The rusty grain beetle (*Cryptolestes ferrugineus*) is one of the most common insect pest of stored products. Phosphine (PH_3) is a major fumigant used for treating various food commodities, and the wrong aplication has led to resistance to phosphine. The development of high levels of resistance to phosphine has been reported in México. For that reason, different doses and exposure times were used to control of *C. ferrugineus* in two stages, larvae, and adult. In a warehouse using a Grainbag (GrainPro®) with 50 kg of wheat (*Triticum aestivum* L.) as support. Three doses of phosphine were used, 1.4 gr/m^3, 2.8 gr/m^3 and 4.2 gr/m^3 and 3, 5 and 7 days to determine the proper dose and exposure time for control *C. ferrugineus*. An application of 2.8 gr/m^3 with 5 days could achieve 100% mortality in both stages.

Efficacy Studies on $ECO_2FUME^®$ Phosphine Fumigant for Complete control of *Sitophilus zeamais* and *Tribolium castaneum* in stored maize in Thailand

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Abstract

$ECO_2FUME^®$ fumigation of maize bag stacks under gas proof sheets was conducted to establish the optimal dosages (application rate) and exposure times (fumigation period) against mixed-age cultures of *Sitophilus zeamais* and *Tribolium castaneum*. The Complete Randomized Design (CRD) experimental design was employed, with 3 replications and 4 treatments. The experiments were divided into three groups: 1) treatment with a 25 g/m^3 $ECO_2FUME^®$ application rate (350 ppm phosphine) for 3, 4, and 5 days and a control treatment; 2) treatment with an $ECO_2FUME^®$ application rate of 50 g/m^3 (700 ppm phosphine) for 2, 3, and 4 days and a control treatment; and 3) treatment with a 70 g/m^3 $ECO_2FUME^®$ application rate (1,000 ppm phosphine) for 1, 2, and 3 days and a control treatment. The three target phosphine concentrations of 350 ppm, 700 ppm and 1,000 ppm were maintained during the whole fumigation period. Results of the studies showed that no insect was alive at all dosages and exposure times. The studies also indicated that fumigation with $ECO_2FUME^®$ could reduce the fumigation period by increasing the phosphine concentration. The effective fumigation protocols on maize against mixed-age cultures of *S. zeamais* and *T. castaneum* were ECO_2FUME application rates of 25 g/m^3 for 3 days, 50 g/m^3 for 2 days and 70 g/m^3 for 1 day. The target phosphine concentration must be maintained throughout the fumigation period to achieve 100% mortality of all stages of insects.

Keywords: $ECO_2FUME^®$ phosphine fumigant, stored-product insects, *Sitophilus zeamais*, *Tribolium castaneum,* fumigation protocols, stored maize

1. Introduction

Maize is a primary ingredient of animal feed. Thailand's maize demand in 2016 was 5.85 million tons, which increased by 2.77% from 5.72 million tons in 2015. Because of the expansion of the livestock industry, demand for maize for animal feed increased. In 2016, Thailand exported 0.58 million tons of maize with total value of 4,855.34 million baht, a significant increase from 0.08 million tons with total value of 716.79 million baht in 2015. Maize production and value increased 7.25 and 6.77 times, respectively, because maize was increasingly exported to ASEAN markets including The Philippines, Indonesia and Vietnam (Office of Agricultural Economics, 2016).

For use as animal feed, maize must be stored for several months to sustain the continuous supply to the feed processors. When maize is stored at the production sites for a period longer than 3 months, insect infestation becomes a common and serious problem. The major insect pests that negatively affect the quality and quantity of stored maize in Thailand are *Sitophilus zeamais*, *Tribolium castaneum* and *Crytolestes ferrugineus* among others.

In Thailand, phosphine has been used as an effective fumigant to control several stored grain insect pests for more than 50 years (Sukprakarn et al., 1996). Formulations of phosphine available in Thailand are tablets or blankets of the metallic salts aluminium phosphide and magnesium phosphide that emit phosphine gas (PH_3) when exposed to air and moisture. Following application of tablets, concentration of PH_3 increases over several hours or days until all of the material is reacted. The increase rate of concentration depends on temperature and relative humidity. It is highly important to establish and maintain the most appropriate concentrations and exposure times in each particular situation. Precise dosing of PH_3 and the assurance of appropriate exposure times are difficult to achieve because of the dynamic release characteristics of the gas from tablet formulations and inherent structural leaks. There is the relative ease and safety in applying tablets to grain mass, but the influence of efficacy due to leaky structures may be a disadvantage (Banks, 1994; Bonjour, 1998).

$ECO_2FUME^®$ fumigant gas is a cylinderised formulation of PH_3 dissolved in liquid carbon dioxide at approximately 2% PH_3 and 98% CO_2 by weight. It is packaged in a high-pressure aluminium steel cylinder, with a net content of 31 kg of PH_3/CO_2 mixture and an equivalent phosphine amount of 620 g (Tumambing et al., 2012). $ECO_2FUME^®$ was established and approved in the U.S.A., which allows a shorter fumigation time of 24 hours for a 500 - 1,000 ppm phosphine concentration at 27°C or higher temperatures. The mixture of PH_3 and CO_2 is not flammable, which eliminates all safety concerns with the dispensing rate or dilution rate. Traditional solid formulations can generate PH_3 concentration above the lower flammability limit for PH_3 thereby creating a hazard (Cavasin et al., 2001).

There are several advantages when applying $ECO_2FUME^®$. The dose of $ECO_2FUME^®$ phosphine fumigant applied to the commodity is rapid delivery, easy maintenance of the required dose during the fumigation period, shorter exposures and ease of application. $ECO_2FUME^®$ does not require the applicator to enter the fumigation space. The ready-to-use cylinders can be dispensed from outside of silos or structures being fumigated. This eliminates the need for entry into confined spaces to apply fumigants and solid waste disposal (Bonjour, 1998; Phillips, 1998).

The controlled application of fumigant gas resulting in less fumigant was introduced in stored product instead of the traditional solid formulation. It relies on the generation of a high initial phosphine concentration followed by a slow deterioration to ensure that the phosphine concentration - time product (CT) - will result in an effective fumigation. With $ECO_2FUME^®$ fumigant gas, the concentration can be easily controlled by the applicator to maintain an efficacious concentration and can be precisely measured by adding the required amount of gas when needed. Disposal of solid waste products from tablets is becoming more difficult every day. $ECO_2FUME^®$ fumigant gas eliminates the concern associated with deactivating unspent metal phosphide residue and disposal of the waste product (Cavasin et al., 2001).

$ECO_2FUME^®$ is currently being considered for registration as an alternative to methyl bromide in Thailand by Genera Asia Co. Ltd. in 2011. Because $ECO_2FUME^®$ has never been used in Thailand therefore the study on this fumigant was needed.

The objective of this study was to establish the optimal dosages (application rate) and exposure times (fumigation period) of $ECO_2FUME^®$ phosphine gas for killing mixed-age of the major stored product insects in stored maize (*S. zeamais* and *T. castaneum*) in Thailand.

2. Materials and Methods

Test insects and preparation of mixed-age cultures

All insects of *S. zeamais* and *T. castaneum* used in this study were obtained from the stored-product insect colonies maintained at the Post-harvest and Processing Research and Development Division of the Thailand Department of Agriculture. The mixed-age cultures were prepared by adding 50 young adults (2-3 week olds) of each species (*S. zeamais* and *T. castaneum*) into a glass bottle

containing 200 g of a culture medium which was different for each species; brown rice for *S. zeamais*, rice bran for *T. castaneum* and covered with filter paper for 3 weeks. Afterwards, all adults were removed and kept in the laboratory for 4 weeks at 30±2°C temperature and 65±5% relative humidity before fumigation. All life stages were examined for their presence in the mixed-age culture glass bottle prior to fumigation.

Fumigant

$ECO_2FUME^®$ phosphine fumigant is a product of Solvay's Niagara Falls, Canada, facility (known by its legal entity name, Cytec Canada).

Fumigation of mixed-age cultures

The experiment was conducted in a concrete warehouse of Bangkok Food Products Co., Ltd. (CPF), located at the Phra Bhuttabat district, Saraburi province, Thailand, in 2014. Maize used for the trials was packaged in jumbo bags (1,000 kg capacity). The concrete floor was thoroughly cleaned, and black polyethylene floor sheets (0.5 mm thickness) were then laid out on the ground as an under-layer sheet. For construction of the stack, 8 jumbo bags of maize were piled on the floor sheet. Stack sizes were between 6.7 to 7.5 m^3. A cage of mixed-age test insects in a culture medium was placed into the maize jumbo bag on top of the stack. Each maize stack with test insects was then covered with a clear polyvinyl chloride (PVC) sheet (0.2 mm thickness) to construct fumigation enclosures. The fumigation sheet was then sealed to the floor with sand snakes (cotton bags of 100 cm x15 cm filled to 80% with sand). Considerable attention was given to the sealing operation to ensure that the fumigation sheet was neatly folded, without wrinkles, that it extended at least 1 meter away from the stack edges, and that overlapping sand snakes were laid in double rows at the corners. This type of setup was taken to ensure minimum gas leakage.

Efficacy trials were designed in Complete Randomized Design (CRD) with 3 replications and 4 treatments. The experiments were divided into 3 groups:

1) Treatment with a 25 g/m^3 $ECO_2FUME^®$ application rate (350 ppm phosphine equivalent) for 3, 4, and 5 days, and a control treatment (untreated).

2) Treatment with a 50 g/m^3 $ECO_2FUME^®$ application rate (700 ppm phosphine equivalent) for 2, 3, and 4 days, and a control treatment.

3) Treatment with a 70 g/m^3 $ECO_2FUME^®$ application rate (1,000 ppm phosphine equivalent) for 1, 2, and 3 days, and a control treatment.

The required amount of phosphine from $ECO_2FUME^®$ was injected inside the trap using a stainless steel hose and a gas injector with a gas flow rate of 6.85 kg/min. The exact amount of dispensed $ECO_2FUME^®$ was determined by the weight change of the cylinder on the top of a 100-kg digital scale with an accuracy of 0.01 kg or 10 g.

Monitoring of gas concentration

Phosphine concentration was monitored at each of the following intervals: 1) 1 and 18 hours for a 1-day exposure time; 2) same as item 1 plus 24 and 42 hours for a 2-day exposure time; 3) same as item 2 plus 48 and 66 hours for a 3-day exposure time; 4) same as item 3 plus 72 and 90 hours for a 4-day exposure time; and same as item 4 plus 96 and 114 hours for a 5-day exposure time. Three target phosphine concentrations of 350 ppm, 700 ppm and 1,000 ppm were maintained during the whole fumigation period. When the phosphine concentration fell below the target concentration, $ECO_2FUME^®$ was topped up to bring back the concentration at or above the target concentration. Phosphine concentration was monitored with calibrated SILOCHEK phosphine monitor (0 - 2000 ppm).

Fumigation was terminated at 1, 2, 3, 4 and 5 days of exposure time, followed by aeration of the slightly opened enclosure until the phosphine concentration reached the threshold limit value (TLV)

of 0.3 ppm or lower. The plastic cover sheet at each of the treatment stacks was completely removed afterwards.

Assessment of insect mortality

Effectiveness of $ECO_2FUME^®$ against the test insect was determined by mortality of mixed-age cultures. After fumigation, the glass bottles with the test insects were retrieved from each stack and the mortality of adults from each experiment was recorded. Dead and alive insects were separated, and each culture medium was returned to the bottles and kept in the laboratory at 30±2°C and 65±5% for 6 weeks. The bottles were observed weekly to determine if any newly emerged adults surfaced from hatched eggs. This period was sufficient for emergence of all insects in the treatment as well as the control. The occasional dead insect from the control treatment was corrected by Abbott's formula (Abbott, 1925).

Monitoring of temperature, relative humidity and moisture content

The temperature and relative humidity in the warehouse were monitored by a thermo-recorder every day during the fumigation period. The moisture contents of maize were measured before and after the treatment by applying the samples to a SB 900 Steinlite moisture meter.

3. Results

The effectiveness of $ECO_2FUME^®$

The effectiveness of $ECO_2FUME^®$ fumigation at different concentrations and exposure periods against mixed-age cultures of *S. zeamais* and *T. castaneum* are shown in Table 1. Results indicated that both insect species were completely controlled (100% mortality), and no live insects were observed immediately after fumigation or throughout the 6 weeks of monitoring. The test insects in the non-fumigated control samples continued to develop and emerged normally.

All of the application rates (25, 50 and 70 g/m³ $ECO_2FUME^®$) at any exposure time in each experiment were equally effective against mixed-age cultures of the two insect species in maize fumigation.

Tab. 1 The survival of insects inside the maize stacks during $ECO_2FUME^®$ fumigation with dosage 25, 50 and 70g/m³ at different exposure times.

Dosages (g/m³)	Times)Days(	The number of insects survival)insect(			
		Sitophilus zeamais		*Tribolium castaneum*	
		Immediately after fumigation	6 weeks after fumigation	Immediately after fumigation	6 weeks after fumigation
25 g/m³)350 ppm)	3	0[1/]	0	0	0
	4	0	0	0	0
	5	0	0	0	0
Unfumigated)control)	5	1,443	1,094	2,336	1,531
50 g/m³)700 ppm)	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
Unfumigated)control)	4	1,358	997	1,853	1,192
70 g/m³)1,000 ppm)	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
Unfumigated)control)	3	1,268	983	1,838	1,015

[1/] Mean of 3 replications

The concentration of phosphine

The phosphine concentration profile on the maize stack fumigated with 25g/m³ $ECO_2FUME^®$ at 3, 4 and 5 days of exposure time is shown in Figure 1. One hour after gas injection, the phosphine

concentration at 3, 4 and 5 days inside the maize stacks were 677, 682 and 527 ppm, respectively. The higher phosphine concentration compared to the target concentration was due to the phosphine gas initially occupying only the free space inside the stack. As the calculation of $ECO_2FUME^®$ dose is based on total empty space volume, the free space volume becomes smaller due to volume occupied by the maize stacks. Phosphine gas will then penetrate through the whole maize stack until equilibrium concentration is reached. After fumigation for 18 hours, the concentrations decreased and dropped down to 302, 318 and 282 ppm, respectively, indicating the distribution of phosphine gas in the entire maize stack. There was high fluctuation in the phosphine concentration inside the maize stack due to the addition of $ECO_2FUME^®$ to maintain the target phosphine concentration.

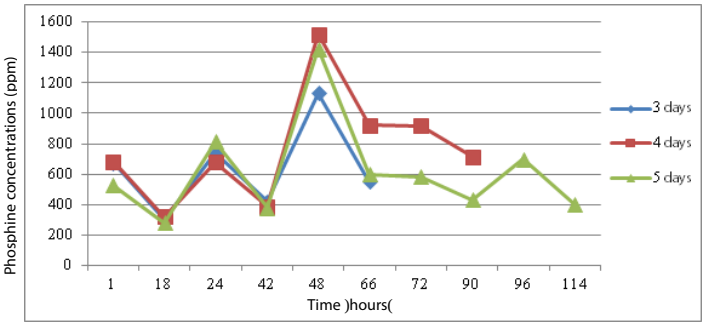

Fig. 1 Average phosphine concentrations inside the maize stacks during fumigation at the different times with $ECO_2FUME^®$ dosage 25 g/m³ (350 ppm).

The phosphine concentration curves of maize stack treated with 50 g/m³ $ECO_2FUME^®$ at 2, 3 and 4 days of exposure periods, as shown in Figure 2, display similar variations of phosphine concentrations with the treatment at 25 g/m³ $ECO_2FUME^®$. The phosphine concentration was below the target concentration after fumigation for 18 hours.

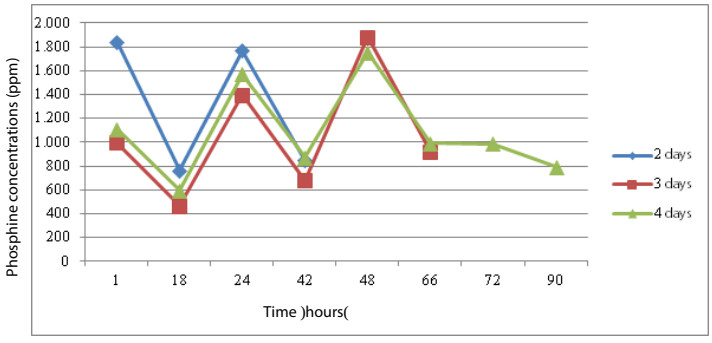

Fig. 2 Average phosphine concentrations inside the maize stacks during fumigation at the different times with $ECO_2FUME^®$ dosage 50 g/m³ (700 ppm).

The phosphine concentration profile of maize stacks fumigated with 70 g/m³ $ECO_2FUME^®$ at 1, 2 and 3 days of exposure periods is shown in Figure 3. The variation of phosphine concentration of this treatment was quite similar to the treatment with 25 g/m³ $ECO_2FUME^®$ in that the phosphine concentration reduction from an initial concentration of 2000 ppm almost hit the target concentration of 1000 ppm after a fumigation period of 18 hours.

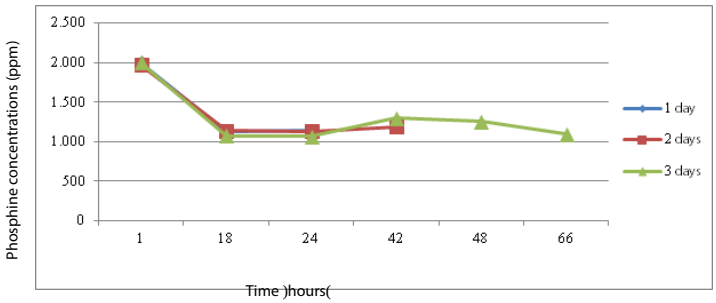

Fig. 3 Average phosphine concentrations inside the maize stacks during fumigation at the different times with $ECO_2FUME^®$ dosage 70 g/m³ (1,000 ppm).

Maintenance of target concentration

As shown in Table 2, there was addition of $ECO_2FUME^®$ conducted at 18 hours and 42 hours for the 3 days and 4 days of fumigation, and at 18, hours, 42 hours and 92 hours for the 5 days of fumigation as top up procedure. This was to maintain the target phosphine concentration at 350 ppm. Every time top up was done, the phosphine concentration was much higher than the target concentration due to the initial occupancy of free space by the gas before equilibrium distribution is reached.

In case of the treatment with 50 g/m³ $ECO_2FUME^®$ (700 ppm phosphine), Table 3 shows that top up was required at 18 hours for the 2 day fumigation, and at 18 hours and 42 hours for 3 and 4 day fumigation. For the treatment with 70 g/m³ $ECO_2FUME^®$ (1000 ppm phosphine), Table 4 shows that there was no top up needed for one day fumigation, and top up was required at 18 hours for the 2 and 3 day fumigation but there was an inconvenient in this experiment, therefore, the top up was done after fumigation for 24 hours instead of 18 hours.

The need for top up meant that maize used in the experiment absorbed some of the phosphine gas inside the stack, which caused the reduction of the phosphine concentration during the fumigation period. In this study, maize could absorb up to 40% of the initial phosphine concentration. As the gas absorption continues during fumigation, the amount increases as the fumigation period is increased. When the phosphine concentration fell below the target concentration, top up of $ECO_2FUME^®$ dosing was conducted to bring back concentration at or above the target concentration. With the ability to safely top up the stacks with $ECO_2FUME^®$, the desired concentration was maintained.

Tab. 2 The top up volumes of ECO_2FUME inside the maize stacks at the different times of fumigation with $ECO_2FUME^®$ dosage 25 g/m³ (350 ppm).

Time)Days(	Top up volume of ECO_2FUME)g)									
	Hours									
	1	18	24	42	48	66	72	90	96	114
3	-	13.44[1/]	-	5.75	-	-				
4	-	12.87	-	7.60	-	-	-	-		
5	-	15.64	-	8.87	-	-	-	4.24	-	-

[1/]Mean of 3 replications

Tab. 3 The top up volume of ECO_2FUME inside the maize stacks at the different times of fumigation with $ECO_2FUME^®$ dosage 50 g/m³ (700 ppm).

Time)Days(	Top up volume of ECO_2FUME)g)							
	Hours							
	1	18	24	42	48	66	72	90
2	-	14.22[1/]	-					
3	-	35.95	-	23.28	-	-		

4	-	27.00	-	9.47	-	-	-	-

1/Mean of 3 replications

Tab. 4 The top up volume of ECO_2FUME inside the maize stacks at the different times of fumigation with ECO_2FUME® dosage 70 g/m³ (1,000 ppm).

Time)Days(	Top up volume of ECO_2FUME)g)					
	Hours					
	1	18	24	42	48	66
1	-	-				
2	-	-	16.44 1/	-		
3	-	-	23.11	-	-	-

1/Mean of 3 replications

Monitoring of temperature, relative humidity and moisture content

The moisture content of maize was 13.2-13.8%. Temperature and relative humidity ranges inside the stacks were 29 - 35°C and 43 - 66%, respectively.

4. Discussion

Fumigation with ECO_2FUME® could reduce the fumigation period by increasing the phosphine concentration. The effective ECO_2FUME® fumigation protocols on maize against mixed-age cultures of *S. zeamais* and *T. castaneum* were 25 g/m³ (350 ppm phosphine) for 3 days, 50 g/m³ (700 ppm phosphine) for 2 days, and 70 g/m³ (1,000 ppm phosphine) for one day.

The phosphine target concentration must be maintained throughout the fumigation period to achieve 100% mortality of all stages of insects. It is necessary to monitor phosphine concentrations during fumigation. If the concentration of phosphine is not regularly monitored during the entire exposure period, the fumigation will be ineffective. Therefore, it is best fumigation practice to monitor the concentration of fumigant inside the sealed stack regularly to keep the phosphine concentration at the recommended minimum concentration.

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