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EFFICACY AND REGISTRATION STATUS OF ECO₂FUME 2 LG AGAINST *TRIBOLIUM CASTANEUM* HERBTS, *SITOPHILUS ZEAMAI*S MOTSCHULSKY AND *ORYZAEPHILUS SURINAMENSIS* LINNAEUS ON MILLED RICE IN INDONESIA

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ABSTRACT

Efficacy study of ECO₂FUME 2 LG against *Tribolium castaneum* Herbts, *Sitophilus zeamais* Motschulsky and *Oryzaephilus surinamensis* Linnaeus on milled rice was conducted in experimental storage in SEAMEO BIOTROP, Indonesia. Using completely randomized design with four levels of dosage (i.e., 70 g/c.m. (1000 ppm), 52.5 g/c.m. (750 ppm), 35 g/c.m. (500 ppm) and 17.5 g/c.m. (= 250 ppm) and control, this study was done in 5 replications. Test insects were 200 adults of *T. castaneum*, *S. zeamais* and *O. surinamensis* per experimental unit. These insects were split into 5 groups and put in small plastic bottles, 40 insects per bottle. Bottles containing test insects were put randomly inside each experimental staple, including control. Parameters observed were mortality of test insects and the number of larvae that emerged from the eggs two weeks after fumigation terminated. Milled rice moisture contents were checked before and after fumigation. Fumigation was conducted for 36 hours under PVC fumigation sheets. Phosphine concentrations were checked at 2, 8, 20, 26 and 32 hours after fumigation. Upon completion of fumigation, mortality of test insects were counted and plastic bottles containing milled rice for test insects food were incubated for 14 days. Results showed that at all dosage levels tested, ECO₂FUME 2 LG effectively controlled *T. castaneum*, *S. zeamais* and *O. surinamensis*. Recommended doses based on this study were 20-70 g/c.m. (= 1000 ppm) and depend on exposure time. The longer the exposure time the lower the dose. However, for using high dose with sorter exposure time still need to be tested. The study results became the basis for the Ministry of Agriculture of Republic of Indonesia to issue a minister decree No.: 4198/Kpts/SR.140/10/2011 permitting the distribution of this fumigant in Indonesia.

Key words: ECO₂FUME 2 LG, *Tribolium castaneum*, *Sitophilus zeamais*, *Oryzaephilus surinamensis*, fumigant, fumigation, dosage, efficacy

INTRODUCTION

Basic management to control stored-product insects requires a combination of fumigation and surface spraying or fogging. In the past, fumigants available for this purpose in Indonesia are methyl bromide and phosphine. However, since Montreal Protocol took effect in 1995 and methyl bromide was phased out for grain storage in Indonesia in 2008, the only fumigant available for commodity maintenance in storage is phosphine.

Improper management to control stored-product insect pests causes high economic damages. Application of phosphine needs longer exposure time than methyl bromide. Fumigators, especially field workers, do not usually follow correct exposure time due to their impatience causing the failure of the control program and rendering the insects to become resistant to the fumigant used. Phosphine formulation that can be applied with shorter time could overcome this problem.

ECO₂FUME is a liquefied gas mixture of 2% phosphine and 98% carbon dioxide (CO₂) (by weight) packaged in high pressure aluminum or steel cylinders (Cavasin et al., 2008). ECO₂FUME application manual mentions that pressurized carbon dioxide serves as a propellant for delivering the product and enhances the fumigant's effectiveness by helping to quickly disperse phosphine into the space to be fumigated. Carbon dioxide retards flammability. Phosphine and carbon dioxide are both gases that, under sufficient pressure, can exist in a liquid state. It is this "liquefied gas" that is stored in the cylinder. The product is withdrawn from the cylinder as a liquid, but dispensed as a gas. In expanding from a liquid to a gas, it increases in volume by hundreds of times. As a new product in Indonesia, the effectiveness of ECO₂FUME 2 LG needs to be confirmed through efficacy trial on commodity.

The objective of this field efficacy trial was to evaluate the effectiveness of ECO₂FUME 2 LG (2% phosphine and 98% CO₂ by weight) against stored-product pests in milled rice, i.e. *Tribolium castaneum* Herbs (Coleoptera: Tenebrionidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* L. (Coleoptera : Silvanidae).

MATERIALS AND METHODS

1. Time and location

The field efficacy trial was conducted from June to July 2011 at SEAMEO BIOTROP's Postharvest Research Warehouse for fumigation and at the Entomology Laboratory for the preparation of insect test, observation of insect mortality and number of insect spawns. SEAMEO BIOTROP is located in Bogor, Indonesia.

The temperature and relative humidity inside the warehouse during the fumigation period were in the range of 24.5 to 29.0°C and 69 to 86%, respectively.

2. Materials and equipments

Materials and equipments used in this trial were: milled rice in polypropylene bag, test insects (*T. castaneum*, *S. zeamais*, and *O. surinamensis*), PVC plastic fumigation sheets, wooden pallets, sand snakes, full face masks, filter canisters for phosphine gas, standard application equipments for ECO₂FUME application, phosphine monitor, phosphine leak detector, thermohygro meter, grain moisture meter and other appropriate equipments.

3. Methods

3.1. Experimental design

Efficacy trial of ECO₂FUME 2 LG was performed using Randomized Complete Design. Four dosage levels of ECO₂FUME 2 LG tested were: 17.5 g/c.m. (= 250 ppm), 35 g/c.m. (= 500 ppm), 52.5 g/c.m. (= 750 ppm), and 70 g/c.m. (= 1000 ppm) and control (0 g/c.m. (= 0 ppm). Each treatment consisted of 5 replications.

Twenty five staples of milled rice were used as trial units. The volume of each unit was 1.15 c.m. All trial units were laid out randomly at Postharvest Research Warehouse.

3.2. Test insects

The test insects used for each experimental unit in this trial were 200 adults of *T. castaneum*, *S. zeamais*, and *O. surinamensis* which were collected from insects reared at BIOTROP's Entomology Laboratory. These insects were split into 5 groups and put in small plastic bottles with milled rice as a culture media. Each bottle contains 40 insects and covered with gauze to allow the fumigant gas to penetrate into the plastic bottles through it. Adult of test insects have been incubated in the milled rice media for one week before fumigation treatment. It was expected that the eggs of each adult test insects could be found in the media. The eggs that were laid by adults also were used for test insects.

3.3. Efficacy trial procedures

Plastic hose was mounted on each staple to measure the phosphine concentration during fumigation. Bottles containing test insects were put randomly inside each experimental staple, including control. PVC plastic cover sheets (thickness 0.15 mm = 150 µm) were used to cover each treatment which was infested by the test insects for fumigation enclosures. To avoid gas leaking, the edge of plastic sheets was pressed with sand snakes and also taped to the concrete floor. Procedure for applying ECO₂FUME followed the application manual issued by Cytec. ECO₂FUME cylinder was placed on top of a digital weighing scale with the capacity of 100 kg.

The ECO₂FUME liquefied gas, then injected into the enclosure using high pressure dispensing hose. Fumigation was conducted for 36 hours under PVC plastic cover sheets. Fumigation was terminated by aerating the enclosure until the phosphine concentration reached the threshold limit value (TLV), i.e. below 0.3 ppm then plastic cover sheets were removed.

Parameters observed were mortality of test insects and the number of larvae that emerged from the eggs two weeks after fumigation terminated. Milled rice moisture contents were checked before and after fumigation and were analyzed with digital grain moisture meter. Phosphine concentrations were checked at 2, 8, 20, 26 and 32 hours after fumigation started.

After fumigation was terminated, the adult test insects were removed from culture media in the plastic bottles for mortality counting, then plastic bottles containing milled rice for test insects food were incubated for 14 days until the eggs hatched. Adult test insects were re-collected and counted to find out how many were alive and dead. Mortality data was then used to calculate the effectiveness of fumigant according to Abbott formula, then analyzed by analysis of variance (ANOVA) with 5% difference level. Fourteen days after fumigation, the presence of individual larval offspring of test insects was observed. This revealed that the eggs were not killed by fumigant applications, so they can still continue their life until they hatch into larvae. The presence of the individual generation of test insects after fumigation was analyzed descriptively.

Treatments of tested fumigant is considered effective based on the following criteria: 1) test insect mortality is significantly more effective compared to the mortality in the control, 2) there is no development of egg into larva after incubation for 14 days on fumigation treatment.

RESULTS AND DISCUSSION

1. Efficacy of ECO₂FUME 2 LG

The effectiveness level of tested fumigant on test insects *T. castaneum*, *S. zeamais*, and *O. surinamensis* can be divided into 1) the effectiveness of the adult stadia and 2) the effectiveness of the eggs stadia.

1.1. Efficacy of ECO₂FUME 2 LG against adult stadia

Efficacy of ECO₂FUME 2 LG against the adults of *T. castaneum*, *S. zeamais*, and *O. surinamensis* based on corrected mortality at all doses tested : 70 g/c.m. (= 1000 ppm), 52.5 g/c.m. (= 750 ppm), 35 g/c.m. (= 500 ppm) and 17.5 g/c.m. (= 250 ppm) was up to 100%. The efficacy of the control (no treatment of fumigant) is 0% meaning there were no dead test insects found. Result of analysis of variance at 5% difference level on the efficacy of fumigant showed a significant difference between control and all doses of fumigant treatment i.e., 70 g/c.m. (= 1000 ppm), 52.5 g/c.m. (= 750 ppm), 35 g/c.m. (= 500 ppm) and 17.5 g/c.m. (= 250 ppm). There was no significant difference ($\alpha = 5\%$) between each dose treatment. It could be concluded that all dose treatments of fumigant are effective in controlling *T. castaneum*, *R. dominica*, dan *S. zeamais* compared to control (without fumigant).

ECO₂FUME application manual mentions that fumigation success depends on the use of appropriate doses, sufficient exposure period, correct application procedures and proper sealed enclosure. The recommended dose by Cytec for ECO₂FUME applications at temperatures above 26°C is 200 to 1000 ppm with a 36 hour exposure period or 500 to 1000 ppm with a 24 hour exposure period. To maximize control, extreme care must be observed in sealing, higher dosages must be used, exposure periods must be lengthened, proper application procedures must be followed, temperature and humidity must be favorable.

1.2. Efficacy of ECO₂FUME 2 LG against eggs stadia

Determination on the effectivity of the fumigant on eggs differed from determination on the effectivity of the fumigant on adults which is based on adults mortality of the test insects. The effectivity of the fumigant on eggs of the test insects was not counted from egg mortality because in this efficacy test did not have the initial count of the eggs. One week before fumigation, the milled-rice to be used for food of test insects was filled with adults of the test insects to allow the adults to lay eggs. Hence it was assumed that in the milled-rice of the test insects, eggs were already available. The number of eggs laid by the adult of the test insects was not counted.

To determine the effectivity of the fumigant on egg stadia, the presence of hatched larva was observed after the used milled-rice test media was incubated for 14 days after fumigation and after the removal of the test adults right after the fumigation finished i.e. at the time of counting of mortality of the test insects.

The number of larvae did not determine the effectiveness of the fumigant to eggs. Effectiveness of fumigant treatment depends on the presence or absence of larvae. The fumigant treatment is considered effective if no live larvae is found in a single treatment, and not effective when live larvae still exist.

The incubation results showed that in all doses of fumigant treatments i.e., either 70 g/c.m. (= 1000 ppm), 52.5 g/c.m. (= 750 ppm), 35 g/c.m. (= 500 ppm) and 17.5 g/c.m. (= 250 ppm), did not indicate any development of eggs into larvae, while the milled rice of untreated fumigant (control) contained larvae. At control, the larvae that emerged from 200 adults averaged 184 larvae of *T. castaneum*, 324 larvae of *S. zeamais* and 266 larvae of *O. surinamensis*. This shows that all test doses of ECO₂FUME 2 LG were effective to control eggs.

2. Milled rice moisture content

The high moisture content of commodity can affect its quality during storage. Therefore, in testing the efficacy of this fumigant, moisture content analysis was conducted both before and

after fumigation to determine the fumigant's influence in increasing the moisture content of milled rice. Based on samples analyzed, the moisture content of milled rice before and after fumigation were 15.9% and 15.7%, respectively. This fact proves that ECO₂FUME 2 LG does not cause an increase in moisture content that can result in decreased quality of stored milled rice.

3. Phosphine gas concentration

Phosphine concentrations were checked to ensure the target concentration was reached. During fumigation, if due to the leaks, phosphine concentration dropped down below the target concentration, topping up should be done. Topping up is done when concentration levels drop more than 10% from the target concentration. The ability to top up the gas is one of the advantages in using liquefied cylinderized phosphine like ECO₂FUME compared to tablets or plates to ensure the success of fumigation. Refumigation after the failure of a fumigation is costly and renders the insects to develop resistance.

The formulation of ECO₂FUME 2 LG can be applied with shorter time exposure than that of tablet, pellet, or plate because there is no need to react with oxygen to produce phosphine gas. Decrease in exposure time will help storage manager to distribute their product faster.

The benefits in using ECO₂FUME are: 1) more effective to control insects due to its ability to maintain minimum target concentration by top up any time during fumigation and reduced risk of target insects experiencing narcosis (hibernation due to localized exposure to very high phosphine concentration), 2) more efficient to utilize the gas with greatly reduced amount of phosphine required to maintain the minimum target concentration or concentration-time (CT) product of 100% efficacy, 3) more friendly to environment due to no waste deactivation and disposal of residue, 4) needs shorter time due to quick establishment of target concentration thereby reducing total effective fumigation time.

Based on this efficacy result, the Ministry of Agriculture Republic of Indonesia has issued a minister decree No.: 4198/Kpts/SR.140/10/2011 permitting the distribution of ECO₂FUME 2 LG in Indonesia.

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