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# PROPYLENE OXIDE AS AN ALTERNATIVE TO METHYL BROMIDE FOR QUARANTINE PURPOSES

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## ABSTRACT

Propylene oxide (PPO) was evaluated at a low pressure of 100 mm Hg for toxicity to different life stages of the Indianmeal moth, *Plodia interpunctella* (Hübner) in the absence and presence of three species of nuts. Eggs and larvae were generally the most tolerant life stages in empty chambers and on the nut crops. Complete mortality of all life stages was achieved at a CT product of 61.2 mg h/L for empty space fumigation. Dosages of 13.9, 60.3, 72.1 and 93.1 mg/L were required to kill 99% of the larvae when fumigation of 4-h duration took place in an empty chamber and in the presence of peanuts, almonds and walnuts, respectively. After an initial dose of 68.7 mg/L and a 5-h exposure time, sorption of PPO by peanuts, almonds and walnuts was relatively high, ranging from 87% of the initial concentration for peanuts to 91% for walnuts. PPO residues measured in peanuts, almonds and walnuts were 111, 46 and 80 ppm, respectively, 1 day after termination of fumigation, all of which were below the 300 ppm maximum tolerance set by the FDA of the United States. These data show that the combination of PPO with low pressure has the potential for replacing methyl bromide fumigation for quarantine and pre-shipment purposes.

## INTRODUCTON

Different kinds of nuts are crops of great commercial importance to many exporting countries including Turkey and Israel. Nuts are very susceptible to infestation by insects during and after harvest, and subject to rancidity deterioration caused by increase in FFA (free fatty acids) levels. Insect infestation can pose a serous threat to quality control particularly the contamination caused by the Indianmeal moth, *Plodia interpunctella* (Hübner) which is both common and widespread. Until now the nut industry has relied on two chemical fumigants, namely methyl bromide (MB) and phosphine for post-harvest insect disinfestation. However, MB is being phased out under the Montreal Protocol because of its ozone depletion potential (UNEP, 1995).

Also, phosphine is under scrutiny because of carcinogenic concerns (Garry *et al.*, 1990; Alavanja *et al.,* 1990), pest resistance (Champ and Dyte, 1976; Zettler *et al.*, 1989; Zettler and Cuperus, 1990) and its requirement of long exposure periods of 5 days or longer, that make this chemical unsuitable for quarantine and pre-shipment (QPS) fumigations. Therefore, there is an urgent need to develop viable alternatives to both these fumigants.

Propylene oxide (PPO) is a liquid fumigant under normal temperature pressure (NTP) with a boiling point of 35ºC and a noticeable ether-like odor (Weast et al., 1986). As a fumigant, PPO has reduced environmental risks compared with methyl bromide. It does not deplete ozone and it degrades into nontoxic propylene glycol in the soil and in the human stomach. PPO is commonly used as a sterilant to kill bacteria, mould and yeast contamination on processed spices, cocoa and processed nutmeats except peanuts. PPO disadvantage as a fumigant is its flammability from 3% to 37% in air and therefore, to avoid combustion it should be applied under low pressure or in a CO2-enriched atmosphere.

Several studies have already been published by Creasy and Hartsell (1999), Griffith (1999), Isikber et al. (2001) and Navarro et al. (2004) that showed that under conditions of low pressure PPO kills all stages of several stored product insects (*Tribolium castaneum* (Herbst)), *P. interpunctella* and *Trogoderma variabile* Ballion) within a short exposure time. These insect toxicity studies provide an initial data-base to confirm that PPO could be an effective replacement for MB in many post-harvest situations.

The loss of MB already has a significant negative impact on the food industry, since no available alternatives currently exist for rapid disinfestation in quarantine and pre-shipment (QPS) situations. The need to develop a new fumigant for QPS purposes is critical. Consequently, the objective of this study was to evaluate the toxicity of PPO against this major pest of stored nuts, and determine its sorption and residues in walnuts peanuts and almonds.

## MATERIALS AND METHODS

***Test insects:*** Toxicity tests were carried out on all life stages of the ubiquitous stored-product pest, *Plodia interpunctella* (Hübner) (Indianmeal moth). All stages were obtained from laboratory cultures reared at 26±1ºC and 70±5% relative humidity (r.h.) using standard culture techniques (8).

***Commodities:*** In-shell almonds (Victoria variety) at a moisture content (m.c.) of 8.6±0.2%, in-shell walnuts (Hartley variety) at 10.0±0.1% m.c., and in-shell peanuts (Shulamit variety) at 9.6±0.3% m.c. were used in the tests.

***Fumigation chambers:*** Test chambers consisted of 2.64 L desiccators, each capped with a ground-glass stopper equipped with glass entry and exit tubing.

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.

***Dosing and fumigation procedures:*** For fumigations at low pressure, the insects were first placed in the empty desiccators, and then 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 ranging from 1 mg/L to 20 mg/L. Each test was replicated at least twice. A 4-h exposure time was used throughout the experiments. For all fumigations, r.h. and temperature were maintained at 65±5% r.h. at atmospheric pressure and 30±1ºC respectively.

For PPO fumigation in the presence of the commodities each desiccator was loaded separately with one kg of almonds, walnuts and peanuts and then 50 larvae, confined, separately, inside the wire-mesh cages were inserted into the commodity.

***Measurement of sorption and residues in the commodities:*** Each commodity weighing 1.0±0.01 kg was placed separately inside a fumigation chamber. Sorption profiles of PPO were determined for each commodity at a dose of 68.7 mg/L applied over a 5 h exposure period. The gas concentration of PPO was measured using a Shimadzu 17A GC fitted with an FID (Flame Ionization Detector) and an ECTM–WAX capillary column (30 m length x 0.25 mm ID x 0.25 µm Film Thickness). The PPO residues in peanuts, almonds and walnuts were measured after 5 h. fumigation at 30ºC at the sole dose of 112 mg/L PPO. Residue levels in each commodity were determined at the end of fumigation and following a 3 d aeration period. The residue levels in the commodities were determined by a commercial analytical laboratory service (Aminolab Ltd. Israel) following an 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:*** Zero dose control and dose-mortality responses were subjected to probit analysis by the POLO-PC computer program to determine $LC_{50}$s, $LC_{99}$s and their respective 95% confidence intervals. Required concentrations x time (Ct) products to obtain 50% and 99% mortality of all insect stages of each insect were calculated using the $LC_{50}$ and $LC_{99}$ concentrations derived from probit analyses.

## RESULTS AND DISCUSSIONS

PPO under 100 mm Hg was toxic to all life stages of *P. interpunctella.* Eggs and larvae of *P. interpunctella* by $LC_{99}$ values of 15.3 and 13.9 mg/liter respectively were more tolerant than the adults and pupae by $LC_{99}$ values of 5.9 and 8.8 mg/liter, respectively (Table 1). The complete mortality of all life stages of *P. interpunctella* was achieved at a Ct product of 61.2 mg/liter/h. It required dosages of 13.9, 93.1, 60.3 and 72.1 mg/l to kill 99% of the larvae of *P. interpunctella* when fumigated in

an empty desiccator and in the presence of walnuts, peanuts and almonds, respectively (Table 2). The results indicated that there was a six and half-fold increase in $LC_{99}$ value of PPO at low pressure when the larvae were fumigated in the presence of walnuts as compared to those fumigated in the empty space. Similarly, there was a four to five-fold increase in the $LC_{99}$ value of PPO at low pressure for fumigation in peanuts and almonds as compared to fumigation in the empty desiccators. Thus, the present study indicates that a much higher dose of PPO is required for fumigation in the presence of walnuts, peanuts and almonds to obtain complete mortality of the larvae of *P. interpunctella.*

TABLE 1
Toxicity of PPO (mg/litre) and C x t products (mg h/litre) for all life stages of *P. interpunctella*

| **Life stage** | **n[a]** | **Slope[b]±SE** | **$LD_{50}$ (Fiducial limit)[c] (mg/liter)** | **$LD_{99}$ (Fiducial limit)[c] (mg/liter)** | **H[d]** | **Ct product for $LD_{99}$ (mg h/liter)** |
|---|---|---|---|---|---|---|
| Egg | 1000 | 6.4±0.66 | 6.7 (6.03 – 7.25) | 15.3 (13.42 – 18.48) | 0.75 | 61.2 |
| Larva | 236 | 9.1±1.52 | 7.7 (7.10 – 8.38) | 13.9 (11.89 – 18.59) | 0.26 | 55.6 |
| Pupa | 276 | 12.3±2.65 | 5.7 (5.01– 6.25) | 8.8 (7.69 – 11.79) | 0.70 | 35.2 |
| Adult | 202 | 4.7±0.71 | 1.9 (1.58 - 2.22) | 5.9 (4.41 - 9.72) | 0.89 | 23.6 |

[a] Number treated, excluding controls.
[b] Slopes among life stages of tested insect are unparallel and unequal where noted.
[c] Numbers in brackets give the 95% confidence range.
[d] Heterogeneity factor, chi-square/degrees of freedom (chi-square is significant, $P < 0.05$)

Sorption of PPO by walnuts, peanuts and almonds after a 4-h exposure time was very high, ranging from 87% to 91% of the initial concentration (Figure 2). In all cases, there was an initial rapid decrease in concentrations of PPO during the first hour of exposure followed by a more gradual subsequent drop. The drop in concentrations during the first hour for walnuts was 86% of the initial dosage applied, while that for peanuts and almonds, was from 77% to 82% of the initial dosage applied indicating a rapid sorption of PPO by all three species of nut.

The PPO residues in walnuts, peanuts and almonds were a maximum average of 80, 111 and 46 ppm respectively at 0-1 day after termination of aeration (Table 3). These were all below the maximum tolerance of 300 ppm. Very low PPO residues ranging from <2 to 26 ppm were detected at 3 days after termination of aeration. These data

indicate that the PPO rapidly desorbs from the commodity under conditions of NAP and 30-35ºC.

TABLE 2
Toxicity of propylene oxide at 100 mm Hg for the larvae of *P. interpunctella* in presence of 0.5 Kg of peanuts, almonds, walnuts, or in empty space

| **Treatments** | **n**[a] | **Slope**[b]**±SE** | **$LD_{50}$ (Fiducial limit)**[c] **(mg/L)** | **$LD_{99}$ (Fiducial limit) (mg/L)** | **H**[d] |
|---|---|---|---|---|---|
| PPO at 100 mm Hg with Peanut | 240 | 21.2±3.16 | 46.8 (44.70 – 48.53) | 60.3 (56.95 – 66.25) | 0.51 |
| PPO at 100 mm Hg with Almond | 238 | 19.4±3.38 | 54.7 (52.12 – 57.01) | 72.1 (67.09 – 82.86) | 0.37 |
| PPO at 100 mm Hg with Walnut | 240 | 19.8±3.77 | 71.0 (67.84– 73.66) | 93.1 (86.67 – 107.60) | 0.03 |
| PPO at 100 mm Hg with Empty Space | 236 | 9.1±1.52 | 7.7 (7.10 – 8.38) | 13.9 (11.89 – 18.59) | 0.26 |

[a] Number treated, excluding controls.
[b] Slopes among life stages of tested insect are unparallel and unequal where noted.
[c] Numbers in brackets give the 95% confidence range.
[d] Heterogeneity factor, chi-square/degrees of freedom (chi-square is significant, $P < 0.05$)

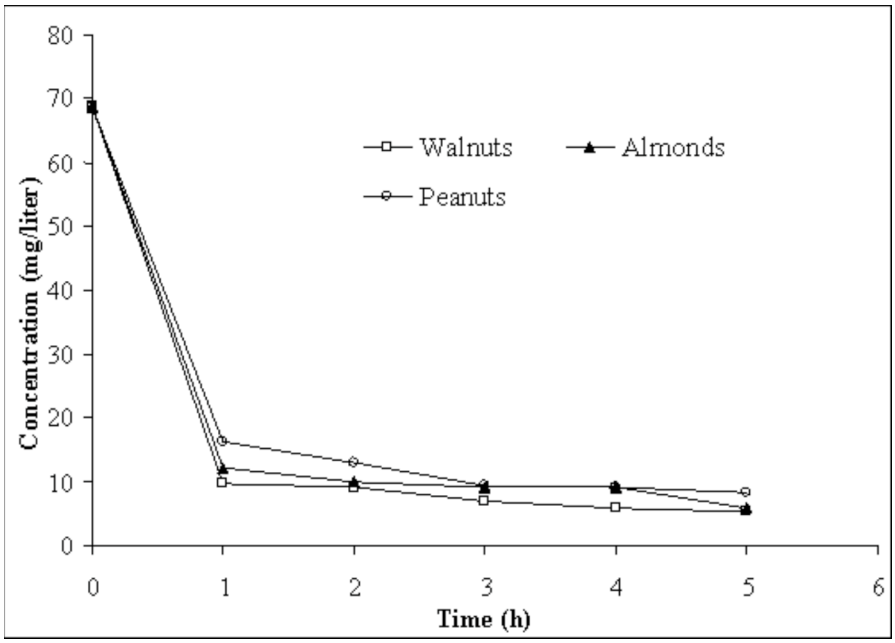

**Figure 1.** Change in concentration of PPO during 4 h fumigation of peanuts, almonds and walnuts, at an initial dosage of 68.7 mg/litre.

TABLE 3
PPO residue level (ppm) in peanuts, almonds and walnuts.

| Commodity | Average PPO Residue (ppm) in sample during aeration | |
|---|---|---|
| | 0-1 day | 3 days |
| Walnuts | 80 | 26 |
| Almonds | 46 | <2 |
| Peanuts | 111 | 8 |

## CONCLUSION

Although sorption of PPO by the nuts tested was relatively high, the fumigation still enables a sufficient build up of gas concentrations to achieve insect mortality. Based on its high and rapid toxicity to insects, and its rapid desorption from the commodities, the combination of PPO with low pressure can become a potential fumigant for replacement of MB for quarantine purposes where rapid disinfestation of the nuts is essential.

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