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Comparative practical application of two novel candidate fumigants

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ABSTRACT

In search for methyl bromide alternatives along with the wide use of phosphine, which its efficacy is in decrease due to resistance development of stored-products insects and the need for longer exposure times, two candidate fumigants have been tested against stored-products insects. Both fumigants have the property to act within short exposure times on storage insects, comparable to methyl bromide. Both fumigants were tested in: (*a*) laboratory scale trials on 2.85 L desiccators, (*b*) semi-commercial scale trials in 1.5 m^3 PE laminated envelopes, and (*c*) commercial trials scale trials on 8 m^3 PE laminated envelopes or woven PVC cocoons. The PE laminated envelopes and the PVC cocoons are suitable for fumigation of stored commodities, can serve as flexible fumigation chambers, and have low fumigant permeability. Ethyl formate (EtF) was tested for 12 h exposure time on the larval stage of *Carpophilus hemipterus* (L.) and *Plodia interpunctella* (Hubner). Propylene oxide (PPO) was tested for 24 h exposure time on adult stage of *Oryzaephilus surinamensis* (L.), *Rhyzopertha dominica* (Fabricus) and *Tribolium castaneum* (Herbst). Since both fumigants are flammable and liquid at room temperature, both of the fumigants were applied using an evaporator device which mixes the gas with the CO_2 on site. Recommended dosage of EtF was 67.2 g m^{-3} in 352.8 g $m^{-3}CO_2$ and that of PPO was 35 g m^{-3}in 437.5 g $m^{-3}CO_2$ were tested. Results show 100% mortality of all tested insects in all trials. Toxicity in LD_{50} values for mammals of both fumigants, compared to phosphine, is higher. EtF is easier for handling because of its higher boiling point than PPO.

Key words: Ethyl formate, Fumigation, PE laminated envelopes, Propylene oxide, Stored-products insects

The use of methyl bromide (MB), after its phase out in 2015, is only permitted globally for quarantine and pre-shipment (QPS) treatments. In Israel and in most parts of the world, the only alternative fumigant for treatment of post-harvest of durables remaining is phosphine. However, phosphine is characterized by the long exposure time needed for successful control and the emerging resistance of insects to the fumigant (UNEP, 2006). For example, in China, Daulin et al. (2007) reported on a resistance survey carried out in 2002, resistant strains to phosphine were detected in 42% of all surveyed samples. The most resistant *Sitophilus oryzae* (L.) strain had a resistance level to PH_3 of x305, and for *Rhyzopertha dominica* (Fabricius) it was x315 (Daulin et al., 2007).

Cellular membranes of insects act as a sieve, and prevent the phosphine molecules from entering the cell. Resistance to phosphine is probably due to reduced uptake or active exclusion of phosphine through the cell membranes. Heritable genetic factors have been shown associated with phosphine in some insects, thus it is clear that selection for resistance by inadequate treatment could occur. Under-dosing and especially insufficient exposure periods over many years are the likely reason for the resistance to phosphine observed in some strains of stored product pests (UNEP, 2006). The potential of stored-product pests to develop phosphine resistance is a severe concern as reported by many researchers (Pimentel et al., 2008).Thus, biological and operational factors contribute to the rate of resistance development in insect (UNEP, 2006).

The global concern from the introduction of new pests or new resistant strains of known grain storage insect pest along with the phase out of MB increased the interest for additional alternatives—a fumigant that will be user friendly to environment, effective,

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rapid in action and at the same time should be on an acceptable cost basis. Therefore, two fumigants of choice considered were ethyl formate (EtF) and propylene oxide (PPO).

Propylene oxide is an organic compound which is a colourless volatile liquid that is produced on a large scale industrially for the production of polyether polyols used in making polyurethane plastics. PPO boils at 35°C and is a liquid at normal temperature and pressure. The United States Food and Drug Administration has approved the use of PPO to sterilize raw almonds [*Prunus dulcis* (Mill.) D.A.] (beginning on 1 September 2007, in response to two incidents of contamination by *Salmonella* in commercial orchards, one incident occurring in Canada and the other in the United States. Pistachio nuts (*Pistacia vera* L.) can also be subjected to PPO to control *Salmonella*. It is a safe fumigant for use on food as a sterilant because it is quickly converted to non-toxic glycols in the stomach. Therefore, it has been used for food sterilization since 1958 (Griffith, 1999). It is also not an ozone depleter and is environmentally benign. However, PPO is flammable from 2.3% to 38.5% by volume in air. Elimination of the flammability hazard of PPO can be achieved by applying it under low pressure or in an inert gas enriched atmosphere. Efficacy of PPO against stored-product pests has been tested in laboratory scale both by Navarro et al. (2004) and Hartsell et al. (2001) which showed a greater efficacy on all life stages of different stored-product pest species under either 92% CO_2 or under 100 mm Hg than with PPO by itself. Its insecticidal properties under low pressure by killing all stages of the confused flour beetle (*Tribolium confusum* Jacquelin du Val) (Navarro et al. 2004), the Indian meal moth [*Plodia interpunctella* (Hubner)] and the warehouse beetle (*Trogoderma variabile* Everts) at concentrations as low as 100 g of PPO m^3 (Griffith, 1999) has been demonstrated. Also, Isikber et al. (2004) showed an efficacy of 26.1 g m^{-3} to control four main stored-product species under low pressure in only 4 h exposure.

Today, the Dried Fruit Association is using PPO as a sterilant in the dried fruit industry in the USA. PPO is an FDA approved fumigant to control microbial contamination in dry and shelled walnuts, cocoa (*Theobroma cacao* L.) powder and spices.

Another candidate fumigant is EtF which occurs naturally in soil, water, vegetation such as orange [*Citrus* × *sinensis* (L.) Osbeck] juice, honey, apples (*Malus pumila* miller), pears (*Pyrus* sp.) and wine and a range of raw and processed foods (from 0.05 to 1 mg kg^{-1}). It is known to breakdown into naturally occurring products — formic acid and ethanol (Ducom, 2010).It is used as a synthetic flavouring agent in the food industry and as fragrances; it is also a GRAS registered food additive in some countries (Ducom, 2010). It decomposes slowly in water, releasing formic acid and ethanol. Laboratory tests as a fumigant against insect pests of food commodities and field trials on bagged cereals, spices, pulses, dry fruits and oilcakes have been carried out (Muthu et al., 1984).

The EtF has been registered in Israel as the product Vapormate™, which is a low human risk fumigant formulated by BOC Australia, a member of the Linde Group, and contains 16.7 wt % EtF in liquid CO_2 (Ryan and Bishop, 2003). The CO_2 in Vapormate™ has been added to eliminate the flammability of the EtF and to enhance efficacy by its synergistic effect in reducing the time required to kill insects (Haritos et al., 2006). However, shipping and handling of the cylinders makes the fumigation very costly.

The aim of this work was to examine efficacy of two flammable fumigants at their liquid phase with CO_2 to reduce their flammability and to increase their effectiveness in controlling stored product pests.

MATERIALS AND METHODS

All test insects were reared at Green Storage Ltd. laboratory at 30±1ºC and 70±2% r.h. Test insect species were: adults of *Rhyzopertha dominica* (Fabricius), *Tribolium castaneum* (Herbst), and *Oryzaephilus surinamensis* (L.) for the PPO tests. Larva of *Carpophilus hemipterus* (L.) and *Plodia interpunctella* were exposed to EtF. In addition, the efficacy of the fumigant was tested compared to the standard fumigant phosphine for 5 d exposure time. Phosphine gas was produced at the laboratory and was injected to the desiccator at a dosage of 2 mg L^{-1}. Gas measurements were taken at the beginning and at the end of the exposure time.

There was only one set of bioassay with one point of gas measurement in the laboratory trials. In the semi-commercial trials there was one measurement point but with three sets of bioassay; at the bottom, middle and top of the big bag as well as at the commercial scale trial. In the commercial scale trials, only two points of gas measurement were taken, at the bottom and top.

Laboratory trials

PPO fumigation: Adults of *R. dominica*, *T. castaneum* and *O. surinamensis* were placed into 22 mL glass vials together with about 3 g ground wheat (*Triticum* sp.). Each glass vial together with the test insects were placed into a 2.85 L gas-tight desiccator used as a fumigation chamber. Each desiccator contained 500 g polished rice (*Oryza sativa* L.) (11.5%

m.c.). The fumigant was introduced into the desiccators to achieve concentration of 35 g m^{-3} at 30±1ºC for 24 h. The CO_2 was first introduced using a gas syringe. The fumigant was introduced at its liquid phase using a syringe from a Aberco Ltd. (USA) PPO bottle. Gas calculations were made according to the barometric pressure of the day; the amount of gas which was introduced into the desiccator was first vacuumed from the air inside the desiccator. At the end of the exposure time, the glass vials with the test insects were removed from the fumigation chamber and placed in an incubator at 30±1ºC for 24 h before mortality counts were made. Gas measurements were taken using PPO ampoules (163SA 0.05–5.0% Sensidyne FL, USA made) for every three test replicates.

EtF fumigation: Larva of *C. hemipterus* and *P. interpunctella* were placed into 22 mL glass vials together with about 0.5 g of date (*Phoenix dactylifera* L.) fruit. Each glass vial together with the test insects were placed into a 2.85 L gas-tight desiccators, used as a fumigation chamber. The fumigant was introduced into the desiccator to achieve concentration of 67.2 g m^{-3} at 30±1ºC for 12 h. The gas was introduced at its liquid phase using a syringe from a Sigma-Aldritch Ltd. bottle. Gas calculations were made according to the barometric pressure of the day; the amount of gas which was introduced into the desiccator was first vacuumed from the air inside the desiccator. At the end of the exposure time, the glass vials with the test insects were removed from the fumigation chamber and placed in an incubator at 30±1ºC for 24 h before mortality counts were made. Gas measurements were taken using EtF monitoring device (G460model, GFG Europe Ltd., UK) for every three test replicates.

Semi-commercial and commercial trials

Three semi-commercial and three commercial trials were carried out using 1.5 and 8–10 m^3, accordingly, on flexible fumigation chambers to test the efficacy of the fumigants. Test insects were placed at the bottom, middle and top of each fumigation chamber. Approximately 30 individuals of each insect species were placed in 22 mL vials with its suitable food. The fumigants were introduced using a special evaporating machine designed to mix the fumigant with CO_2 on site. After pouring the calculated dosage, the $CO_2$was allowed to evaporate the fumigant into the flexible fumigation chamber. At the end of each fumigation process and before end of exposure time, fumigant's concentrations were taken. Only at the commercial scale two points of gas measurements were taken, at the bottom and top. At the end of the exposure time, the glass vials with the test insects were removed from the fumigation chamber and placed in an incubator at 30±1ºC for 24 h before mortality counts were made.

At the semi-commercial trials, there was one measurement point but with three sets of bioassay; at the bottom, middle and top of the big bag as well as at the commercial scale trial.

Propylene oxide fumigation: Adults of *R. dominica*, *T. castaneum* and *O. surinamensis* were placed into 22 mL glass vials together with about 3 g ground wheat. Each glass vial together with the test insects were placed into either 1.5 m^3 GrainPro Inc. product laminated PE made fumigation bubble containing approximately 1 tonne of polished rice or 8 tonnes of polished rice in a 9.8 m^3 capacity PVC made flexible fumigation chamber. The fumigant was introduced into the bubble/flexible fumigation chamber to achieve concentration of 35 g m^{-3}along with 437.5 g m^{-3} CO_2at 30±1ºC for 24 h.

Ethyl formate fumigation: Larva of *C. hemipterus* and *P. interpunctella* were placed into 22 mL glass vials together with about 0.5 g of date fruit. Each glass vial together with the test insects were placed into either 1.5 m^3 laminated PE made fumigation bubble containing approximately 1 tonne of 'Medjool' dates or 8 tonnes of 'Deglet Noor' dates in a 8 m^3 capacity laminated PE made flexible fumigation chamber. The fumigant was introduced into the bubble to achieve concentration of 67.2 g m^{-3} along with 352.8 g m^{-3} CO_2at 30±1ºC for 12 h.

RESULTS AND DISCUSSION

Propylene oxide fumigation: Table 1 shows the summary of the tests which were carried out on laboratory, semi-commercial and commercial scale trials to test the efficacy of the fumigant propylene oxide (PPO) on *T. castaneum, R. dominica* and *O. surinamensis* adults for 24 h exposure on polished rice. The data shown is the average of three replicates in each trial. Gas concentrations were measured using test tubes for PPO. In addition, since the goal was 8% PPO with 92% CO_2, measurements of CO_2 were carried out as well. Gas distribution at the beginning of the exposure time was uneven because stratification created. The bottom layer had most of the gas while the upper layer had significantly less. With time, distribution was achieved and, thus, at the end of exposure time the gas concentration was more uniform. The total time of gas flow was 1–1:30 min for the 1.5 m^3 and 10–12 min for the 9.8 m^3 fumigation cubes.

Phosphine exposure time was 5 d using phosphine generated from UPL tablets. Average temperature of the commodity at the semi-commercial and commercial scale trials was 22±3˚C.

Ethyl formate fumigation:

Table 2 shows the summary of the tests which were carried out at laboratory, semi-commercial and commercial scale trials to test the efficacy of the fumigant ethyl formate (EtF) on *P. interpunctella* and *C. hemipterus* larva for 12 h exposure on dates. The data shown are the average of three replicates in each trial. Gas concentrations were measured using EtF monitoring device which measuresV/V and converted to gm^{-3}. In addition, since the goal is 16% EtF with 84% CO_2, measurements of CO_2 were carried out as well. Gas distribution at the beginning of the exposure time was uneven and stratification was observed. Therefore, the concentration of EtF at the beginning of exposure time was the applied dosage.The bottom layer holds most of the gas while the upper layer has significantly less. Within time it is distributed, thus, at the end of exposure time it was more uniform. The total time of gas flow was 1–1:30 min for the 1.5 m^3 and 12 min for the 8 m^3 fumigation cubes.

Many studies have been done testing the efficacy of both fumigants (Griffith, 1999; Annis and Graver, 2000; Navarro et al., 2004; Isikber et al., 2004; Finkelman et al., 2012) and as shown in Tables 1 and 2, in all trials we managed to obtain complete mortality of adults and larva of the different pests in short exposure times of 12 h for EtF and 24 h for PPO. In this work, laboratory trials were carried out for registration purposes, to avoid their flammability, first the CO_2 was introduced then the fumigants were injected at their liquid phase. On semi-commercial and commercial scale, the fumigant was applied through a heating unit to enable evaporation of the gas, thus to obtain adequate distribution. None of these works discussed the mode of application of the fumigants at commercial scale using liquid fumigant except the work carried out by Ren and Mahon (2006) on 125 tonnes grain bin with liquid EtF.

The common practice of PPO today is in vacuum chambers where the pressure is lowered to 100 mm Hg then the fumigant is introduced (Aberco Inc., USA). Use of vacuum chambers for insect control makes the

Table 1 Average of propylene oxide (PPO) concentration (gm^{-3}), CO_2(%) and mortality (%) of *Tribolium castaneum, Rhyzopertha dominica* and *O. surinamensis* adults on laboratory, semi-commercial and commercial scale trials of PPO for 24 h exposure on polished rice

Trial		*O. surinamensis*	*R. dominica*	*T. castaneum*	PPO T_0 (gm^{-3})	PPO T_{24} (gm^{-3})	CO_2T_0 (%)		CO_2T_{24} (%)	
							Top	Bottom	Top	Bottom
Laboratory	PPO T	100.00	100.00	100.00	34.60	18.23	45.33		42.33	
	PPO C	8.33	3.33	5.00						
	PH_3 T	100.00	100.00	100.00	1,497.33*	1,079.33*				
	PH_3 C	6.45	3.33	30.00						
Semi-commercial	PPO T	100.00	100.00	100.00	36.75	26.46	95.67		70.66	
	PPO C	7.03	9.71	25.28						
Commercial	PPO T	100.00	100.00	100.00	35.00	19.20	3.4	81.3	44.83	47.6
	PPO C	1.66	21.41							

*, PH_3 concentration; C, control; T, treatment

Table 2 Average of ethyl formate (Etf) concentration (gm^{-3}), CO_2(%) and mortality (%) of *Plodia interpunctella* and *Carpohilus hemipterus* larva on laboratory, semi-commercial and commercial scale trials of EtF for 12 h exposure on dates

Trial		*C. hemipterus*	*P. interpunctella*	$EtFT_0$ (gm^{-3})	$EtFT_{12}$ (gm^{-3})	CO_2T_0 (%)		CO_2T_{24} (%)	
						Top	Bottom	Top	Bottom
Laboratory	EtF T	100.00	100.00	67.2	66.6	21.25		21.50	
	EtF C	3.33	8.33						
Semi-commercial	EtF T	100.00	100.00	70.0	34.53	1.23	51.00	12.33	13.00
	EtF C	6.06	5.00						
Commercial	EtF T	100.00	100.00	70.0	30.29	4.10	80.50	24.00	22.50
	EtF T	4.55	4.17						

treatment very expensive which may not be afforded in many cases.

In atmospheric pressure fumigation chambers, as in all the trials carried out in this work, we used flexible fumigation chambers. First step to ensure gas tightness of the structure is to have the half time pressure decay test in which could hold at least 5 min (Navarro, 1998). Then the gas injection point needs to be close to floor level. During gas injection, excess air needs to be expelled from the chamber. This air needs to be vented outside to atmosphere through at least 160 mm diameter vent pipe in the ceiling at the opposite end of the chamber. Approximately 25% of the chamber air will need to be vented out during gas injection due to the expansion of the gaseous fumigant and carbon dioxide. Gas Apps Australia P/L recommends when gas injection is complete, vent needs to be switched off and a circulation fan needs to be run for 1 h. in all these trials, since we dealt with small volume that did not exceed 10 m^3, we allowed convection currents to distribute gas, as seen from Tables 1 and 2.

Although USA's PPO Label allows the use of cylindered PPO, its use is extremely limited. Today PPO is applied extensively under vacuum in the USA. The same limitation is relevant to EtF which is applied at large scale only from cylinders. In both methods, costs are high; the vacuum chamber is expensive, also the shipping and handling of the cylinders of both fumigants are expensive.

Although this work on PPO had been carried out on rice, it should be noted that there is no MRL established for rice. On the other hand, EtF has MRL's for many products. It is also regarded by some registration authorities as GRAS, and has higher boiling temperature which is preferable to work with.

CONCLUSION

EtF and PPO serve as good alternative candidates for phosphine fumigation. They are highly effective fumigants which act in short exposure times of 12 to 24 h but are flammable and need to be applied with an inert gas. When treating large volumes, to avoid stratification of the gas, a fan must be installed for the first h of exposure time. MRL levels of PPO need to be determined for cereals and grains.

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