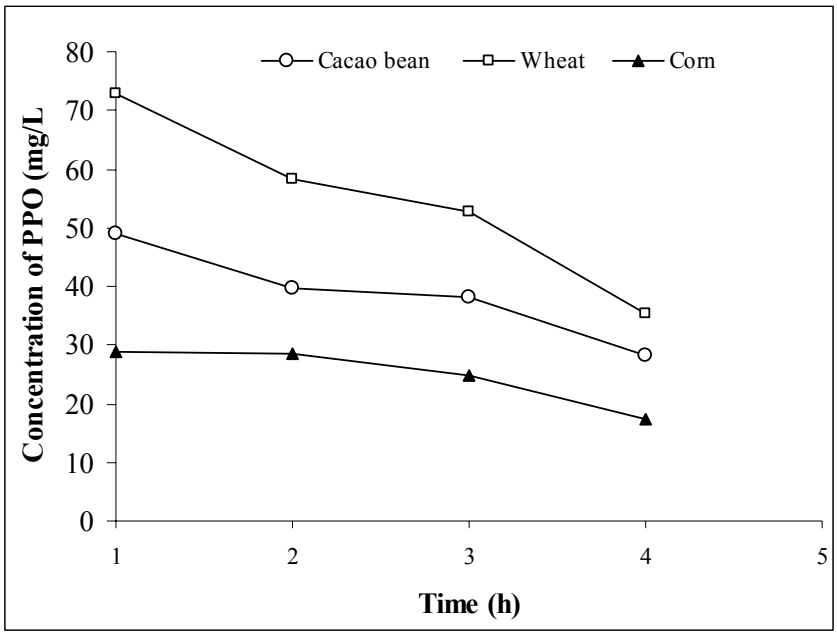

Fig. 1. Concentrations of PPO (mg/l) in fumigation chamber of 2.64 l during four hours of exposure after the application of PPO dose of 82 mg/l to 1 kg of wheat, corn and cacao beans at 30°C and 60±5% relative humidity.

Based on its high and rapid toxicity to insects, its reasonable sorption by the commodities and its rapid desorption from the commodities, the combination of PPO with low pressure can be a potential as fumigant for replacing alternative methyl bromide for quarantine purposes where rapid disinfestation of commodities is essential. However, more research is needed to obtain data on its penetration through the mass of commodities and its phytotoxicity of PPO.

Table 2. PPO residues ppm on wheat, corn and cocoa beans after 4-h fumigation at 30ºC and atmospheric pressure with a dose of 112 mg/l PPO.

Commodity	Average PPO Residue (ppm) in sample during aeration	
	Start	3 days
Wheat	133	14
Corn	157	6
Cocoa beans	117	8

Acknowledgements

We thank the Ministry of Foreign Affairs of Israel (MASHAV) and the University of Kahramanmaras Sutcu Imam Turkey, for providing the funds that enabled Dr Ali A. Isikber to participate in this study during a post-doctorate fellowship undertaken at the Agricultural Research Organization Volcani Center. This work was funded in part by a grant from the United States – Israel Science and Technology Foundation (USISTF), ARO project number 5288.

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