New prospects for ethyl formate as a fumigant for the date industry

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Abstract

Date infestation of nitidulid beetles poses a serious contamination problem for which methyl bromide (MB) provided a solution. However, because of the phase out of MB, alternatives were investigated. Thermal disinfestation method has been successfully applied to some dry date varieties except to Deglet-Noor, Zahidi, and Ameri which are handled in crates of 200 kg to 400 kg. Therefore, thermal disinfestation was not successful because of delayed heating due to the resistance of the dates to hot airflow. The fumigant formulation Vapormate™ was tested as alternative to MB for the disinfestation (proportion of insects found outside the feeding sites) and control of nitidulid beetles from artificial feeding sites at laboratory and for dates in crates at semi-commercial conditions. Vapormate™ contains 16.7% ethyl formate mixed with carbon dioxide. At laboratory conditions the effect of various dosages of Vapormate™ was tested at 30°C and at fixed exposure time of 12 h. Exposure of infested artificial feeding sites by larvae of *Carpophilus* spp. to the concentration of 280 g m^{-3} of Vapormate™ caused 69.3% disinfestation and 79.9% mortality, 350 g m^{-3} resulted in 72.7% disinfestation and 98.8% of mortality and the optimal results were obtained at 420 g m^{-3} that caused 69.6% disinfestations and 100% mortality.

Commercial pilot-plant tests were carried out by applying 420 g m^{-3} Vapormate™ for 12 h in a 9 m^3 flexible liner made of laminate composed of polypropylene/aluminum/polyethylene to cover crates containing infested dates. Disinfestation was tested on naturally infested dates that resulted in an average 100% disinfestation and 95% mortality, while with the artificially infested dates, disinfestation was 97% and mortality 96%. In a second series of tests, a commercial rigid fumigation chamber of 95.6 m^3 was used. After 12 h exposure, 100% mortality was recorded in all date samples. Following the promising results, Vapormate™ was registered in Israel for use by the date industry as an alternative to MB.

Keywords: Date, Nitidulid beetles, Ethyl formate, Vapormate™, Fumigation.

1. Introduction

Field infestations of nitidulid beetles pose a serious contamination problem of dates that requires their treatment immediately after harvest (Navarro, 2006). Until now, this problem has been addressed successfully using Methyl bromide (MB) because it causes a high proportion of larvae and adults to emigrate from the fruit before they succumb. This emigration phenomenon is associated with the disinfestation effect that leaves the fruit free from insect presence and more important than the toxic effect of the treatment. Since MB, under the terms of the Montreal Protocol, was phased out in 2005 the date industry needed to find alternative technologies to MB. Over the last two years the Israeli date industry has adopted thermal disinfestation to control nitidulid beetles infestation (Finkelman et al., 2006; Navarro et al., 2003; 2004; Navarro, 2006). A constant hot airflow can be achieved only when the dates are handled in trays of 3 kg or boxes containing 13 kg. However, date varieties Deglet-Noor, Zahidi, and Ameri are handled in two types of crates each containing 200 kg or 400 kg dates for which thermal disinfestations cannot be implemented. An option considered was the use of ethyl formate (EF) in its commercial form known as Vapormate™ in existing fumigation chambers. Ethyl formate occurs naturally in orange juice, honey, apples, pears and wine. It is used as a synthetic flavoring agent in the food industry and as fragrances; it is also a GRAS registered food additive. 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). Ethyl formate is currently registered as a fumigant in Australia as ERANOL® by Orica Chemnet for the elimination of insect pests in packed dried fruits like raisins. It is toxic to storage

insects including psocids (Annis et al., 2000). Vapormate™ is a low human risk fumigant formulated by BOC Australia, a member of the Linde Group, and contains 16.7 wt% EF in liquid carbon dioxide (CO_2) (Ryan and Bishop, 2003). The CO_2 in Vapormate™ has been added to eliminate the flammability of the EF and to enhance efficacy by its synergistic effect in reducing the time required to kill insects (Haritos et al., 2006). Vapormate™ is now fully registered for use in grain and horticultural products in Australia, in New Zealand for use in grain and for quarantine treatment of bananas (Krishna et al., 2002) and in Israel for dates and stored grains.

2. Materials and methods

2.1. Test insects

Carpophilus hemipterus (L.) (Coleoptera; Nitudilidae) and *C. maculatus* (Murray) were reared on media described by Donahaye and Navarro (1989) in an incubator at 30±1°C. To test infested dates, mixed populations of larvae from both species obtained from the field were incubated at 30°C for about 1 month.

2.2. Laboratory experiments

2.2.1. Evaluation of emigration from artificial feeding sites

The effectiveness of Vapormate™ in causing emigration of *Carpophilus spp*. larvae from artificial feeding sites was tested (Donahaye et al., 1992). The proportion of insects found outside the feeding sites was used to measure response and termed as percent of disinfestation. The artificial feeding sites consisted of cardboard rectangles placed on media in Petri dishes. Circles (9.5-cm diameter) were cut from polyethylene film (0.1-mm thickness) and these were used to line the lids of 9-cm-diameter plastic Petri dishes. The food medium was reheated and diluted as required to obtain a consistency at which it could be poured over the polyethylene to form a layer 5-mm deep. Cardboard rectangles (2 by 4 cm) were placed on top of the food medium, four rectangles to each Petri dish. After the media was solidified, the Petri dishes were opened and 30 larvae (4-5 d old) were introduced, so that each rectangle contained food medium bounded on its upper surface by cardboard and on its lower surface by polyethylene film. The proportion of larvae in feeding sites beneath the cardboard averaged 46% of the number placed in the Petri dishes 24 h earlier. Each feeding site generally held two to four larvae.

Humidity of the microenvironment within the desiccators ranged from 70 to 80% r.h. as determined by a humidity sensor (Hydrolog-D, Rotronic Instrument Ltd., Crawley, West Sussex, UK). Larvae in feeding sites were exposed to different treatments in 2.54-L desiccators. Each treatment was exposed at 30±1°C for 12 h to a series of doses of 280, 350, and 420 mg L^{-1} of Vapormate™ supplied from a pressurized cylinder (83% CO_2 and 17% EF w/w). In addition, the effect of 420 g m^{-3} and 12 h exposure to Vapormate™ on larvae emigration and mortality was studied at: 16ºC, 18ºC, 24ºC, 26ºC and 28ºC. Dosage calculations were converted to the gaseous phase and the required volume of Vapormate™ was obtained by evacuating the desiccator to the desired pressure, followed by restoration of atmospheric pressure using Vapormate™ supplied from a pressurized cylinder. The desired pressure was first calculated by converting the dose into a percentage of the desiccator volume to be treated, then desiccator was evacuated to the desired absolute pressure using a laboratory vacuum pump and the pressure measured using a portable transducer manometer (SE-2000, Celesco, Chatsworth, CA, USA), and then the equivalent to the partial pressure of in air was supplied by restoration of atmospheric pressure using the Vapormate™ supplied from the pressurized cylinder. The same process of evacuating to the desired pressure was carried out in the control desiccator but instead of the gas mixture, the pressure was restored using ambient air at atmospheric pressure. Before treatment, the desiccators were cleaned of any external infestation and the feeding sites were placed separately on the wire mesh floor. Each desiccator was loaded with ten feeding sites taken at random from the supply of feeding sites and during exposure were held in the dark 30±1°C. Upon completion of the exposure period they were removed from the desiccators and the larvae (dead or alive) present on the surface of the feeding sites and at the base of the desiccators were counted. Each feeding site was then opened length-wise using a scalpel and the adults and larvae (dead or alive) still present in each feeding site were counted. Each treatment was replicated four times and for each set of experiments, a control desiccator was exposed to the normal atmosphere for the same time period.

2.2.2. Egg mortality tests

Eggs were obtained by allowing 30 adult *C. hemipterus* to lay eggs over 24 h in the crevice between a microscope cover glass and glass slide formed by gluing them with a paper between both slides (Fig. 1). Eggs were exposed to 420 mg L^{-1} of Vapormate™ in 2.54-L desiccators for 12 h as described above. Mortality counts were made after 3 d and compared with the control group kept under the same temperature and humidity.

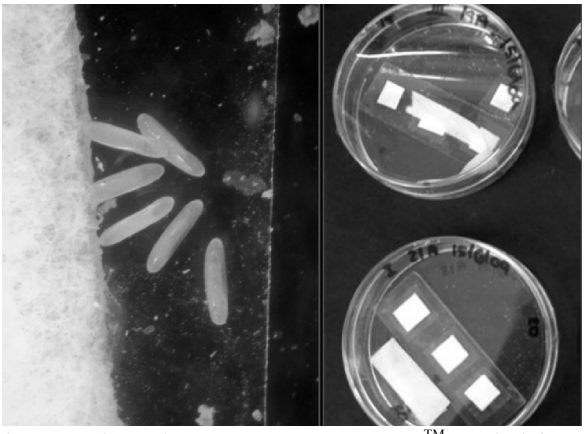

Figure 1 Experimental test exposing *C. hemipterus* eggs to Vapormate™: On the left: eggs under a cover slide. On the right: Slides with eggs placed in Petri dishes ready for exposure to the fumigant.

2.3. Commercial scale pilot trials on date fumigation

2.3.1. Date fumigation in portable flexible chamber

Four pallets containing Halawy variety dates were used for fumigation. Each pallet contained 20 rows and five columns of trays 60 x 40 x10 cm high. Each tray contained about 3 kg dates. Laboratory infested dates (50 fruits in each tray) were placed in pallets 1 and 4 at three layers (top, middle and bottom). Similarly field infested trays were marked in pallets 2 and 3.

The proportion of insects found after fumigation outside the dates and at the trays located below the test trays containing 50 laboratory-infested dates was used to measure percent of disinfestation. Mortality was calculated on the total number of insects found inside and outside the dates in the test trays and below the test trays.

The total volume to be fumigated was calculated as 9.5 m^3. Temperature of the dates was 24°C and ambient was 26°C during the trial. Vapormate™ supplied from the pressurized cylinder was mounted on a scale while the pressure tube was hold inside the sealed liner and secured to prevent movement of the injection tube due to back pressure of the gas. During the injection the opposite top of the chamber was kept open to release excessive pressure and to prevent sudden ballooning of the fumigation chamber (Fig. 2).

Figure 2 The portable disposable fumigation chamber made of flexible liner laminate on the right connected to a Vapormate™ cylinder on the left.

2.3.2. Date fumigation in commercial rigid fumigation chamber

A commercial rigid fumigation chamber of 95.6 m^3 located in Tzemach packing house was connected with a Vapormate™ pressurized cylinder directly to the existing system that was used for fumigation with MB (Fig. 3). Each of the 51 crates contained 400 kg Zahidi variety dates and 12 crates of 200-kg capacity containing Ameri variety dates were used for fumigation.

Figure 3 The commercial rigid fumigation chamber of 95.6 m^3 using Vapormate™ for dates stored in large crates each containing 200 kg or 400 kg dates.

Bioassays consisted of four small glass vials containing 30 laboratory-reared nitidulid larvae and 1 g of date (food) placed in four locations in the fumigation chamber. Two locations were in the top and bottom of crates near the door and two in the top and bottom crates at the far side of the chamber. Each glass vial was inserted 20-cm deep into the date pile. A gas-sampling opening in the chamber was used to measure gas concentration. An initial dosage of 420 g m^{-3} was used and concentrations were measured immediately after the gas release using a CO_2 gas analyzer.

3. Results

3.1 Laboratory experiments

Exposure to 280 mg L^{-1} for 12 h provided the lowest dosage with mortality values (79.9%) and disinfestation of (69.3%). Although mortality values at 350 mg L^{-1} averaged 98.8% and disinfestation was 72.7%, a higher dosage was also tested for complete control. Mortality at exposure to 420 mg L^{-1} resulted in complete kill with disinfestation value of 69.6% (Table 1). Table 2 shows the effect of 420 g m^{-3} and 12 h exposure to Vapormate™ on larvae emigration and mortality at 16ºC, 18ºC, 24ºC, 26ºC and 28ºC. Total larvae mortality was achieved at all temperatures above 18ºC, with the greatest larvae emigrations at 26ºC and 28ºC (Table 2). Table 3 shows the effect of 420 g m^{-3} and 12 h exposure to Vapormate™ on egg mortality at 24ºC and 30ºC. Total egg mortality was achieved at both temperatures.

Table 1 Effect of various dosage (mg L^{-1}) treatments of Vapormate™ on emigration of nitidulid larvae from artificial feeding sites simulating infested dates expressed in percent disinfestation, mortality and survival in control after 12 h at 30ºC (4 replicates for each dose).

Dose (mg/L)	Mortality (%)	SD	Disinfestation (%)	SD	Survival in control (%)	SD
280	79.9	16.26	68.3	15.34	66.7	24.58
350	98.8	1.39	72.7	9.81	76.0	26.82
420	100.0	0.0	69.6	12.85	74.9	21.31

Table 2 Larvae mortality and larvae emigration after 12 h exposure with 420 g m^{-3} of Vapormate™ at 16, 18, 24, 26 and 28ºC (4 replicates for each treatment).

	Mortality (%)					
Temperature (ºC)	Inside dates	SD	Outside dates	SD	Emigration (%)	SD
16	100	0.0	90.4	7.5	74.3	22.7
18	100	0.0	100	0.0	26.9	9.4
24	100	0.0	100	0.0	76.7	8.7
26	100	0.0	100	0.0	88.7	5.1
28	100	0.0	100	0.0	88.2	9.7

Table 3 Egg mortality after 12 h exposure with 420 g m^{-3} of Vapormate™ at 30ºC and 24ºC (4 replicates for each treatment).

	Control			Treatment		
Temperature (ºC)	Average number of eggs	Mortality (%)	SD	Average number of eggs	Mortality (%)	SD
24	21	65.9	1.2	113	100	0.0
30	33	53.0	4.2	123	100	0.0

3.2. Commercial scale pilot trials on date fumigation

Table 4 shows results using the flexible chamber on laboratory-infested dates after 12 h exposure with 420g m^{-3} of Vapormate™ ; there was with 97% disinfestation and 96% mortality calculated on total number of insects found inside and outside the dates. Table 5 shows results using the flexible chamber on naturally infested Halawy dates after treatment. All dates were clean from pests with a value of 100% disinfestation while mortality value of the beetles outside dates was 95%. The second type of fumigation chamber was a commercial rigid-fumigation chamber. All the bioassay test larvae in all four locations were killed and the dates collected from the same locations were free from insect contamination.

Table 4 Actual number of nitidulid beetles found outside and inside Laboratory infested dates after 12 h exposure with 420 g m^{-3} of Vapormate™ inside the flexible fumigation chamber. Calculated average disinfestation 97% and mortality 96%.

	Average number of insects outside date				Average number of insects inside date			
Layer of exposure in	Larvae		Adults		Larvae		Adults	
the experimental stack	Live	Dead	Live	Dead	Live	Dead	Live	Dead
Top	1	143	0	1	0	6	0	0
Middle	22	387	3	60	0	6	0	0
Bottom	10	297	6	39	0	13	0	0

Table 5 Actual number of nitidulid beetles found outside and inside naturally infested Halawy dates after 12 h exposure with 420 g m^{-3} of Vapormate™ inside the flexible fumigation chamber. Calculated average disinfestation 100% and mortality 95%.

	Average number of insects outside date				Average number of insects inside date			
Layer of exposure in	Larvae		Adults		Larvae		Adults	
the experimental stack	Live	Dead	Live	Dead	Live	Dead	Live	Dead
Top	0	0	0	0	0	0	0	0
Middle	21	456	0	76	0	0	0	0
Bottom	12	111	0	15	0	0	0	0

4. Discussion

The use of Vapormate™ as a fumigant to control nitidulid beetles has never been implemented before in the date industry. In order to comply with the registration demands in Israel there was a need to perform studies in the laboratory and to perform commercial scale trials. Under controlled laboratory conditions exposure to 420 g m^{-3} of Vapormate™ at 30ºC and 12 h exposure time resulted in 100% mortality of the nitidulid beetles and 69.6% disinfestations of the dates. Tests on larvae emigration and mortality at four temperatures resulted in total mortality at all temperatures (18º to 28ºC) with greatest larvae emigration at 26ºC and 28ºC. Vapormate™ was found to be effective fumigant to control nitidulid beetles including eggs, larvae and adults at exposure of 420 g m^{-3} of Vapormate™ for 12 h at temperatures above 24ºC.

The commercial trials using the flexible 9.5 m^3 chamber and commercial rigid fumigation chamber of 95.6 m^3 resulted in 100% mortality of nitidulid beetles and disinfestations greater than 95%. Vapormate™ was registered in Israel for the use by the date industry as an alternative to MB for the control nitidulid beetles at dosage of 420 g m^{-3} of Vapormate™ for 12 h at temperatures above 24ºC.

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