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VAPORMATE®AS A FUMIGANT FOR THE CONTROL OF MEALYBUGS IN PINEAPPLE AND PESTS OF STORED RICE AND MAIZE

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ABSTRACT

Experiments were carried out at the National Plant Quarantine Service at Katunayake and at the Food Research Unit at Gannoruwa, Peradeniya, Sri Lanka to evaluate the efficacy of Vapormate® (Ethyl formate 16.3% w/w in liquid carbon dioxide 83.7% w/w), against mealybugs in pineapples [*Ananas comosus* (L.) Merr.] and stored grain pests of rice (*Oryza sativa* L.) and maize (*Zea mays* L.). Vapormate® is an eco-friendly safer alternative for Methyl Bromide, and accepted worldwide for quarantine and non-quarantine treatment of food items, packaged and stored foods and processing equipment. Fumigation standards recommended by the manufacturer for pineapples (4h exposure of 360g Vapormate®/m^3) resulted 100% mortality in mealybugs, (*Dysmicoccus brevipes*), without deteriorating the organoleptic and physicochemical properties of the fruits. The recommended fumigation standards by the manufacturer for stored grains (24h exposure of 420 g Vapormate®/m^3) found to be highly effective in controlling both larvae and adults of stored grain pests of rice and maize namely, maize weevil (*Sitophilus zeamais*), rice weevil (*Sitophilus oryzae*), red flour beetle (*Tribolium castaneum*), confused flour beetle (*Tribolium confusum*) and rice moth (*Corcyra cephalonica*). Therefore, Vapormate® can be recommended for pre-shipment fumigation of pineapples and for pre-entry and non-quarantine treatment of stored rice and maize in Sri Lanka as recommended by the manufacturer.

***Key words*:** Carbon Dioxide, Ethyl Formate, fumigation, stored grain pests, Pineapple Mealybug, Vapormate®

INTRODUCTION

Sri Lanka needs to explore possibilities of introducing internationally accepted novel techniques for quarantine treatments in order to increase opportunities for export market of agricultural products. In addition, efforts need to be taken to minimize pest related losses incurred in stored grains such as rice and maize through environmental- and user- friendly technologies. Sri Lanka as a signatory to the Montreal Protocol has committed to develop alternatives to minimize the use of Methyl Bromide (MeBr) (Bond, 1985; Ekanayake and Sumathipala, 2010). Furthermore, identification of safer alternatives to replace the currently used Phosphine, which poses a number of negative issues like resistance development in exposed pests and residues in treated food, has become a necessity.

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Pineapple mealybug (*Dysmicoccus brevipes*) is considered as one of the main quarantine pests of pineapple [*Ananas comosus* (L) Merr.]. Field control of pineapple mealybugs using expensive physical and chemical methods does not satisfy the international quarantine standards. Hence, pre-shipment fumigation before cool storage (at 12 °C) transport is considered as the best option available. MeBr fumigation, the currently used practice, causes internal browning of pineapples, which is considered as one of the biggest draw backs.

Losses incurred in stored cereals and pulses due to pest damages in Sri Lanka meant for the consumption and as seed material are high (Wasala *et al.,* 2016). Phosphine and other insecticides used for postharvest pest control has resulted in several drawbacks including risk to the applicator, pesticide residues, resistance development, *etc*. (Nugaliyadde, 2001). Therefore, a demand exists for a low-toxic alternate fumigant for quarantine and non-quarantine treatment of stored grains in Sri Lanka.

Vapormate® (Ethyl formate16.7% w/w; volume 11%+ carbon dioxide 83.3% w/w; volume 89%), which is available in compressed liquid form is an eco-friendly, safer, non-residual fumigant (Damcevski *et al.,* 2003, Lawrence, 2005; Finkelman *et al*., 2012). It can be used to protect post-harvest products, packaged and stored foods and processing equipments effectively and efficiently. It is already approved for quarantine treatment of a long list of fruits, vegetables and grains (Zhang *et al*., 2003; Damcevski *et al.,* 2003; Lawrence, 2005; Finkelman *et al*., 2012; Bessi *et al*., 2016). The active ingredient ethyl formate (EF) degrades to naturally occurring substances. It is a proven substitute for MeBr and Phosphine and has already been accepted by many countries including Australia (AIQS), New Zealand, Japan, EU and USA for pre- and post- quarantine fumigation (Ryan and Bishop, 2003; Damcevski *et al*., 2003; Lima, 2010; Sung, 2008 Saayman, 2011).Therefore, experiments were conducted to determine the efficacy of the standardized doses of Vapormate® for the control of mealybugs in pineapple and pests of stored rice and maize.

MATERIALS AND METHODS

Quarantine Fumigation of Pineapples for the control of Mealybugs

The experiments were conducted at the National Plant Quarantine Service (NPQS) at Katunayake and the Food Research Unit (FRU) at Gannoruwa, Peradeniya, Sri Lanka. A total of 96 Pineapples (variety 'Mauritius'), moderately infested to mealybugs (*Dysmicoccus brevipes*), were used for the experiment. The pineapples were randomly placed inside 12 corrugated cartoon boxes (40 x 48 x 24 cm) at the rate of eight fruits per box. All the fruits were labeled according to the box number and fruit number, and their weights (g) and visual counts of mealybug colonies were recorded.

Six pineapple boxes were fumigated using Vapromate® (in 1m^3 fumigation chambers) at NPQS using the recommended dose for pineapple mealybugs (360gVapromate®/m^3 for a period of 4h), and the remaining boxes were kept untreated. Of the six treated pineapple boxes, three were stored in a cool chamber (at 15^{o}C) and the remaining three were kept under ambient condition in the laboratory (27 -31°C, RH 65-75 %). Similar procedure was adopted for the untreated pineapple boxes (three boxes in a cool chamber at 15^{o}C and the rest under ambient condition in the laboratory). Pineapples were withdrawn randomly from each box at the rate of two per box before treatment and 1h and 7 and 14 days after treatment (DAT). The surface of the sampled pineapples was cleaned with fine brushes to remove the dead and live mealybugs (adults and juveniles) for counting under a magnifying glass (x10). The surface-cleaned pineapples were then given to NPQS and FRU at the rate of one pineapple per box to assess the physicochemical and organoleptic properties as per standard procedures.

Munsell color system was used to assess skin and internal color, and the internal browning was rated visually. Fruit hardness was measured using a handheld fruit Penetrometer (Wagner). The brix values of the pineapples were measured using a digital laboratory refractometer (Portable Optical Refractometer: 45 - 80 °Brix, Eclipse Professional) and the pH values of the pineapple juice were obtained through a handheld portable laboratory pH meter (Orion). The taste of the pineapples was evaluated by a panel composing around 10 staff members using a 9-point hedonic scale (for sensory evaluation).

During fumigation period EF and CO_2 concentrations in the center of the fumigation chambers were measured at 1h intervals with the help of a gas monitor (G450 Multigas monitor, Linde Group) to determine the stability of the gas concentration inside the chamber, and to detect any leakages of gas from the chamber. Chi-Square Test (p=0.05) was performed to determine the independence of treatment effect on pest populations. The ANOVA was performed using SAS 9.1.3 portable soft-ware for quantitative data obtained in physicochemical and organoleptic properties.

Quarantine Fumigation of Rice and Maize for the Control of Stored Grain Pests

A 76.0 m^3container (12.06 x 2.34 x 2.68 m) stationed at the FRU at Gannoruwa was used for the experiment. The container had 4,440 kg commercial grade red rice (*Oryza sativa*) imported from India and 4,500 kg commercial grade locally produced maize (*Zea mays*), heavily infested with grain weevils and borers. Using a 45 cm seed sampler (Nobel Trier) a total of six samples of rice and four samples of maize seeds (weighing approximately 250 g each) were drawn randomly from the polysack bags to estimate the pest populations at the Plant Protection Service (PPS) of the Department of Agriculture at Gannoruwa. In addition, three bulk samples (approx. 1kg each) were taken to study the pest profile in the stored grains. Vapormate® was applied as recommended by the Linde Group, Germany stored grain pests at the rate of 420 g Vapormate®/m^3) and the container was kept sealed for 24 h. As the container volume was 72 m^3, the estimated weight of Vapormate® delivered was 32 kg. Using a gas monitor (G450 Multigas Monitor, Linde Group), ethyl formate and carbon dioxide concentrations at three points inside the container (front, middle and rear) were measured at 6h intervals to determine the stability of the gas concentration inside the container and to detect any leakages of gas from the container. After degassing the container, rice and maize seeds were sampled as done prior to fumigation to estimate the live and dead insects. Chi-Square Test (p=0.05) was performed to determine the independence of treatment effect on pest populations.

RESULTS AND DISCUSSION

Quarantine Fumigation of Pineapples

The weight of the pineapples used for the experiment found to be within the acceptable range for Mauritius pineapples (929 $\pm$ 19 g/ fruit). The pineapples selected for the experiment were moderately infested with mealybugs (average 17.2 $\pm$ 3.5 colonies/fruits). All the treated pineapples either kept under 12 °C or ambient temperature did not have live mealybugs throughout the two-week experimental period as compared to untreated pineapples. The untreated pineapples kept under 12°C had a lower number of mealybugs compared to those kept under ambient conditions (Table 1).

Hardness and pH of the four groups of fruits evaluated at NPQS and FRU indicated no significant difference (p>0.05) in these characters within each sampling day, indicating that Vapormate® treatment had no effect on these characters. However, the Brix value of the treated fruits stored at 12 °C was lower than the untreated and treated fruits stored under ambient conditions (Table 2).

The skin color, flesh color and internal browning of the treated and untreated fruits did not show any significant difference (p>0.05) indicating that the fumigant used had no effect on the pigmentation of the treated fruits (Table 3). The only exception observed was the internal browning of untreated pineapples stored under ambient conditions. Some of the untreated fruits rot during storage and had to be discarded from quality evaluations.

The EF and CO_2 concentrations as detected by the EF monitor was at an acceptable level indicating that the chamber is air tight and hence no leakages occurred (Table 4). This is the first time that fumigation standards for Vapormate has been made to control mealybug in pineapple meant for export market.

Fumigation of Rice and Maize

The rice and maize grains were heavily infested with four common grain pests and almost all insects (larvae and adults) were found dead after treatment (Tables 5 and 6). The bulk sample, which was observed 7 days after fumigation were also free from living insects indicating 100% mortality of pests exposed to Vapormate®. These mortality records under different dose / time regimes found to be similar to the observations reported earlier by Damcevski *et al.*, (2003) and Damcevski *et al.*, (2010).

The EF and CO_2 concentrations as detected by the EF monitor was marginally lower than the expected range from 6h after treatment, in spite of no gas leakage detected outside the container. This could be due to the absorbance of the fumigants by the rice and maize grains that occupied > 60% of the container volume (Ryan and Bishop, 2003; Damcevski *et al*., 2003; Damcevski *et al*., 2010) (Table 7).

CONCLUSIONS

The recommended dose and exposure period of Vapormate® (360 g/m^3 and 4 h exposure) for the control of mealybugs (*Dysmicoccus brevipes*) in pineapple resulted in 100% mortality of the insects within 1h after treatment. Cold storage of pineapples at 12 °C, after fumigation, further minimizes the population growth of mealybugs. The above dose did not show any negative effect on the physiochemical and organoleptic properties of fruits compared to the untreated pineapples. The standard treatment of Vapormate® recommended for the control of general stored grain pests of

Table 1: Mealybug populations in pineapples before and after the treatment

Treatment	*Average number of live and dead mealybugs (adults and juveniles)/pineapple(n=6)*			
	Before treatment	*1h after treatment*[1]	*1st week after treatment*[2]	*2nd week after treatment*[3]
Vapormate® & stored at 12 °C	21	0 (18)	0 (0)	0 (0)
Vapormate® & stored under ambient conditions	15	0 (13)	0 (0)	0 (0)
Without Vapormate® & stored at 12 °C	19	15 (0)*	06 (5)*	08 (0)*
Without Vapormate® & stored under ambient conditions	14	11 (0)*	18 (0)*	26 (0)*
	ns	S[1]	S[2]	S[3]

*** Values within parenthesis are number of dead mealybugs**
[1]Chi-Square = 23.1, p <0.001: $X^2 = _{0.001,4} = 14.2$;**
[2]Chi-Square = 54.1, p <0.001: $X^2 = _{0.001,4} = 23.1$**
[3]Chi-Square = 65.1, p <0.001: $X^2 = _{0.001,4} = 31.6$**

Table 2: Hardness (H), brix value (Bx) and pH of the treated and untreated pineapples before treatment and 1 and 2 weeks after treatment (combined results from NPQS and FRU) (n=6)

Treatment	*Before treatment*			*1 week after treatment*			*2 weeks after treatment*		
	H	*Bx*	*pH*	*H*	*Bx*	*pH*	*H*	*Bx*	*pH*
V + 12 °C	1.41	10.4	4.01	1.39	10.0a	4.1	0.8	11.0a	4.5
V + Room temp.	1.36	10.2	3.86	1.81	12.1b	3.9	0.9	16.1b	3.9
No V + 12 °C	1.32	10.7	4.01	1.62	14.0b	4.6	0.4	15.5b	3.8
No V + Room temp.	1.39	10.5	3.89	1.70	14.0b	3.7	0.3	17.3b	3.8
	ns	ns	ns	ns		ns	ns		ns

V = Vapormate®; H = hardness, Bx = brix value; Within a column, means followed by the same letter are not significantly different by the DMRT at p=0.05; ns = non-significant (p>0.05)

rice and maize (420 g/m^3 and 24h exposure) also resulted in 100% mortality to both larvae and adults of maize weevil (*Sitophilus zeamais*), rice weevil (*Sitophilus oryzae*), red flour beetle (*Tribolium castaneum*), confused flour beetle, (*Tribolium confusum)* and rice moth (*Corcyra cephalonica*). There was no tolerance limit for quarantine fumigation of

Table 3: Skin color (SC), flesh color (FC) and internal browning (IB) of the pineapple samples before treatment. (combined results from NPQS and FRU) (n=6)

Treatment	*Before treatment*			*1 week after treatment*			*2 weeks after treatment*		
	SC	*FC*	*IB*	*SC*	*FC*	*IB*	*SC*	*FC*	*IB*
V + 12 °C	Y/O	Y/O	No	Y/O	Y/O	No	Y	Y/O	No
V + Room temp.	Y/O	Y/O	No	Y/O	Y	No	Y	Y	No
No + 12 °C	Y/O	Y/O	No	Y/O	Y/O	No	Y	Y/O	No
No V + Room temp.	Y/O	Y/O	No	Y/O	Y	No	Y	Y	Yes

V = Vapormate®; SC= Skin color, FC= flesh color, IB= internal browning; Y= yellow; O= orange

Table 4: Calculated (provided by the supplier) and detected EF and CO_2 (detected by the monitor) concentrations in the container just after treatment (0h) and hourly reading after treatment (Treated dose = 360g Vapormate®/m^3(volume of the chamber)

EF and CO_2 levels	*ET (% volume)*		*CO_2 (% Volume)*	
	Chamber 1	*Chamber 2*	*Chamber 1*	*Chamber 2*
Calculated	1.8	1.8	7.5	7.5
Detected				
0h	1.88	1.84	7.5	7.6
1h	1.08	1.82	7.6	7.6
2h	1.70	1.8	7.7	7.7
3h	1.72	1.7	7.7	7.6
4 h	1.60	1.7	7.7	7.6

EF = ethyl formate

Table 5: Pest profile observed in the bulk sample (1 kg) drawn from the container at the Food Research Unit

Pest	*Before treatment*		*1 day after treatment**	
	No. adults	*No. larvae inside grains*	*No. live adults (dead adults)*	*No. live larvae (dead larvae)*
Maize weevil (*Sitophilus zeamais*)	141	47	0 (235)	0 (39)
Rice weevil (*Sitophilus oryzae*)	341	35	0 (280)	0 (56)
Red flour beetle (*Tribolium castaneum*)	89	18	0 (72)	0 (61)
Confused flour beetle (*Tribolium confusum)*	12	17	0 (3)	0 (40)

***The bulk sample was observed 7 days after fumigation**

agricultural commodities

The treatment standards for Vapormate® stated above are thus, recommended for pre-shipment fumigation of pineapple and for pre-entry and non-quarantine treatment of stored rice and maize under commercial scale use in Sri Lanka.

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Table 6: Pest profile observed in the bulk sample drawn from the container at FTU (n=3)

Commodity	***Pest***	***Av. number of live adults before treatment (rounded to whole number)***	***Number of live adults after treatment***
Maize	Beetles	75	0
	Weevils	9	0
Rice (raw rice)	Beetles	54	0
	weevils	3	0
Rice (par boiled rice)	Beetles	88	0
	weevils	6	0

Table 7: Calculated and detected EF and CO_2 concentrations in the container just after treatment, and at 6 hourly intervals after treatment (at the Front, middle and bottom levels of the container) with 420 g Vapormate®/m^3 (32 kg/container)

EF and CO_2 levels		**EF (% Volume)**			**CO_2 (% Volume)**		
		Front	**Middle**	**Back**	**Front**	**Middle**	**Back**
Calculated		2.17			18.8		
Detected 17/3/2016	4 pm	3.2	2.9	2.8	24.0	23.5	23.0
	10 pm	1.08	1.04	1.03	18.0	18.1	18.0
18/3/2016	4 am	0.65	0.68	0.62	15.8	15.4	15.4
	10 am	0.63	0.64	0.64	15.3	15.0	15.2
	4 pm	0.61	0.62	0.63	14.8	14.6	14.6

NS=Non-significant

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