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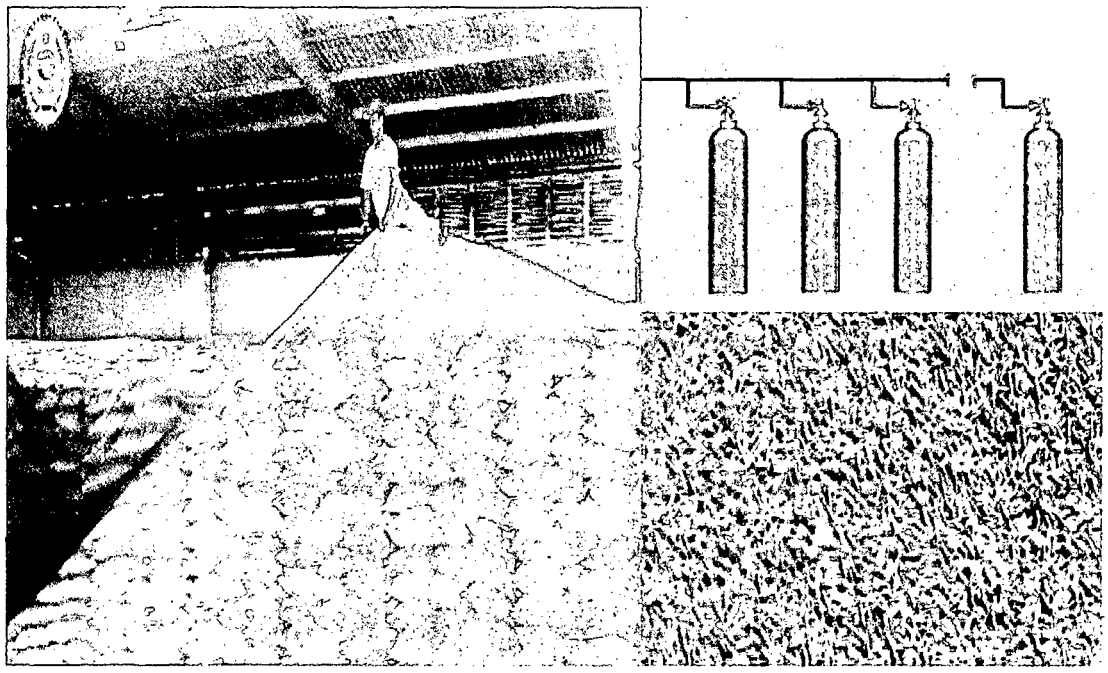
Ammendment to
UNIDO Contract No.: 99/174/VK
Project No.: MP/INS/98/107

DEMONSTRATION PROJECT :
ALTERNATIVES TO THE USE OF METHYL BROMIDE IN STORED PRODUCTS IN INDONESIA

FINAL REPORT



THE NATIONAL LOGISTICS AGENCY (BULOG)
DEPARTMENT OF PLANT PESTS AND DISEASES, IPB
SOUTH EAST ASIAN REGIONAL CENTRE FOR TROPICAL BIOLOGY, BOGOR

JANUARY, 2002

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EXECUTIVE SUMMARY

In the era of open and free market exporting countries have to have the capability to penetrate and compete with international traders. One of the factors to win in the fierce competition in the global market is quality assurance of exported commodities. Quality products certainly play an important role in order to capture the opportunity in international market. Therefore, Indonesia like any other agricultural exporting countries has to improve its agricultural products to meet quality and safety standards as demanded by consumers in importing countries.

In this respect the use of methyl bromide for maintaining quality of exported products is frequently practiced, as pre-shipment and quarantine treatments, controlling insect in storage and soil treatment. However, due to methyl bromide potency to deplete ozone layer, the use of this fumigant should be reduced and subsequently be phased-out at certain time frame. With such a rigid time schedule as stipulated in the Montreal Protocol, alternative technology to methyl bromide has to be found.

Three methods of technology alternative to methyl bromide, namely phosphine tablets, Eco2Fume and hermetic system were demonstrated for maintaining quality of paddy and corn and testing was carried-out in Bulog Research facilities in Tambun and Seameo Biotrop in Bogor. The two commodities were observed for six months and their quality was verified using various quality indicators such as moisture contents, percentage of broken kernels, number of insect and population of fungi.

Results of the observations indicated that out of the three methods demonstrated fumigation using Eco2Fume gave the most promising systems in terms of technical considerations based on indicators described previously. The number of insects, fungal invasion as well as aflatoxin production, was lower as compared to grain treated with phosphine tablets or hermetic systems. Eco2Fume also showed a quick dispersal of phosphine gas throughout the stack increased its effectiveness to control insects. It should be pointed-out, however, technical possibility of Eco2Fume should be supported by economic feasibility of this system in order to make such a treatment compatible to methyl bromide.

Hermetic systems actually could be feasible as alternative to methyl bromide providing the sealed conditions to ensure oxygen concentration less than 2% could be maintained throughout storage period. As soon as oxygen concentration increase due to leakage or inability to hold vacuum conditions, re-infestation and fungal invasion will become serious problems. During the first month of storage hermetic system was able to maintain quality of grain (both paddy and corn) in good conditions, no significant changes in moisture contents of the grain, percentage of broken kernels, invasion of fungi and insects. The observation had to be discontinued due to leakage of the plastic cover that directly increased the oxygen concentration constantly, until reaching normal level similar to ambient air.

Type of treated commodity played a key role in determining the adsorption rate of phosphine gas during exposure period of fumigation using phosphine tablets or Eco2Fume. In addition, moisture contents of the treated commodity had significant effect on phosphine gas adsorption; and high moisture (above 14%) tended to increase adsorption rate of phosphine gas , which reduce the effectiveness of fumigation.

The fungal infection test indicated that all treatments had been able to retard the development of fungi and production of aflatoxin. Hermetic systems gave a good control over fungi during storage period as far as vacuum conditions could be maintained. In the other hand Eco2Fume provided a better control of fungi and aflatoxin production throughout the observation, as compared to phosphine tablet treatment or hermetic systems. In this respect, the development of fungi and production of aflatoxin during storage could be kept minimal using three alternative methods.

The test to verify the susceptibility of three dominant species of storage insects **Rhyzopertha dominica, Tribolium castaneum and Sitophilus zeamais** to fenithrothion and phosphine, using FAO testing standard, gave indication that **R. dominica** had developed resistancy to both phosphine and fenithrothion at certain degree. The other two species, only slightly showed a potency of resistant towards fenithrothion but not to phosphine, meaning this fumigant still provided a hundred percent mortality. Recognising these two controlling systems are applied routinely in Bulog

storages, it is suggested that more thorough study be conducted to check the resistancy of all species of stored products in Bulog warehouses.

A short survey to verify the consumption of methyl bromide in Indonesia during 1998-2000 was carried-out to complement the previous survey. The results indicated that methyl bromide uses for fumigation declined slightly from 149 MT in 1998 to 146 MT in 2000. The reduction in methyl bromide uses probably due to less quantity of agricultural products exported by Indonesia within that period. In addition, rapid turn-over of Bulog rice from normally more than six months to three months had contributed to the decline in the use of methyl bromide for controlling insect in the storage.

From the three demonstrated technology applied to paddy and corn, confirmed that all the three systems have the potency to be developed further as technology alternative to methyl bromide. There was no technical difficulty in applying the methods, providing all supporting elements such as piping systems for Eco2Fume application, vacuum conditions for hermetic systems can be guaranteed.

It should be pointed-out, however, in order to provide a better picture on the application of all alternatives technology, observation on the implementation of all systems in actual operation have to be conducted. Such an effort is indeed necessary to get a better picture of the alternative to methyl bromide, recognising the danger of interpreting the results of relatively 'controlled' conditions into actual operation in the field.

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INTRODUCTION

It is generally known that in the era of liberalized market, quality product plays determinant role in winning the fierce competition in the global market. Various requirements have to be met in order to capture the opportunity in international market. Therefore agricultural countries like Indonesia have to improve their agricultural export commodities to meet quality and safety standards as demanded by consumers in importing countries.

Methyl bromide (MeBr) is still considered the most popular fumigant due to its effectiveness, short exposure period, easy to handle and not too complicated in its application. The methyl bromide popularity is declining due to its negative effect to ozone layer, and scientific evidence indicated that this fumigant has much stronger ozone depleting substance than chlorine from CFC. Therefore, technology alternative to this fumigant has to be found-out, since MeBr scheduled to be phased-out gradually as stated in the Montreal Protocol.

A demonstration project on alternative technology to methyl bromide was carried-out in Indonesia since early 2000. The activities conducted under this endeavour consisted of evaluation on technical and financial feasibility of alternative technologies such as the use of pressurized phosphine in combination with carbon dioxide (Eco_2Fume), the use of cotton sheet as physical protection against re-infestation, fumigation using phosphine tablet as fumigant and implementation of integrated storage pest management (ISPM). The assessments were conducted on milled rice, coffee and timber and the demonstrations were carried-out in three locations : Surabaya, Jakarta and Lampung. In addition, workshops and training were also conducted as a measure to disseminate outcome of the demonstrated technologies.

The results of demonstration project have proven that alternative technologies, namely: the use of solid and liquefied phosphine, cotton sheet barrier and ISPM are compatible to methyl bromide, providing some pre-requisites are met. Those pre-requisites, among others the initial quality of commodity stored has to be good, economically the alternatives technology are feasible and suited to the need of particular commodity. It should be pointed-out, however, adopting the technology in a large scale could be misleading, recognizing conditions in the actual operation are

relatively uncontrollable as compared to trial phase. In addition data on the use of alternatives to maintain quality of grains other than rice such as corn, and paddy are deemed necessary, since these two products are important commodities in domestic as well as international markets.

Information collected from various sources provides other alternatives technology with bright prospect such as hermetic storage system. Therefore in the additional activities related to the MP/INS/98/107 further demonstration works on the use of hermetic storage is conducted in addition to liquid and solid phosphine as fumigant. Testing the susceptibility of dominant insect to phosphine is also important data to support the use of this fumigant for controlling insect of stored grains. To get the latest data on the use of methyl bromide in Indonesia a short survey was conducted to collect figures on the use of this fumigant carried-out by fumigators and importers of methyl bromide.

MATERIALS AND METHODS

The continuation of demonstration projects on alternatives technology to methyl bromide were carried-out in two locations; one in a small storage test located in the Seameo-Biotrop complex in Bogor and in actual operational storage located in Bulog Food Technology Research complex in Tambun, near Jakarta. Basically in both location demonstration activities were similar, the only different was on the number of stacks for each commodity since the length of observation would be set-out three months in Bogor whereas in Tambun nine months for paddy and three months for corn. With such arrangements in Tambun the total number of stacks for corn was 6 stacks and paddy was 10 stacks. In Bogor there were 12 stacks altogether in which both corn and paddy had 6 stacks each and period of observation for three months.

a. Materials

Two commonly stored agricultural commodities were used for this purpose, namely corn and paddy (un-husked rice), with quantity of 12 metric tons (MT) each. Both commodities were placed in polypropylene bag of 50 kg capacity per bag but for this purpose each bag was filled with 35 kg corn or paddy in order to make them easy to handle.

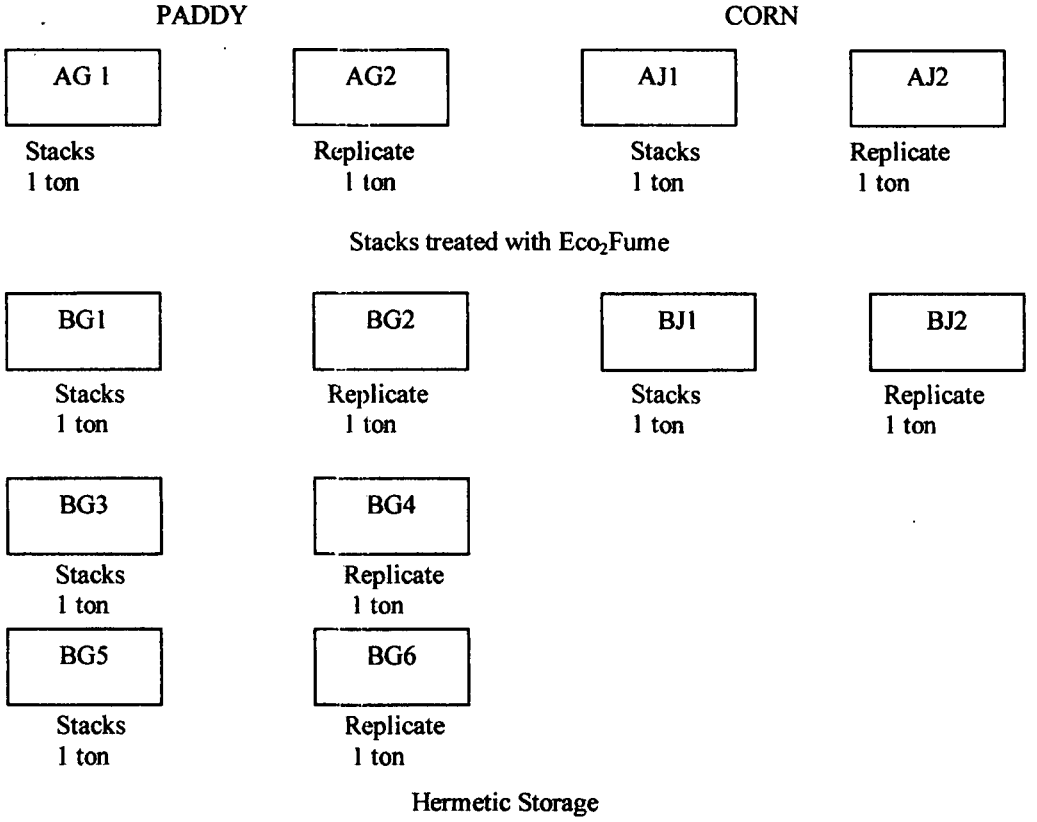
In Tambun the number of stacks were 16, with approximately one MT per stack and arrangement for both corn and paddy was set-out as follow: corn six stacks and paddy ten stacks, thus altogether there were 16 stacks.
In Bogor number of stack was 12 stacks consisted of corn and paddy which had similar number of stacks : six and the quantity was approximately one MT per stack.

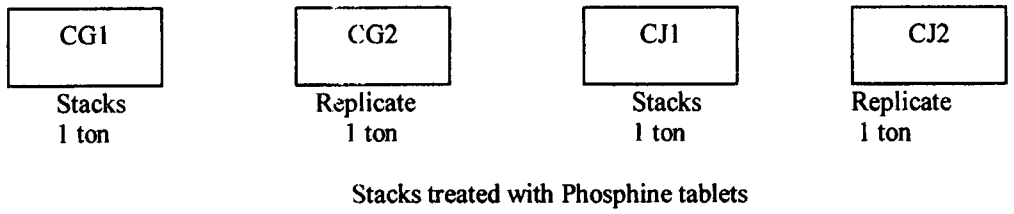
Newly harvested paddy was used for this project and purchased from local farmers near Tambun and paddy was then dried using sun-drying until the moisture content reached 14% to 14.5 % and then placed in plastic bags prior to stacking.

Corn was purchased in the form of un-sheeted corn on the cobs and dried using sun drying until the moisture reached 14%-14.5% and then unshelled and placed into plastic bags with quantity of 35 kg per bag and these bags were stacked in the storage.
Lay-out of bags and stacking in each location as shown in Figures 1 and 2.

Figure 1. LAY OUT of STACKS in TAMBUN RESEARCH CENTER

A. 3 months Storage





B. 4-9 months Storage

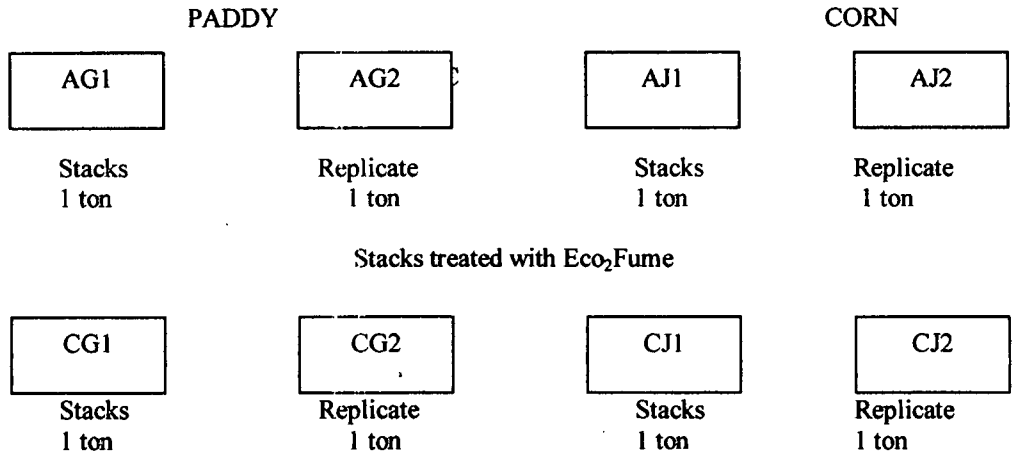
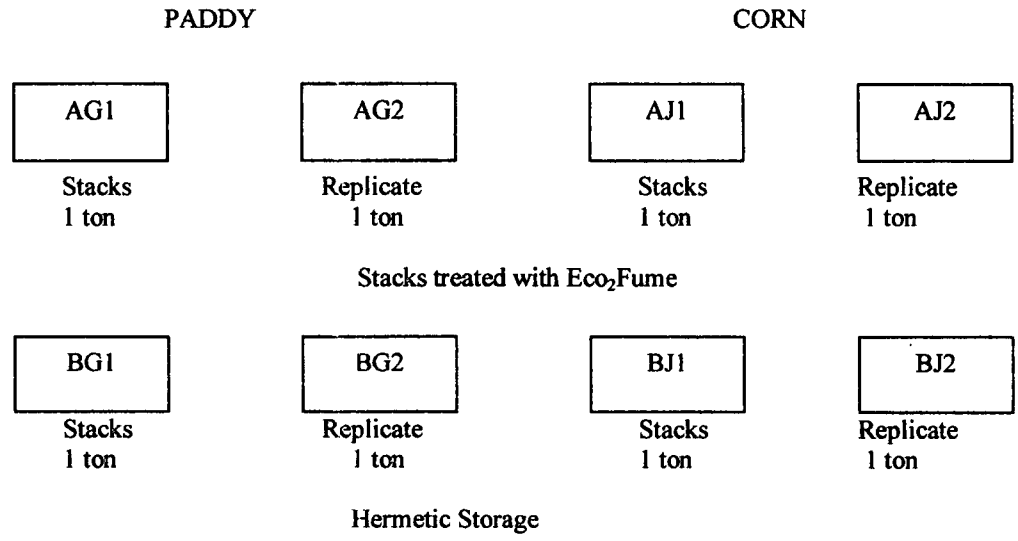
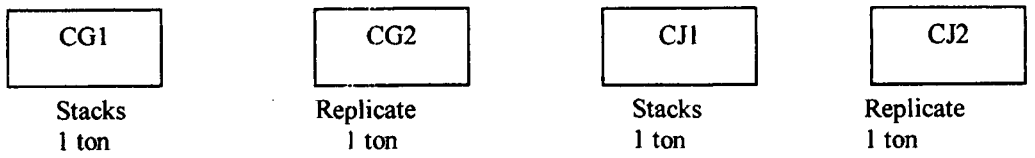


Figure 2. LAY OUT of STACKS in BIOTROP

A. 3 months Storage





Stacks treated with Phosphine tablets

Sheeting of the stacks

A good quality of gas tight poly-vinyl chloride (PVC) plastic sheet with 0.25 mm thickness was used as plastic cover and 0.76 mm thickness for base plastic placed under the stacks The permeability of these plastic had been tested in Industrial Testing Material Laboratory under the Ministry of Trade and Industry in Bogor and the report indicated that all of the plastic intended for fumigation and vacuum plastic enclosure to simulate 'hermetic storage' had passed the minimum permeability test done for this purpose.

The plastic sheet suited to the stack and the dimension of each plastic sheet was (2 x 1.7 x 1.8) m and the number of plastic sheets needed for treatment A (fumigation with phosphine tablet as control) and C (fumigation using Eco_2Fume @) was 16, for both paddy and corn, 8 sheets per commodity. For treatment B (hermetic treatment) 12 sheets were used; 8 for paddy and 4 for corn.

Prior to construction of stacks storage was cleaned and repaired to minimize crack and crevices which were considered potential source of insect infestation. Cleaned storage then sprayed with contact insecticide with fenitrothion as active ingredient at the rate of 0.75 cc/m2, intended to control residual insects and to provide protection against incoming insects.

Surface spraying was also carried-out at the peripheral of the stack and walls of the storage intended to control crawling insects on the surface of the stacks . Spraying was repeated at interval of four weeks until end of observation.

b. Methods

As indicated earlier both paddy and corn were dried to lower the moisture to reach 14%-15%, and then packed in plastic bags ready for stacking and further treatment.

Treatment

There were three treatment applied in this project and the description as follows:

a. Treatment A : grain fumigated using pressurized fumigant Eco_2Fume at recommended dosage rate which produced at least 200 part per million (ppm) phosphine gas throughout the exposure period minimum of four days.
b. Treatment B: grains were placed under plastic enclosure and vacuumed using vacuum cleaner apparatus and oxygen concentration was maintained at maximum less then 2%
c. Treatment C: grain fumigated using phosphine tablet at dosage rate 2 tablets per metric ton and exposure period minimum of five days Treatment C served as control for this activities

Each treatment had two replicates and observation period minimum three months except for paddy in Tambun where observation period was extended to reach nine months, to simulate normal Bulog storage period for paddy.

It was planned that all activities both in Tambun and Bogor could be started at the same period in April 2001, however due to difficulties in getting corn, the demonstration in Bogor was slightly delayed for almost four weeks. Time schedule of all activities in two sites as depicted in Annex 1 and Annex 2.

Bioassay and measuring gas concentration.

To evaluate the effectiveness of fumigations, bioassay using a 50 g of brown rice infested with 25 ***Sitophilus zeamais,*** and and another 50 g of brown rice infested with 25 ***Tribolium castaneum.*** Both insect tests four tubes of each species were placed near gas concentration monitoring tubing-line: therefore in each stack there were 8 bio-assay tubes in place prior to fumigation. After fumigation, bio-assay tubes were removed and examined for insect mortality. The number of live and dead adult insects were recorded and compared with the control. Evaluation on effectiveness of fumigation were continued by examining bio-assay tubes until four weeks after fumigation. The results of these examination would ensure that fumigation had controlled insects of pre-adult stages (egg, larvae, and pupae). The number of adults emerged in the control

samples provided an indication of the level of infestation of pre-adults at the time of fumigation.

Gas concentration under the plastic sheet was measured using a gas monitoring tubing-line, placed in top, middle and bottom levels adjacent to the corner of each stack, that made of three monitoring points per stack. A phosphine -and oxygen- meters were connected directly to the monitoring tubing-line and reading was carried-out one hour after fumigation and measurement of phosphine gas and oxygen concentrations was repeated in the following days during working hours at interval of four hours.

During fumigation physical conditions such as temperature and relative humidity in the storage were recorded

Bait-traps

To monitor the possible development of insect population in all treated stacks, bait traps were placed in three places in each stack. The bait traps were observed at monthly interval to figure out species of insects found and to monitor the population of insect in each stack.

Bait trap was made of perforated plastic mesh filled with brown rice which had relatively high percentage of broken kernels, intended to attract insect to harbor in the trap. The number of insects trapped in each bait trap was counted to predict the level of insect infestation in each stack.

Sampling and sample analyses

Samples for assessing various parameters were collected from determined bags at each stack from three different levels (top, middle and bottom levels) of all treatment. Sampling spear was used to draw samples from the pre-determined bags and mixed and then divided for sample analyses using Boerner divider. Analyses including the followings: moisture contents of the grains, percentage of broken kernels, number of live or dead insects found, number of yellow grains and chalky kernels (for paddy), and percentage of damaged grains and percentage of foreign matters for corn. Physical analysis of all samples collected was conducted in Bulog laboratory in Tambun.

Mycology and aflatoxin tests

Samples of both corn and paddy were tested for possibility of fungal infestation and testing was conducted at the beginning of the demonstration and the tests were repeated at interval of four weeks. Samples were plated in agar media and species of fungi infected the grain were then identified using standard fungi identification method. Mycology and aflatoxin tests were conducted in Seameo Biotrop laboratory in Bogor.

Standard procedure for aflatoxin test using HPLC was conducted to verify the possibility of aflatoxin contamination in both corn and paddy. Aflatoxin tests were conducted at interval of four weeks.

Susceptibility tests

To verify the possibility of dominant insects resistant towards phosphine, a susceptibility tests were carried-out. Three species of insects ***Rhyzopertha dominica, Sitophilus zeamais*** and ***Tribolium castaneum*** were used for testing susceptibility of these insects to phosphine gas. A FAO standard for susceptibility test was used for this purpose. The number of insect per species was 50 with known age (1-14 days).

Technical analysis

To verify the feasibility of alternatives technology to methyl bromide demonstrated in the project, a technical analysis would be carried-out, based on various parameters such as efficacy of each treatment, technical requirement etc. On technical analysis advantage and disadvantage of each alternative technology would be assessed based on quality changes of the grains during storage period, complexity of its application, and implementation procedures as compared to methyl bromide. Since in this demonstration methyl bromide was not applied, therefore the results of previous works done in the first phase of this project would be used as reference.

RESULTS AND DISCUSSION

a. Results from Tambun Research Center

- **Phosphine and Eco_2Fume Concentration**

The gas concentrations during the first, second, third, fourth, and fifth days of fumigation are presented in Table 1.

Table 1. Daily changes in phosphine and Eco_2Fume concentrations (ppm) in paddy and maize during fumigation at BULOG warehouse, Tambun

Commodity/fumigant		FUMIGATION									
		First		Second		Third		Fourth		Fifth	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Paddy											
Phosphine	1	128	138	716	821	205	400.5	52.5	162.5	9	25.5
	2	125	132	724	857	172.5	390.5	49	154.5	8.5	24.5
	X	**126.5**	**135**	**720**	**839**	**188.75**	**395.5**	**50.75**	**158.5**	**8.75**	**25**
Eco_2Fume	1	560	2000	1321.5	785	712	315	350	161.5	85.5	36
	2	680	2000	1725	765	693	350	254.5	162.5	82.5	36
		620	**2000**	**1523.3**	**775**	**702.5**	**332.5**	**302.25**	**162**	**84**	**36**
Maize											
Phosphine	1	170	165	1962	1634	1224.5	1104	910	699.5	308	167.5
	2	155	134	2000	1673	1335	1049.5	905.5	676.5	264	172
		162.5	**149.5**	**1981**	**1653.5**	**1279.8**	**1076.8**	**907.75**	**688**	**286**	**169.75**
Eco_2Fume	1	976	2000	2000	1661.5	1700	1190	1172.5	819	549	439
	2	570	2000	2000	1785	1658	1183.5	111.5	772.5	512	427.5
		773	**2000**	**2000**	**1723.3**	**1679**	**1186.8**	**642**	**795.75**	**530.5**	**433.25**

The phospine gas in Eco_2Fume fumigation released much faster compared to phosphine fumigation possibly due to the present of CO_2 in Eco_2Fume, which functions as carrier to speed up phosphine released. During fumigation period the concentrations of phosphine and Eco_2Fume on maize stacks were higher than those in paddy stacks (Figure 3), due to the difference in gas absorption of the commodities. All fumigants are, to some extent, sorbed by commodities. According to AFHB and ACIAR (1998) the rate of absorption was depended primarily on the type of fumigant and the commodity fumigated. In addition prevailing temperature and humidity also influenced gas absorption.

Figure 3 also shows that during fumigation, either in paddy or maize stacks, the decrease of phosphine concentrations was slower in Eco_2Fume than that of phosphine.

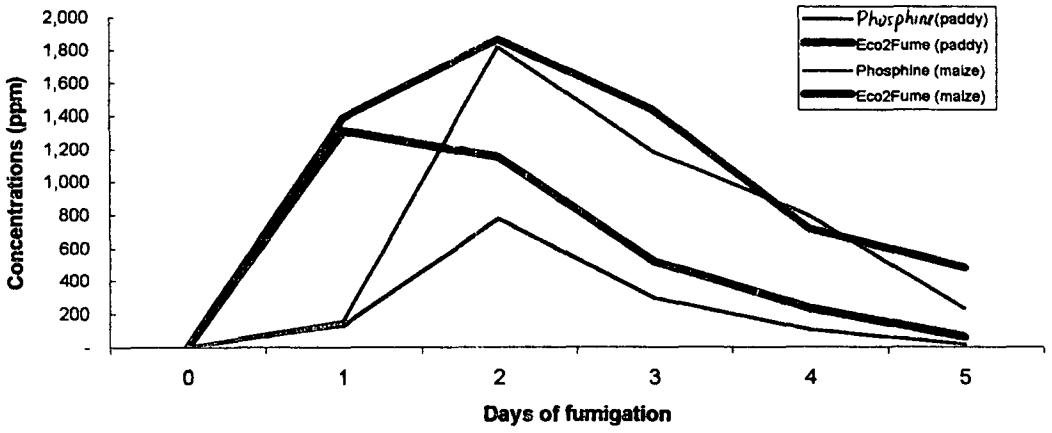


Figure 3. Daily changes in phosphine concentrations in paddy and maize during fumigation

- **Temperature and relative humidity in the storage**

The daily average temperature and relative humidity of the storage during 5 days of fumigation. ranged between 27.5 – 32 °C and 61 – 73 %, respectively (Table 2).

Table 2. The daily average temperature during phosphine and Eco_2Fume fumigation at BULOG warehouse in Tambun

1	32	61.5
2	31	61
3	29.5	68.5
4	30	68.5
5	27.5	73

- **Moisture content**

Moisture content of either paddy or maize fumigated with phosphine and Eco_2Fume had a same pattern. It increased after fumigation (7 days of storage) and then decreased after 90 days of storage (Figures 4 and 5), except moisture content of maize fumigated with phosphine. The moisture content of maize fumigated with phosphine was relatively constant (Figure 5).

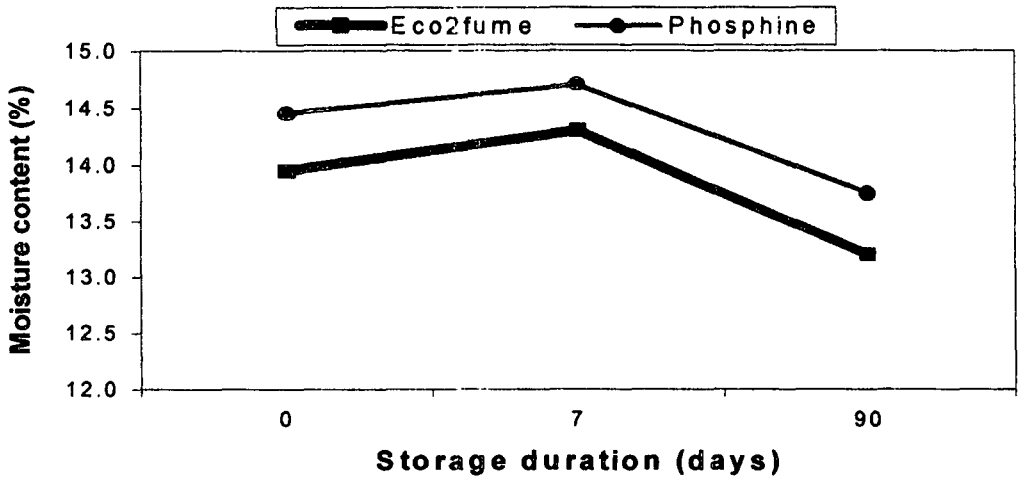


Figure 4. Moisture content of **paddy** fumigated with phosphine and Eco_2Fume during storage

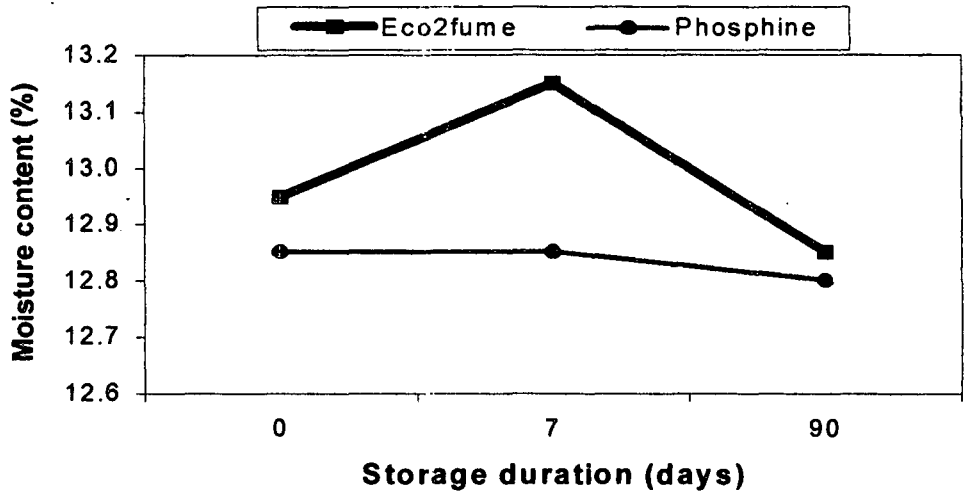


Figure 5. Moisture content of **maize** fumigated with phosphine and Eco_2Fume during storage

- **Species and total fungal population**

Eighteen fungal species were isolated from **paddy** treated with the two fumigants during storage. They were *Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. penicillioides, A. tamarii, Chaetophoma* sp., *Curvularia lunata, C. pallescens, Eupenicillium cinnamopurpureum,*

Eurotium chevalieri, E. repens, Fusarium semitectum, Penicillium citrinum, P. islandicum, P. oxalicum, Wallemia sebi and 1 unidentified species.

The number of fungal species on paddy decreased after fumigation (after 7 days of storage). The number of fungal species on paddy at the beginning of storage and after Eco_2Fume fumigation were 8 and 7 species, respectively, while on paddy at the beginning of storage and after phosphine fumigation were 8 and 6 species, respectively (Annex 3).

A. candidus, A. flavus, A. fumigatus and *A. tamarii* were always isolated from paddy treated with Eco_2Fume, while *A. candidus, A. flavus, A. niger* and *A. tamarii* were always isolated from paddy treated with phosphine tablets.

Thirteen fungal species were isolated from **maize** treated with the two fumigants during storage. They were *Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. penicillioides, A. tamarii, Eurotium chevalieri, E. repens, Mucor praini, M. saturnius, Penicillium citrinum, Synchephalastrum racemosum* and *Wallemia sebi.*

The number of fungal species on maize increased after Eco_2Fume fumigation (after 7 days of storage), however, it was constant after phosphine fumigation. The number of fungal species on maize at the beginning of storage and after Eco_2Fume fumigation were 7 and 8 species, respectively, while on maize at the beginning of storage and after phosphine fumigation were both 7 species (Annex 4).

A. candidus, A. flavus, A. fumigatus, A. niger and *Eurotium chevalieri* were always isolated from maize treated with Eco_2Fume, while *A. candidus, A. flavus, A. niger* and *E. chevalieri* were always isolated from maize treated with phosphine.

The effect of phosphine and Eco_2Fume on total fungal population of paddy had a same pattern. The total fungal population on paddy increased after fumigation (after 7 days of storage) and after 90 days of storage, but the increase caused by phosphine fumigation was higher than that of Eco_2Fume fumigation (Figure 6). The population of fungi on maize decreased after phosphine fumigation and then increased after 90 days of storage, while the population on maize fumigated with Eco_2Fume increased after fumigation and after 90 days of storage (Figure 7). Dharmaputra *et al* (1993) reported that total fungal population on soybean meal treated with phosphine decreased after treatment, and then increased during storage (190 days).

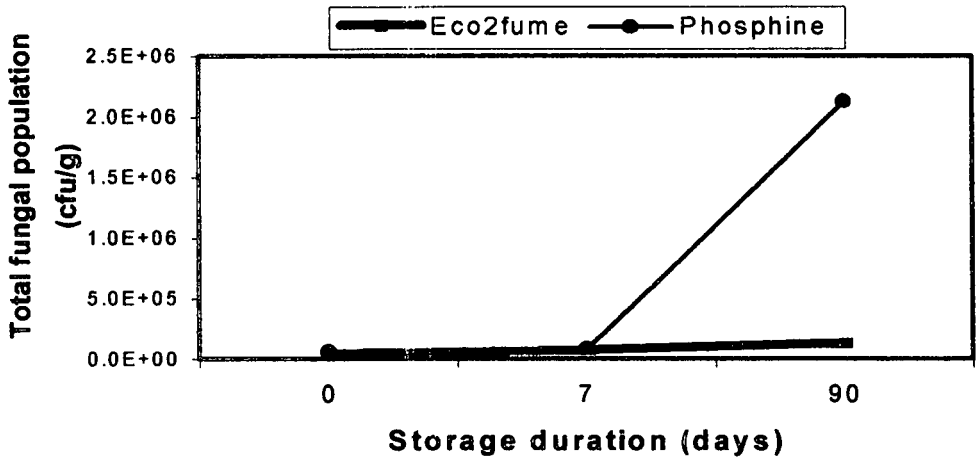


Figure 6. Total fungal population on **paddy** fumigated with phosphine and Eco_2Fume during storage

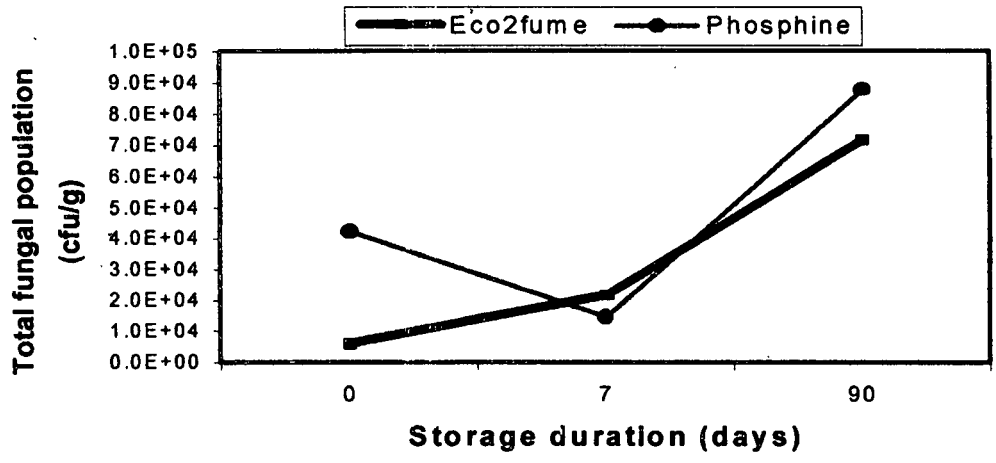


Figure 7. Total fungal population on **maize** fumigated with phosphine and Eco_2Fume during storage

- ***Aspergillus flavus* population**

The effect of phosphine on the population of *A. flavus* on paddy and maize had a different pattern. *A. flavus* population on paddy fumigated with phosphine decreased after fumigation (after 7 days of storage) and 90 days of storage, while that on maize increased after fumigation and 90 days of storage (Figures 8 and 9). It was assumed that the decrease in *A. flavus* population on paddy fumigated with phosphine was due to the

effect of the fumigants. Dharmaputra *et al.* (1991) reported that mycelium growth of 3 isolates of *A. flavus* was almost totally inhibited after phosphine fumigation at concentration of 3.5 mg/L. According to Dharmaputra and Putri (1999) phosphine at concentration of 3.5 mg/L caused 99% of inhibition to the growth of *A. flavus in vitro.*

The effect of Eco_2Fume on the population of *A. flavus* on paddy and maize had a same pattern. *A. flavus* population on paddy or maize fumigated with Eco_2Fume increased after fumigation and decreased after 90 days of storage (Figures 8 and 9).

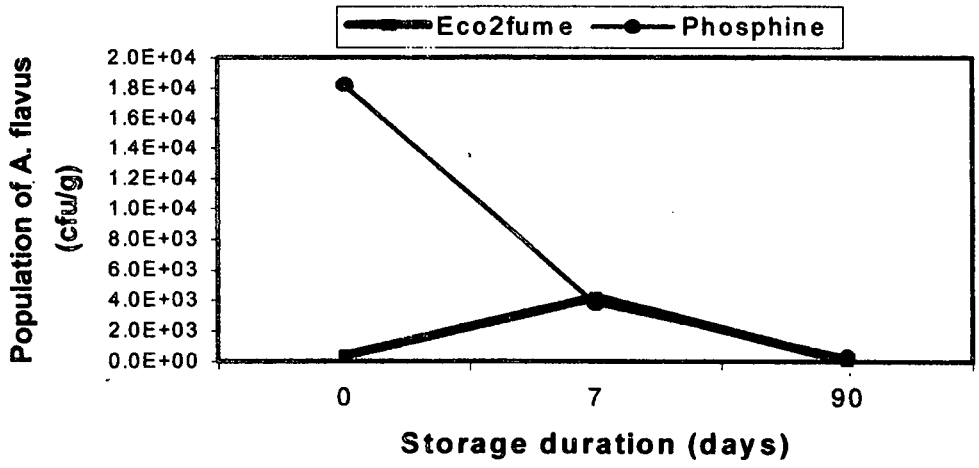


Figure 8. Population of *A. flavus* on **paddy** fumigated with phosphine and Eco_2Fume during storage

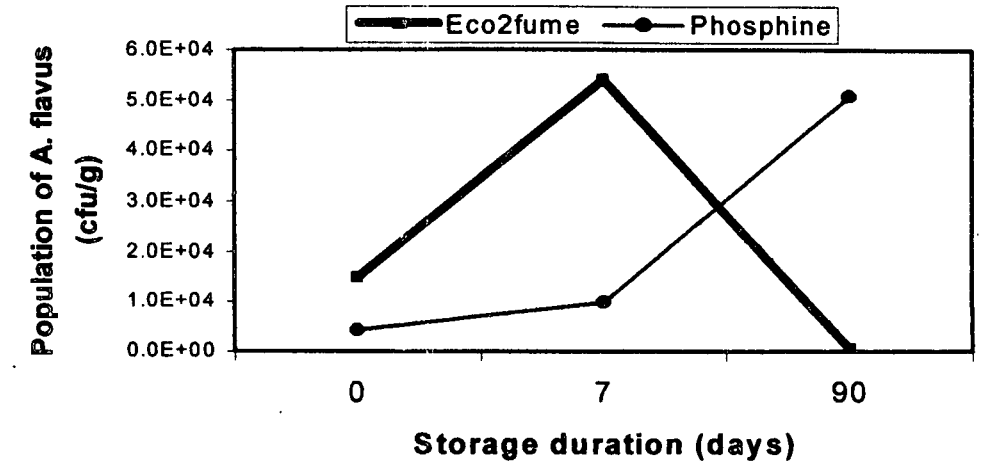


Figure 9. Population of *A. flavus* on **maize** fumigated with phosphine and Eco_2Fume during storage

- **Total aflatoxin content of maize**

Four types of aflatoxin were detected on maize during storage, namely aflatoxin B_1, B_2, G_1 and G_2. Aflatoxin B_1 was always detected during storage. Total aflatoxin content on maize treated with the two fumigants increased after fumigation and decreased after 90 days of storage (Figure 10).

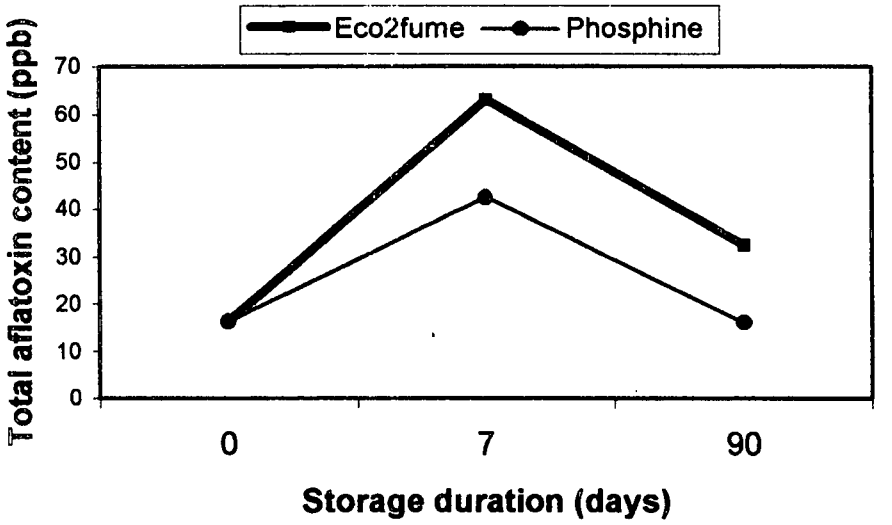


Figure 10. Total aflatoxin content on maize fumigated with phosphine and Eco_2Fume during storage

- **Bait Trap**

The number of insects found in the bait traps is shown in Table 3. There was no insect found in bait traps placed on paddy and maize stacks fumigated with phosphine as well as Eco_2Fume after 30 days of storage, but after 60 and 90 days of storage a relatively low number of insect was observed on paddy and maize stacks fumigated with the two fumigants. The number of insects in the bait traps placed either on maize or paddy stacks fumigated with phosphine was higher than the one fumigated with Eco_2Fume. The number of trapped insects found in the bait traps indicated insect population level at the storage environment.

Table 3. Number of live insects in bait traps during storage at BULOG warehouse in Tambun

Commodity/ fumigant			DURATION OF STORAGE (days)							
			30	60	90				30	60	90
Paddy						**Maize**					
Phosphine	I	1	0	1	1	Phosphine	I	1	0	0	1
		2	0	0	3			2	0	0	2
		3	0	0	1			3	0	0	3
		4	0	1	2			4	0	0	1
Total			**0**	**1**	**7**	**Total**			**0**	**0**	**7**
Average			**0**	**0.25**	**1.75**	**Average**			**0**	**0**	**1.75**
Phosphine	II	1	1	2	5	Phosphine	II	1	0	1	2
		2	0	0	3			2	0	0	1
		3	0	1	1			3	0	0	2
		4	0	1	5			4	0	0	1
Total			**1**	**4**	**14**	**Total**			**0**	**1**	**6**
Average			**0.25**	**1**	**3.5**	**Average**			**0**	**0.25**	**1.5**
Eco_2Fume	I	1	0	0	1	Eco_2Fume	I	1	0	0	2
		2	0	0	2			2	0	0	1
		3	0	0	2			3	0	0	3
		4	0	1	1			4	0	1	1
Total			**0**	**1**	**6**	**Total**			**0**	**1**	**7**
Average			**0**	**0.25**	**1.5**	**Average**			**0**	**0.25**	**1.75**
Eco_2Fume	II	1	0	1	2	Eco_2Fume	II	1	0	0	2
		2	0	0	1			2	0	2	3
		3	2	2	0			3	0	0	1
		4	0	0	2			4	0	0	1
Total			**2**	**3**	**5**	**Total**			**0**	**2**	**7**
Average			**0.5**	**0.75**	**1.25**	**Average**			**0**	**0.5**	**1.75**

- **Bioassay**

The result of the bioassay test is shown in Table 4. After 7 days of fumigation using phosphine as well as Eco_2Fume , some of *R. dominica* were still alive, while all *S. zeamais* was dead. There was an indication that *R. dominica* become resistant to phosphine, however, further study is necessary to prove this assumption.

Table 4. Number of live control insects on stacks during fumigation

Commodity/fumigant		Insect species	
		R. dominica	*S. zeamais*
Paddy:			
Phosphine	1	20,5	0
	2	22	0
Total		**42,5**	**0**
Average		**21.25**	**0**
Eco_2Fume	1	22	0
	2	17,5	0
Total		**39,5**	**0**
Average		**19,75**	**0**
Maize:			
Phosphine	1	0	0
	2	0,25	0
Total		**0,25**	**0**
Average		**0,125**	**0**
Eco_2Fume	1	3,75	0
	2	12,25	0
Total		**16**	**0**
Average		**8**	**0**

- **Insects population inside of the bags**

Six insect species were found in paddy and maize fumigated with phosphine as well as Eco_2Fume during 90 days of storage. These insects were *Cryptolestes* spp., *Liposcelis entomophilus* (Enderlein), *Oryzaephilus surinamensis* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) (Table 5).

Table 5. Population of each insect species (insects/kg) on paddy and maize during storage at BULOG warehouse in Tambun

Insect	Duration of storage (days)					
	0	45	90	0	45	90
	PADDY			MAIZE		
PHOSPHINE						
Cryptolestes	0	3	6.5	0	6	12.5
Liposcelis entomophilus	0	2.5	5	0	2	5.5
Oryzaephilus	0	1	3	0	0	0
surinamensis	0	1	0	0	0	0
Rhyzopertha dominica	0	0	0	0	0.5	0.5
Sitophilus zeamais	0	2	3		0.5	0.5
Tribolium castaneum						
TOTAL	0	9.5	17.5	0	9	19
Eco_2Fume						
Cryptolestes	0	0	1.5	0	0.5	1.5
Liposcelis entomophilus	0	2	3.5	0	1.5	4
Oryzaephilus	0	1.5	0	0	0	1
surinamensis	0	1	1.5	0	0	0
Rhyzopertha dominica	0	0	1	0	2	0.5
Sitophilus zeamais		1	7		1	3.5
Tribolium castaneum						
TOTAL	0	5.5	14.5	0	5	10.5

The total insects population on paddy and maize stacks fumigated with phosphine was higher as compared to fumigated with Eco_2Fume after 45 and 90 days of storage (Figure 11). The total insects population on paddy stacks treated with phosphine after 45 and 90 days storage was 9.5 and 17.5 insects/kg respectively, while for those treatment with Eco_2Fume was 4.5 and 14.5 insects/kg. The total population of insects on maize stacks fumigated with phosphine after 45 and 90 days of treatment was 9 and 19 insects/kg, while those treated with Eco_2Fume was 5 and 10.5 insects/kg.

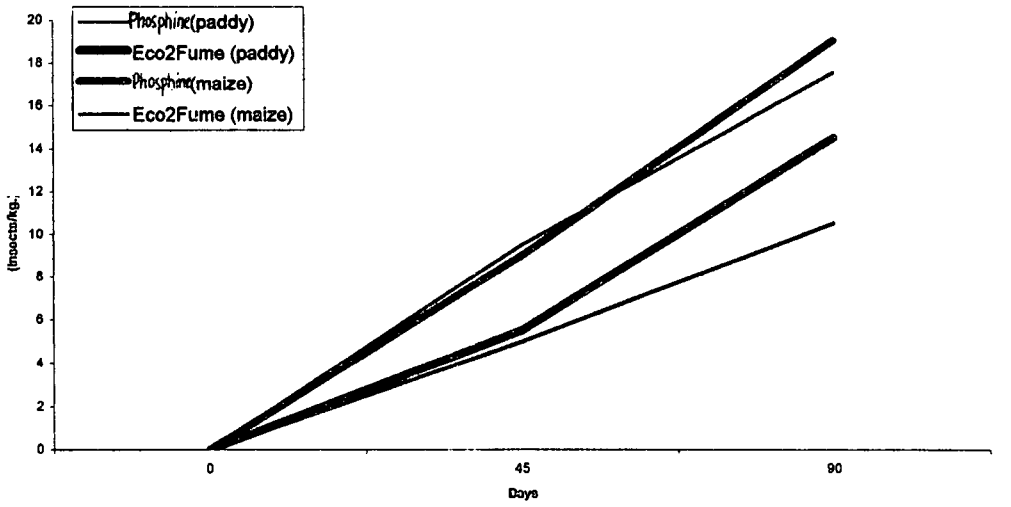


Figure 11. Total population of insect (insects/kg) on paddy and maize during storage

From the increase of insects population on the stacks 90 days after Eco_2Fume as well as phosphine treatments, it could be concluded that there was an overall increase in insects reinfestation.

- **Physical properties of paddy and maize**

The effect of phosphine and Eco_2Fume on the percentage of physical properties of paddy and maize had a same pattern. The percentage of physical property of **paddy**, in terms of empty, damaged and chalky/green kernels found in treated either with phosphine or Eco_2Fume increased after 90 days of storage (Figure 12). In general, the percentage of physical property of maize, in terms of damaged, broken, wrinkle and hole kernels found in treated either with phosphine or Eco_2Fume increased after 90 days of storage (Figure 13).

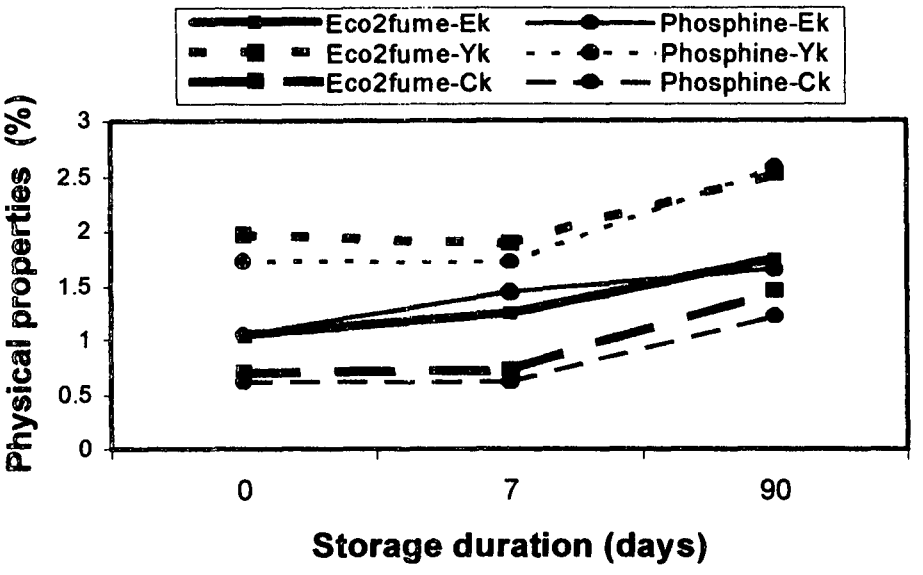


Figure 12. Physical properties of **paddy** fumigated with phosphine and Eco_2Fume during storage
(Ek = Empty kernels; Dk = Damaged kernels; Ck = Chalky kernels)

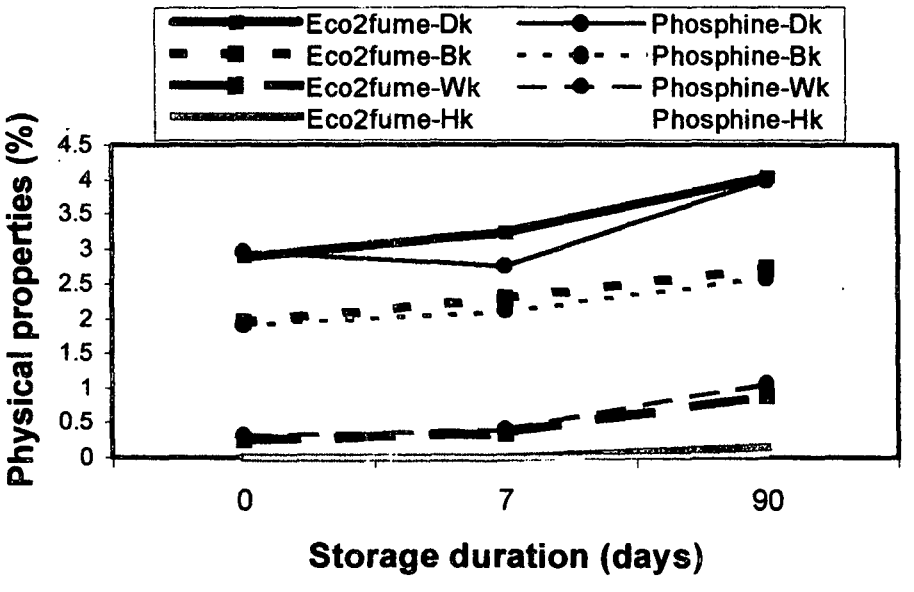


Figure 13. Percentage of physical properties on **maize** fumigated with phosphine and Eco_2Fume during storage
(Dk = Damaged kernels; Bk = Broken kernels; Wk = Wrinkle kernels; Hk = Hole kernels)

The increase of percentage of physical properties was caused by the increasing of total population of insects (Figure 11). Dharmaputra *et al.* (1994) reported that there was a correlation between population of *Sitophilus zeamais* and percentage of damaged kernels of maize.

b. Results from SEAMEO-BIOTROP

Treatment conducted in Seameo Biotrop was slightly delayed due to difficulties in collecting fresh paddy and corn from surrounding areas. The treatment was started in June 2001 almost two months behind the schedule. Fumigation with phosphine tablets was conducted soon after all stacks were ready for treatment. Fumigation had been done with similar protocol as described in the previous section in Tambun .

As indicated earlier hermetic stacks were broken down due to inability to maintain low oxygen concentration, as substitution, hermetic systems was tried in laboratory scale instead of operational scale. Small plastic bags (5 kg) were filled with rice and then vacuumed using special vacuum suction pump to make oxygen concentration less than 2%. The bags then stored to stimulate hermetic systems, and all procedures of quality testing, insects population and other tests were conducted similar to another treatments. Lab-scale test was only carried-out in SEAMEO BIOTROP laboratory.

The results of new hermetic systems indicated that moisture contents of both paddy and corn under low oxygen concentration (less than 2%) were relatively better than the one stored under phosphine tablets and Ecofume treatments. These findings had proven that oxidation process or another internal chemical reaction within paddy kernels and corn kernels seemed to be stopped due to lack of oxygen. Oxidation and chemical changes within grain kernels usually considered as primary source of grain quality deterioration during storage, could be minimized and the grains quality could be maintained relatively well. It gave indication that hermetic systems could be used as one alternative to maintain grain quality during storage without the use of chemical treatment such as fumigation or insecticide spray.

- **Phosphine and Eco_2Fume concentrations**

Concentrations during day 1-7 after fumigation are presented in Table 6.

Table 6. Daily changes in phosphine and Eco_2Fume concentrations (ppm) in paddy and maize stacks during fumigation

Commodities/ Fumigants		Fumigation (days)													
		First		Second		Third		Fourth		Fifth		Sixth		Seventh	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Paddy															
Phosphine	1	566	607	356.5	370.5	375	191	124	153	88.5	63	61	43	37	27
	2	567	615	363	214	215.5	201.5	153	88.5	86	70	61	37	40	60.5
		566.5	**611**	**359.75**	**292.25**	**295.25**	**196.25**	**138.5**	**120.75**	**87.25**	**66.5**	**61**	**40**	**38.5**	**43.75**
Eco_2Fume	1	1523	440	1698	1174.5	422	236	321	134	214.5	210	153	24	101.5	19
	2	2000	2000	1725	1007.5	484	240	314	136.5	61	63.5	12.3	25.5	101.5	19.5
		1761.5	**1220**	**1711.5**	**1091**	**453**	**238**	**317.5**	**135.25**	**137.75**	**136.75**	**82.65**	**24.75**	**101.5**	**19.25**
Maize															
Phosphine	1	850	1330	651	1148	494.5	942	351.5	820.5	267.5	721.5	227	627	175.5	538.5
	2	819	1310	654	1128	490.5	908	306.5	783.5	271	698	225.5	630	177	539
		834.5	**1320**	**652.5**	**1138**	**492.5**	**925**	**329**	**802**	**269.25**	**709.75**	**226.25**	**628.5**	**176.25**	**538.75**
Eco_2Fume	1	923	975	1404	2000	1393	1420	1251.5	1145.5	1094	880.5	983	692.5	838.5	586
	2	2000	2000	2000	2000	1429	1331	1257.5	1150	1108	999.5	981.5	662.5	830.5	523.5
		1461.5	**1487.5**	**1702**	**2000**	**1411**	**1375.5**	**1254.5**	**1147.75**	**1101**	**940**	**982.25**	**677.5**	**834.5**	**554.75**

The release of phospine gas from Eco_2Fume fumigation recorded faster compared to phosphine fumigation. The presence of carbon dioxide as carrier in Eco_2Fume, seemed to speed up phosphine gas released.

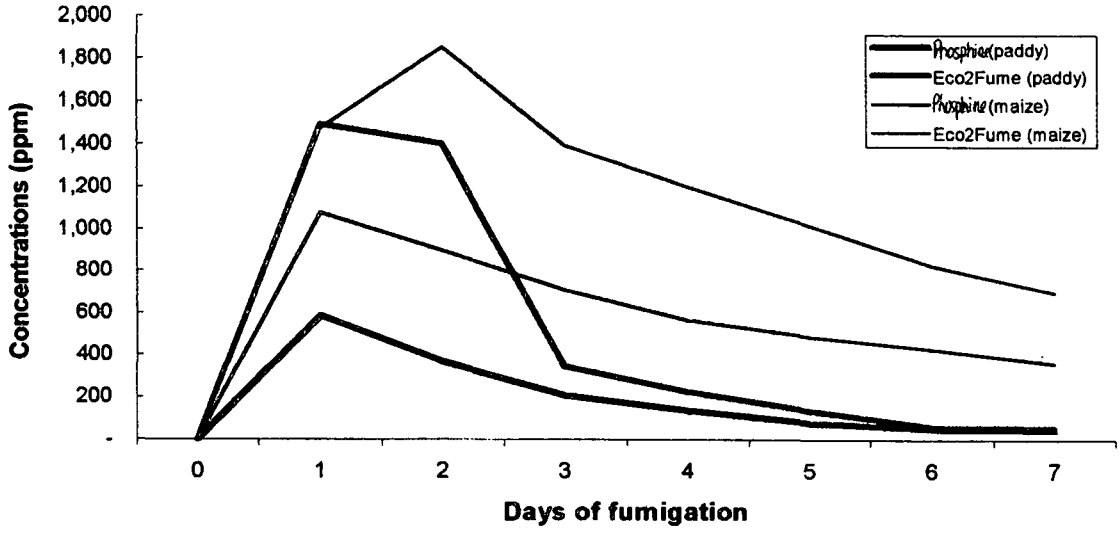


Figure 14. Changes in phosphine gas concentrations in the treated paddy and maize stacks during fumigation

During fumigation the concentrations of phosphine in the treated maize stacks were higher than those in paddy stacks (Figure 14). The difference in gas absorption rates of the two commodities might be responsible for this situation. All fumigants were, to some extent, sorbed by commodities, as reported by AFHB and ACIAR (1989) that the rate of gas absorption depended primarily to the fumigant itself and the commodity fumigated. Physical factor such as ambient temperature and humidity had significant influence to gas absorption during fumigation. Figure 14 shows that during fumigation, either in paddy or maize stacks, the decrease of phosphine gas concentrations in Eco_2Fume treated stack was higher than that of phosphine tablets.

- **Temperature and relative humidity of the storage**

The daily average temperature and relative humidity of the storage during 90 days of storage ranged between 25 – 28° C and 63.5 – 90 %, respectively. In general the daily average temperature and relative humidity seemed to be stable (Figures 15 and 16), the experiment was conducted in May – July 2001 (during the dry season).

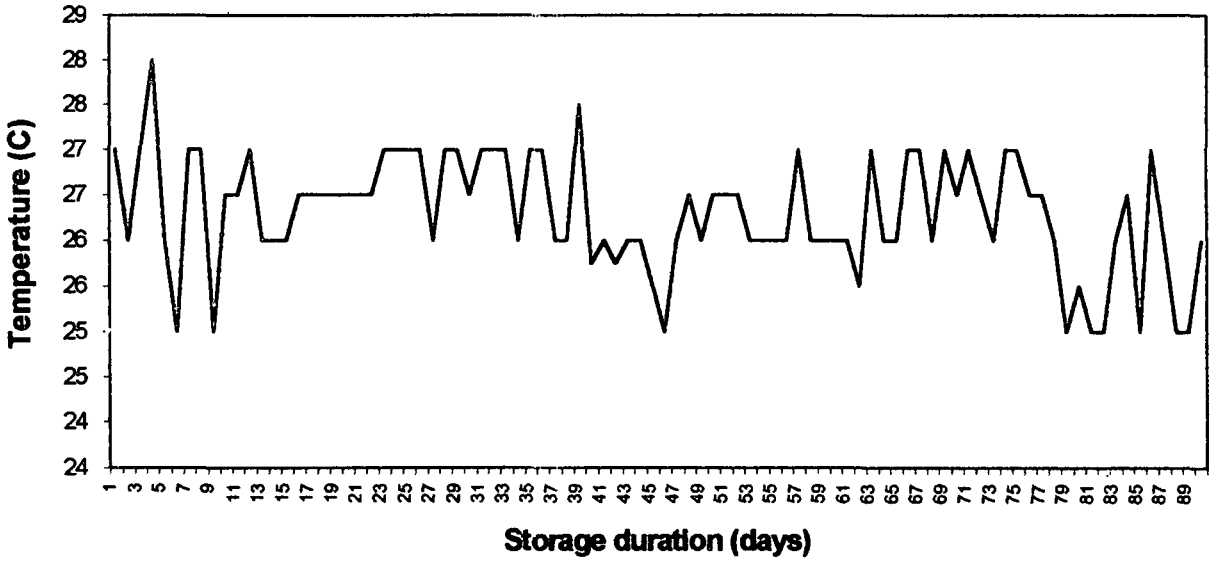


Figure 15. The daily average temperature during storage at BIOTROP warehouse

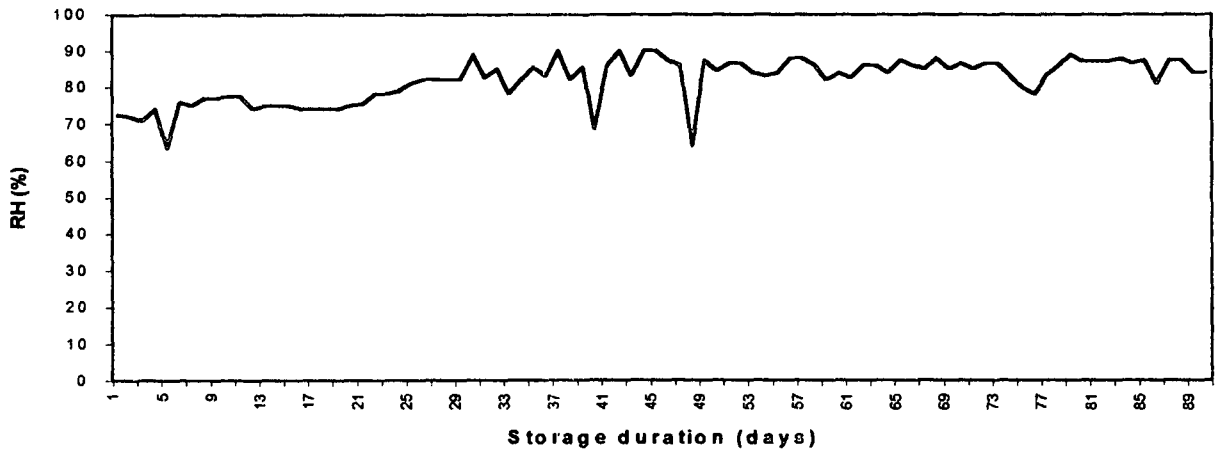


Figure 16. The daily average relative humidity during storage at BIOTROP warehouse

- **Moisture content**

Moisture content of either paddy or maize fumigated with phosphine tablets and Eco_2Fume had the same pattern. It increased until 45 days of storage and then decreased after 90 days of storage (Figures 17 and 18).

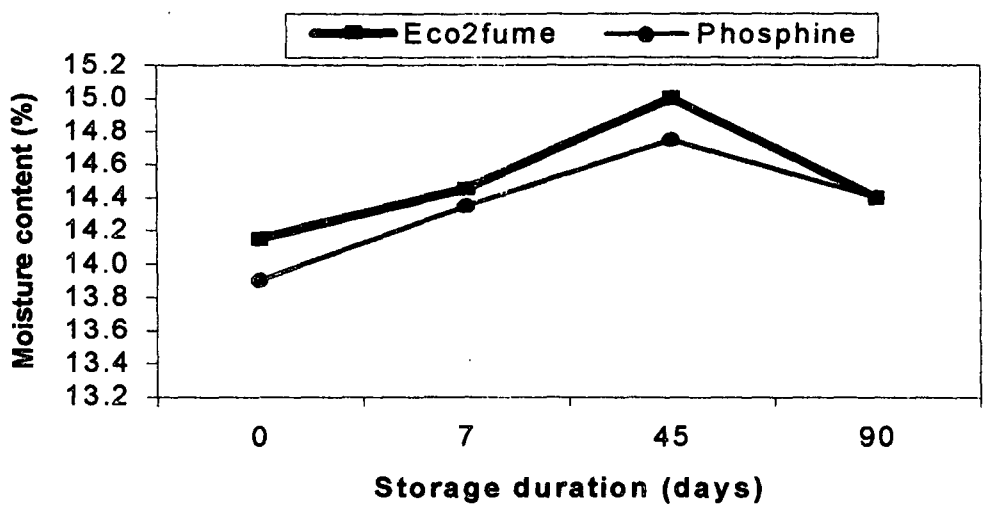


Figure 17. Moisture content of paddy fumigated with phosphine and Eco_2Fume during storage

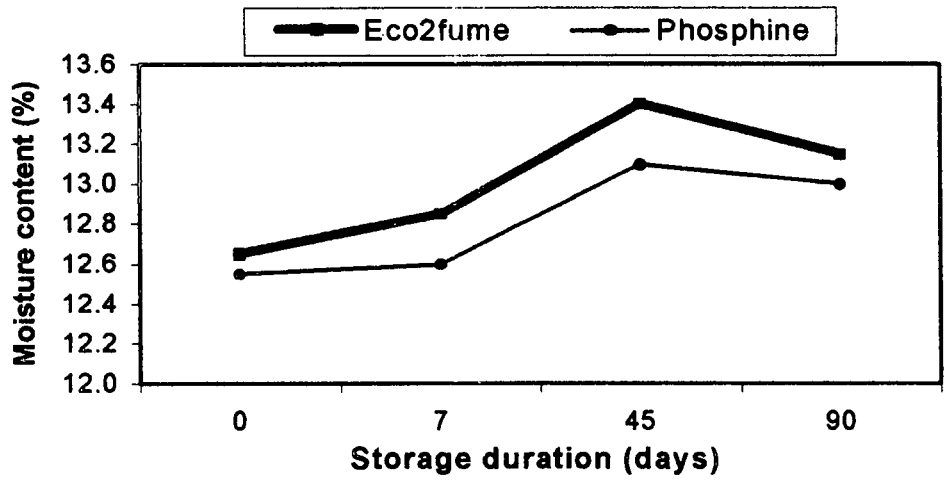


Figure 18. Moisture content of maize fumigated with phosphine and Eco_2Fume during storage

- **Species and total fungal population**

Twenty-seven fungal species were isolated from **paddy** treated with the two fumigants during storage. They were *Alternaria tenuis, Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. penicillioides, A. tamarii, Chaetophoma* sp., *Cladosporium cladosporioides, Curvularia lunata, C. pallescens, Endomyces fibuliger, Eurotium chevalieri, E. repens, Fusarium semitectum, Mucor rouxii, Penicillium citrinum, P. islandicum, P. simplicissimum, Phoma shorgina, Pithomyces chartarum, Syncephalastrum racemosum, Trichoniella padwickii, Wallemia sebi* and 3 unidentified species.

The increase of number of fungal species on paddy after Eco_2Fume fumigation (after 7 days of storage) was higher than that of phosphine fumigation. The number of fungal species on paddy at the beginning of storage and after Eco_2Fume fumigation were 7 and 12 species, respectively, while on paddy at the beginning of storage and after phosphine fumigation were 7 and 10 species, respectively (Annex 5).
A. candidus, A. flavus, Curvularia lunata and *Eurotium repens* were always isolated from paddy treated with Eco_2Fume, while *A. candidus, A. flavus* and *C. lunata* were always isolated from paddy treated with phosphine during storage.

Eleven fungal species were isolated from **maize** treated with the two fumigants during storage. They were *Aspergillus candidus, A. flavus, A. niger, A. penicillioides, A. tamarii, Cladosporium cladosporioides, Eurotium chevalieri, E. repens, Penicillium citrinum, Synchephalastrum racemosum* and *Wallemia sebi.*

The increase of number of fungal species on maize after Eco_2Fume fumigation (after 7 days of storage) was lower than that after phosphine fumigation. The number of fungal species on maize at the beginning of storage and after Eco_2Fume fumigation were 5 and 7 species, respectively, while on maize at the beginning of storage and after phosphine fumigation were 4 and 8 species, respectively (Annex 6).
A. flavus, A. tamarii and *Eurotium repens* were always isolated from maize treated with Eco_2Fume, while *A. flavus* and *E. repens* were always isolated from maize treated with phosphine during storage.

The effect of phosphine and Eco_2Fume on fungal population either on paddy or maize had a same pattern. The total fungal population on paddy decreased after fumigation (after 7 days of storage), and then increased until 90 days of storage (Figure 19). The population on maize decreased after fumigation, and then increased slightly (Figure 20). Dharmaputra *et al* (1993) reported that total fungal population on soybean meal treated with phosphine decreased after treatment, and then increased during 190 days of storage.

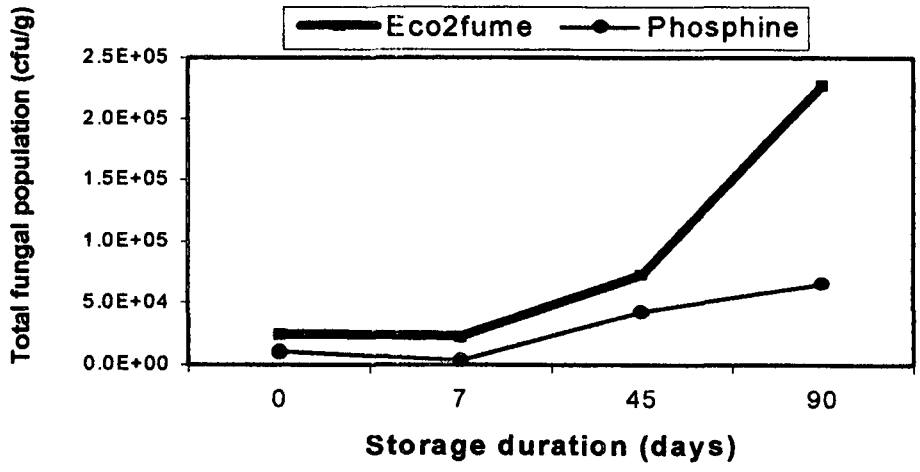


Figure 19. Total fungal population on **paddy** fumigated with phosphine and Eco_2Fume during storage

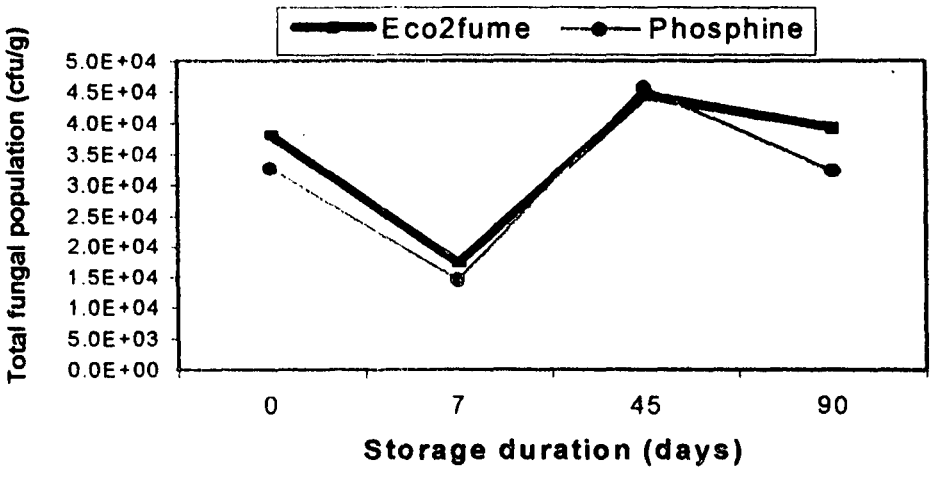


Figure 20. Total fungal population on **maize** fumigated with phosphine and Eco_2Fume during storage

- ***Aspergillus flavus* population**

The effect of phosphine and Eco_2Fume on the population of *A. flavus* either on paddy or maize had a same pattern. *A. flavus* population decreased after fumigation (after 7 days of storage), and then it fluctuated (Figures 21 and 22). It was assumed that the decrease in *A. flavus* population was due to the effect of the fumigants. Dharmaputra *et al.* (1991) reported that mycelium growth of 3 isolates of *A. flavus* was almost totally inhibited after phosphine fumigation at concentration of 3.5 mg/L. According to Dharmaputra and Putri (1999) phosphine at concentration of 3.5 mg/L caused 99% of inhibition to the growth of *A. flavus in vitro.*

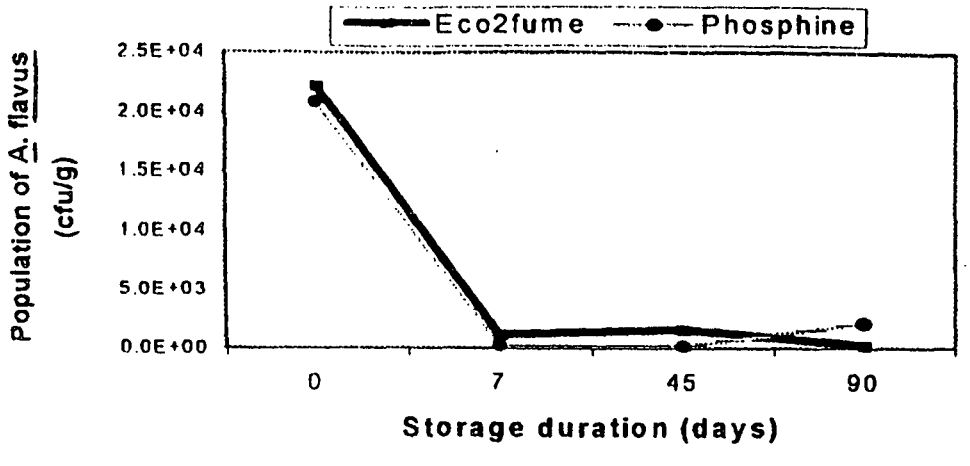


Figure 21. *Aspergillus flavus* population on **paddy** fumigated with phosphine and Eco_2Fume during storage

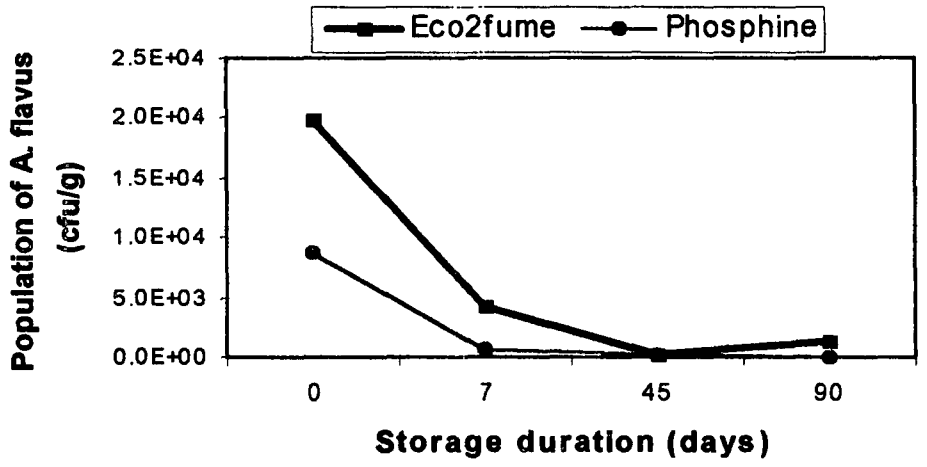


Figure 22. *Aspergillus flavus* population on **maize** fumigated with phosphine and Eco_2Fume during storage

- **Total aflatoxin content of maize**

Four types of aflatoxin were detected on maize during storage, namely aflatoxin B_1, B_2, G_1 and G_2. Aflatoxin B_1 was always detected during storage. Total aflatoxin content on maize treated with Eco_2Fume decreased during storage, and it was not detected (< 5 ppb) after 45 and 90 days of storage, while the content on maize treated with phosphine was not detected after 7 and 45 days of storage and increased after 90 days of storage (Figure 23). It was assumed that the decrease of aflatoxin content on maize treated with Eco_2Fume was due to CO_2. Dharmaputra *et al.* (1990) reported that aflatoxin production of maize treated with CO_2 at 80% concentration was lower than that of untreated maize. According to Dharmaputra *et al* (1992) CO_2 at concentration of 80% reduced aflatoxin production of 3 isolates of *A. flavus in vitro.*

It was assumed that the increase of aflatoxin content on maize treated with phosphine after 90 days of storage was due to the strains of *A. flavus* and the resistance of antagonistic fungi to phosphine. The antagonistic fungi on maize could inhibit aflatoxin production. Dharmaputra *et al.* (1991) reported that aflatoxin production of two isolates of *A. flavus* treated with phosphine at concentration of 3.5 mg/L were lower and significantly different with that of untreated isolates (Dharmaputra *et al.* 1991).

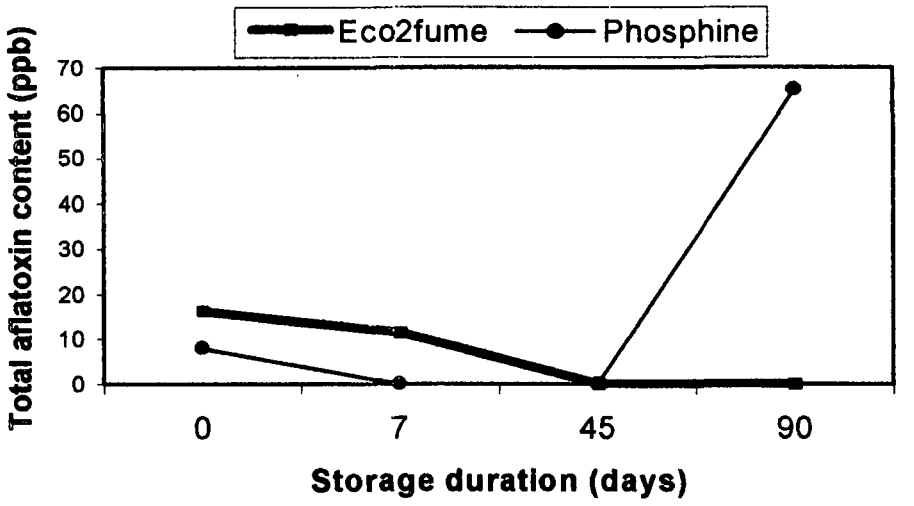


Figure 23. Total aflatoxin content of maize fumigated with phosphine and Eco_2Fume during storage

- **Bioassay test**

The result of the bioassay test is shown in Table 7. After 7 days of fumigation using phosphine as well as Eco_2Fume in paddy, some of *R. dominica* were still alive, while all *S. zeamais* and *T. castaneum* were totally killed. There was an indication that in fumigated paddy, *R. dominica* become resistant, but to prove this assumption further experiment is necessary. In fumigated maize, the three insect species were all killed.

Table 7. Number of alive insects on paddy and maize stacks after fumigation

Commodity/Fumigant		Insects species		
		R. dominica	*S. zeamais*	*T. castaneum*
Paddy:				
Phosphine	1	2	0	0
	2	8	0	0
Total		**10**	**0**	**0**
Average		**5**	**0**	**0**
Eco_2Fume	1	0	0	0
	2	7	0	0
Total		**7**	**0**	**0**
Average		**3.5**	**0**	**0**
Maize:				
Phosphine	1	0	0	0
	2	0	0	0
Total		**0**	**0**	**0**
Average		**0**	**0**	**0**
Eco_2Fume	1	0	0	0
	2	0	0	0
Total		**0**	**0**	**0**
Average		**0**	**0**	**0**

- **Bait Trap**

The number of insects found in the bait traps is shown in Table 8. There was no insect found in bait traps placed on paddy and maize stacks fumigated with Eco_2Fume as well as with phosphine after 30 days of storage, but after 60 and 90 days of storage a relatively low insect infestation could be observed at the maize and paddy stacks fumigated with Eco_2Fume as well as phosphine. The number of insects on all the maize and paddy stacks fumigated with phosphine was higher than that of fumigated with Eco_2Fume. The number of trapped insects found showed the level of insect population in the storage environment.

Table 8. Number of live insects in bait traps during storage at BIOTROP warehouse

Commodity/Fumigant			DURATION OF STORAGE (days)							
			30	60	90			30	60	90
Paddy						Maize				
Phosphine	I	1	0	6	19	Phosphine	I 1	1	9	22
		2	0	7	31		2	1	7	25
		3	1	5	5		3	0	10	34
		4	0	5	23		4	0	12	18
Total			**1**	**23**	**78**	**Total**		**2**	**38**	**99**
Average			**0.25**	**5.75**	**19.5**	**Average**		**0.5**	**9.5**	**24.75**
Phosphine	II	1	3	7	31	Phosphine	II 1	2	15	20
		2	4	9	31		2	2	16	39
		3	1	8	32		3	2	13	16
		4	0	8	43		4	0	10	30
Total			**8**	**32**	**137**	**Total**		**6**	**54**	**105**
Average			**2**	**8**	**34.25**	**Average**		**1.5**	**13.5**	**26.25**
Eco_2Fume	I	1	0	4	44	Eco_2Fume	I 1	1	4	28
		2	0	3	35		2	3	3	9
		3	0	2	9		3	1	2	8
		4	0	2	15		4	2	3	14
Total			**0**	**11**	**103**	**Total**		**7**	**12**	**59**
Average			**0**	**2.75**	**25.75**	**Average**		**1.75**	**3**	**14.75**
Eco_2Fume	II	1	0	3	32	Eco_2Fume	II 1	0	3	46
		2	2	2	27		2	0	2	31
		3	1	1	57		3	1	5	30
		4	3	3	17		4	2	6	22
Total			**6**	**9**	**133**	**Total**		**3**	**16**	**129**
Average			**1.5**	**2.25**	**33.25**	**Average**		**0.75**	**4**	**32.25**

- **Insects population inside of the bags**

Five insect species have been found in paddy and maize fumigated with phosphine as well as Eco_2Fume during 90 days of storage. They are *Cryptolestes* spp., *Liposcelis entomophilus* (Enderlein), *Oryzaephilus surinamensis* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) (Table 9).

Table 9. Population of each insect species (insects/kg) on paddy and maize during storage at BIOTROP warehouse

INSECTS	Duration of storage (days)					
	0	45	90	0	45	90
	PADDY			MAIZE		
PHOSPHINE						
Liposcelis entomophilus	0	0	7.5	0	0	12
Oryzaephilus	0	0	2	0	0	2.5
surinamensis	0	1.5	0	0	1.5	3.5
Rhyzopertha dominica	0	3.5	0.5	0	2	6
Sitophilus zeamais	0	1.5	3.5	0	2	6
Tribolium castaneum						
TOTAL	**0**	**6.5**	**13.5**	**0**	**5.5**	**30**
Eco_2Fume						
Liposcelis entomophilus	0	0	0	0	0	6.5
Oryzaephilus	0	0	1.5	0	0	1
surinamensis	0	0.5	2.5	0	0	3
Rhyzopertha dominica	0	2	0.5	0	2.5	0.5
Sitophilus zeamais	0	1.5	2.5	0	1	3.5
Tribolium castaneum						
TOTAL	**0**	**4**	**7**	**0**	**3.5**	**14.5**

The total insects population on paddy and maize stacks fumigated with phosphine was higher compared to fumigated with Eco_2Fume after 45 and 90 days of storage (Figure 24). The total insects population on paddy stacks fumigated with phosphine after 45 and 90 days storage were 7 and 14 insects/kg, respectively, while those fumigated with Eco_2Fume were 4 and 7 insects/kg. The total population of insects on maize stacks fumigated with phosphine after 45 and 90 days of storage was 6 and 30 insects/kg, respectively, while those fumigated with Eco_2Fume were 4 and 15 insects/kg.

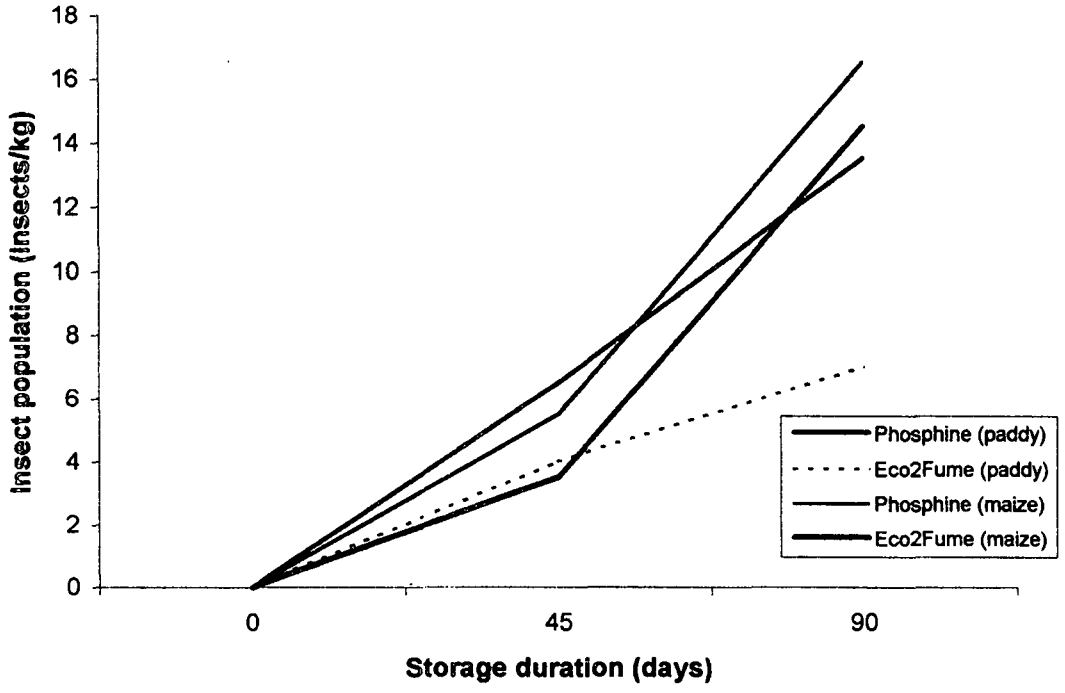


Figure 24. Total population of insect on paddy and maize during storage

The increase of insects population on the stacks treated with Eco_2Fume as well as phosphine after 45 and 90 days of storage indicated that there was an overall increase in insects reinfestation.

- **Physical properties of paddy and maize**

The percentage of physical property of **paddy**, in terms of empty, damaged and chalky/green kernels treated either with phosphine or Eco_2Fume increased after 90 days of storage (Figure 25).

In general, the percentage of physical property of **maize**, in terms of damaged, broken and wrinkle kernels treated either phosphine or Eco_2Fume increased after 90 days of storage (Figure 26).

The increase of the percentage of physical properties was caused by the increase of total population of insects (Figure 24). Dharmaputra *et al.* (1994) reported that there is correlation between population of *Sitophilus zeamais* and damaged kernels of maize.

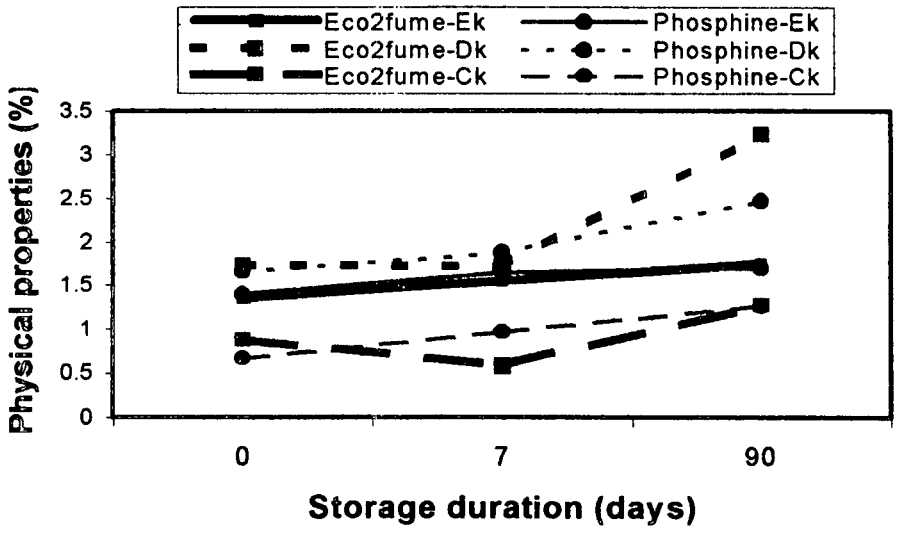


Figure 25. Physical properties of paddy during storage
(Ek = Empty kernels; Dk = Damaged kernels; Ck = Chalky kernels)

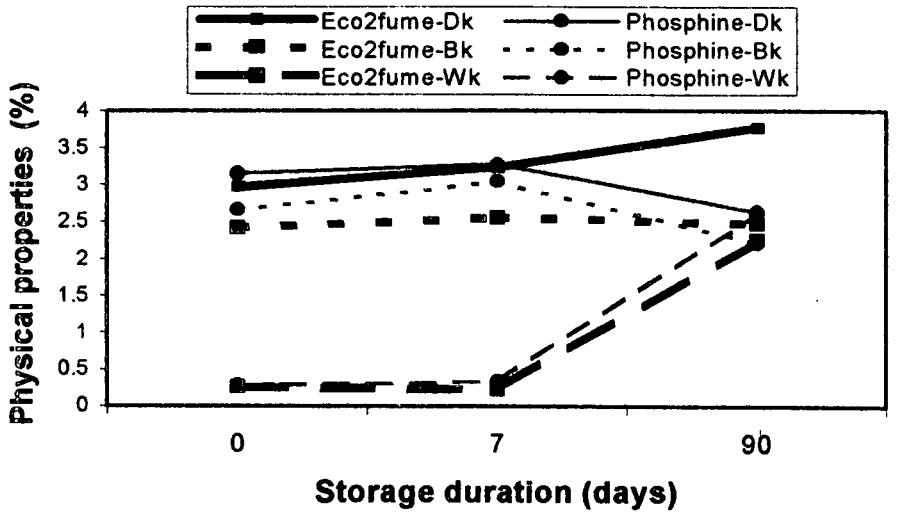


Figure 26. Physical properties of maize during storage
(Dk = Damaged kernels; bk = Broken kernels; Wk = Wrinkle kernels)

The effects of hermetic storage on the quality of paddy and maize stored under laboratory conditions at SEAMEO BIOTROP

- **Temperature and relative humidity of the laboratory**

The daily average temperature and relative humidity in the laboratory during 90 days of storage ranged between 25 – 28° C and 57 - 74 %, respectively In general the daily average temperature and relative humidity of the ambient air in the storage fluctuated slightly throughout the observation period (Figures 27 and 28).

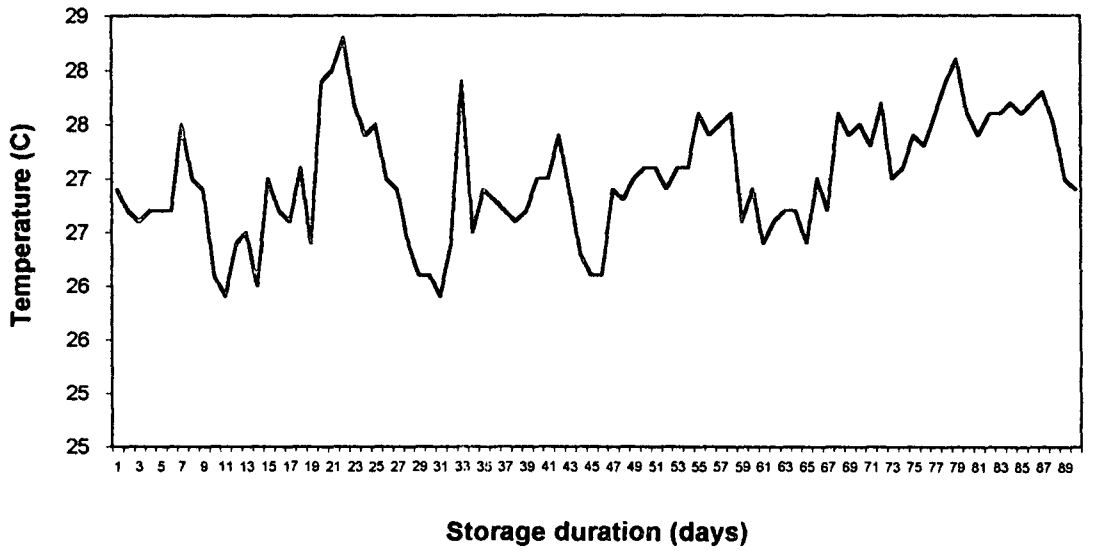


Figure 27. The daily average temperature during storage at BIOTROP Laboratory

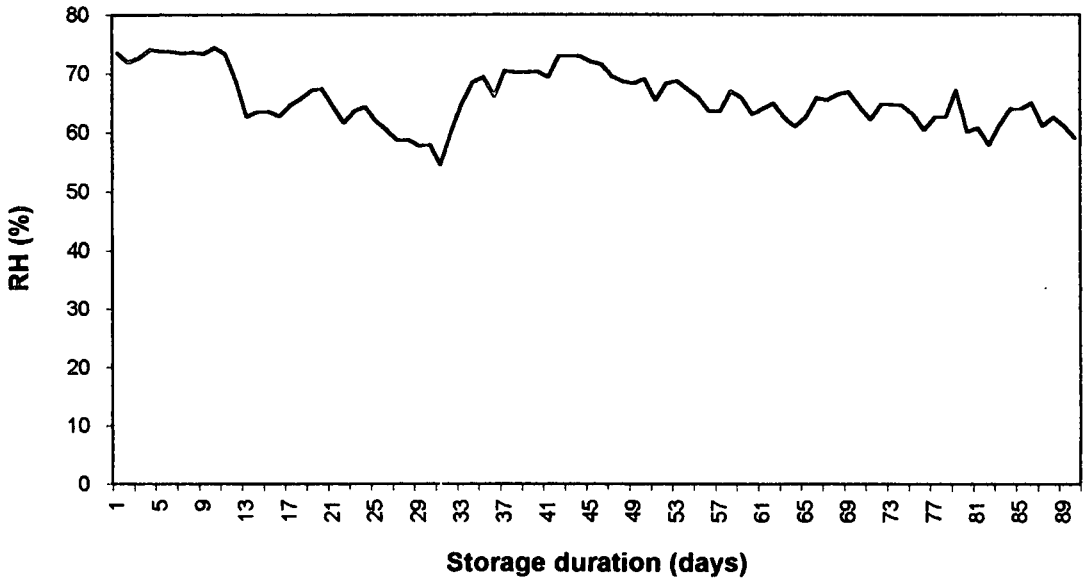


Figure 28. The daily average relative humidity during storage at BIOTROP Laboratory

- **Moisture content**

Moisture content of either paddy or maize packed under hermetic or normal conditions decreased until 45 days of storage and then increased after 90 days of storage (except for paddy packed under hermetic condition) (Figures 29 and 30).

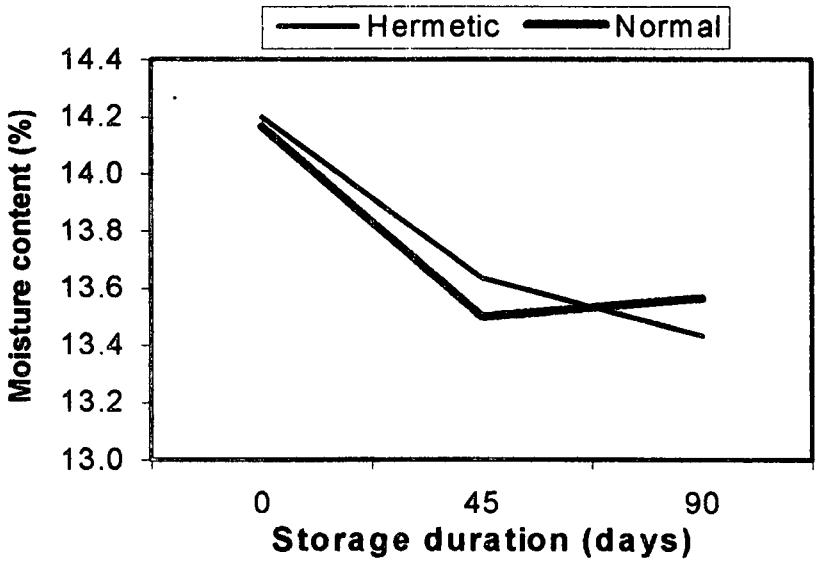


Figure 29. Moisture content of **paddy** packed under hermetic and normal conditions during storage

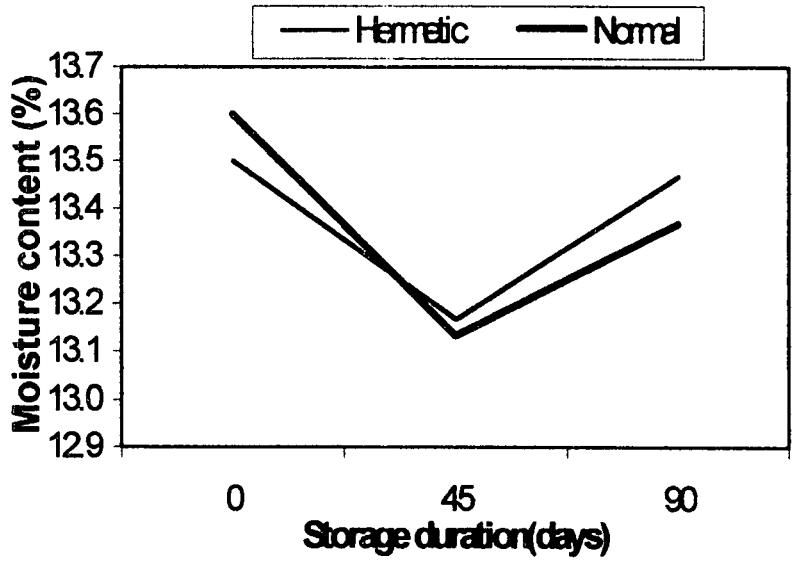


Figure 30. Moisture content of **maize** packed under hermetic and normal conditions during storage

- **Species and total fungal population**

Fourty six fungal species were isolated from **paddy** packed under hermetic and normal condition during storage. They were *Acremonium strictum, Aspergillus clavatus, A. flavus, A. fumigatus, A. niger, A. penicillioides, A. tamarii, A. versicolor, Chaetophoma* sp., *Cladosporium cladosporioides, Curvularia lunata, C. pallescens, Endomyces fibuliger, Eurotium chevalieri, E. repens, Fusarium moniliforme, F. oxysporum, F. proliferatum, F. semitectum, Mucor circinellioides, Nigrospora oryzae, Paecylomyces lilacinus, Penicillium camemberti, P. corylophilum, P. citrinum P. islandicum, Phoma shorgina, Phoma* sp., *Plenodomus* sp., *Syncephalastrum racemosum, Trichocladium* sp., *Wallemia sebi,* and 14 unidentified species (Annex 7).

A. flavus, A. fumigatus, A. niger, A. tamarii, and *C. cladosporioides* were always isolated from paddy packed under hermetic condition, while *A. flavus, Cladosporium cladosporioides, Curvularia pallescens, F. moniliforme* and *F. semitectum,* were always isolated from paddy under normal condition.

Nineteen fungal species were isolated from **maize** packed in hermetic and normal condition during storage. They were *Acremonium strictum, Aspergillus flavus, A. fumigatus, A. niger, A. penicillioides, A. tamarii, A. terreus, A. wentii, A. versicolor, Cladosporium cladosporioides, Endomyces fibuliger, Fusarium moniliforme, Mucor* sp., *Nigrospora oryzae, Penicillium citrinum, Phytomyces chartarum, Synchephalastrum racemosum* and 1 unidentified species (Annex 8).

A. flavus, A. niger, A. tamarii, and *P. citrinum* were always isolated from maize packed under hermetic condition, while *A. flavus, A. niger, A. tamarii, F. moniliforme* and *P. citrinum,* were always isolated from maize under normal condition.

The effect of either hermetic or normal condition on total fungal population of paddy had a same pattern. It decreased after 45 days of storage, and then increased until 90 days of storage (Figure 31). Total fungal population of maize packed under hermetic or normal condition increased after 45 days of storage and then decreased until 90 days of storage (Figure 32).

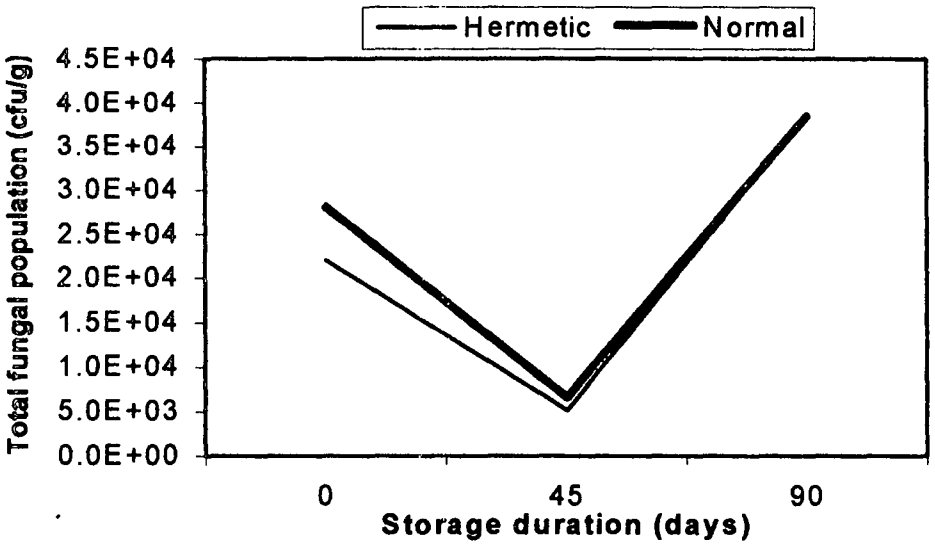


Figure 31. Total fungal population on **paddy** packed under hermetic and normal conditions during storage

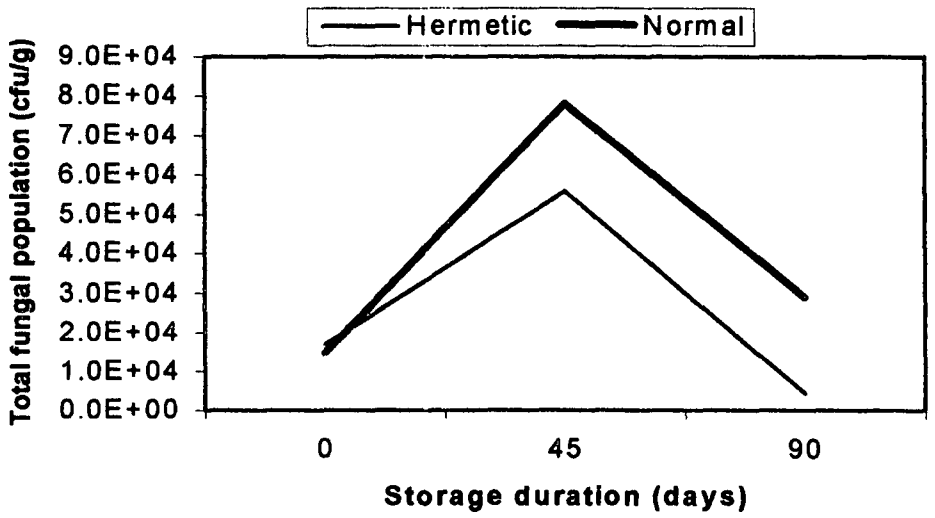


Figure 32. Total fungal population on **maize** packed under hermetic and normal conditions during storage

Sukprakarn *et al.* (2000) found that the levels of mould infection in maize kernels stored under airtight conditions (using Volvani cubes) for 3 and 9 months had only slightly increased. Another study conducted by Bruin (2001) revealed that fungal infection in paddy with initial moisture contents of 10.0 to 13.5%, stored under hermetic conditions for 78 to 183 days, remained stable.

According to Dharmaputra *et al.* (2000) the total fungal population of maize with initial mosture content of 14% stored under airtight condition (initial O_2 content was 1.4% for 6 months), was lower than those of samples stored under normal conditions (initial O_2 content was 21%).
Sidik (2000) reported that on milled rice stored under vacuum conditions for 16 months, the development of fungi and yeast were inhibited.

- ***Aspergillus flavus* population**

The effect of hermetic storage on the population of *A. flavus* either on paddy or maize had a same pattern, i.e. decreased during storage (Figures 33 and 34). It was assumed that the decrease was due to the low oxygen concentration or the presence of fungi antagonistic to *A. flavus*.

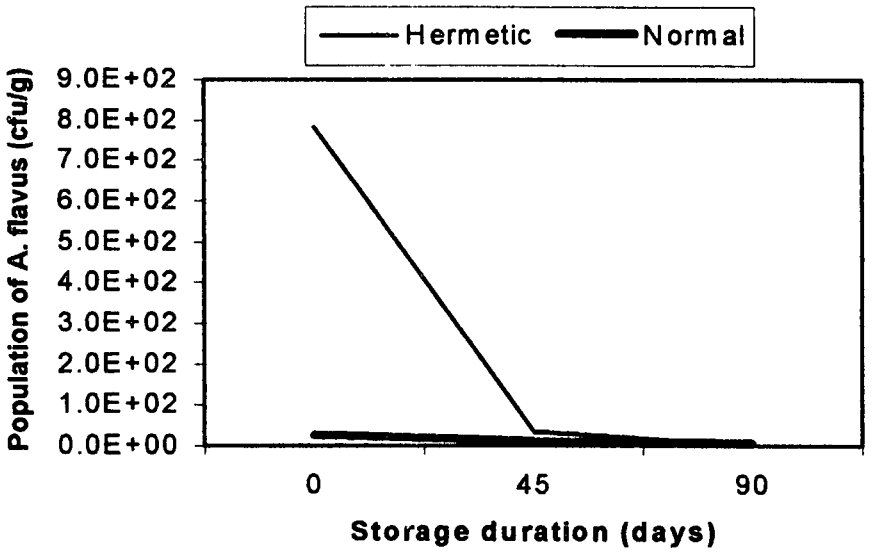


Figure 33. *Aspergillus flavus* population on **paddy** packed under hermetic and normal conditions during storage

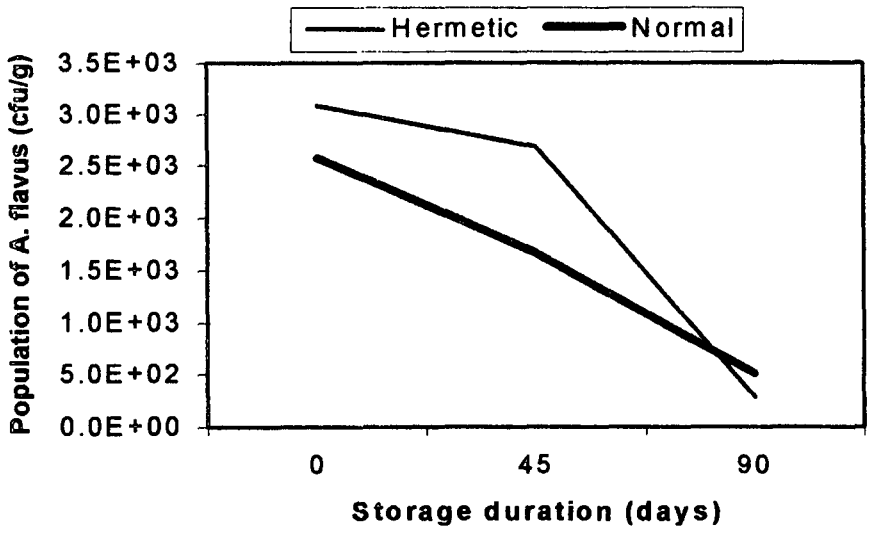


Figure 34. *Aspergillus flavus* population on **maize** packed under hermetic and normal conditions during storage

Dharmaputra *et al.* (2000) revealed that the population of *A. flavus* onmaize with initial moisture content of 14% and stored under hermetic condition for 6 months was lower than that stored under normal condition.

- **Total aflatoxin content on maize**

Three types of aflatoxin were detected on maize packed under hermetic condition after 45 days of storage, namely aflatoxin B_1, G_1 and G_2. Total aflatoxin content on maize under hermetic condition after 45 days of storage was 3.67 ppb, while no aflatoxin was detected in maize under normal condition (Figure 35).

The increase of total aflatoxin content of maize stored under hermetic condition for 45 days of storage was due to aflatoxin accumulation on the substrate. Aflatoxin production depends among other on the strains of *A. flavus* Dharmaputra *et al.* (2000) reported that the total aflatoxin B_1 content on maize with initial moisture content of 14%, stored under hermetic conditions for 6 months was lower than that of stored under normal conditions.

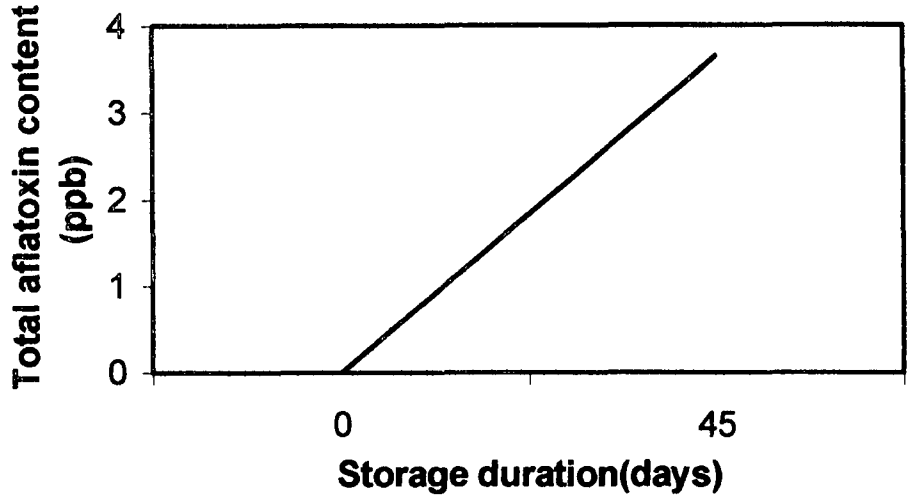


Figure 35. Total aflatoxin content of maize packed under hermetic condition during 45 days of storage

- **Insect population**

The effect of hermetic storage on insects population is shown in Table 5. On paddy and maize stored under hermetic condition, the growth of *R. dominica, S. zeamais* and *T. castaneum* population was inhibited after 45 days of storage. In the control, the population of the three insect species increased with the increase of storage duration (Figure 36).

After 45 days of storage insect population of paddy and maize packed under hermetic condition did not increase. The initial introduced insects were all dead, but after 90 days of storage the insects appeared again. It was due to the oxygen concentration of the hermetic storage which could not be maintained during storage, so that after 45 days of storage, the treatment was only effective for adult insects, but not for the egg. At the beginning of storage the oxygen concentration for paddy and maize were 2.2 and 2.5% respectively, while after 90 days of storage they were 3.8 and 12.0% respectively. The increase in oxygen concentration was due to the quite low permeability of the used plastic bags. If the plastic bags used in this experiment is airtight enough, the oxygen concentration could be maintained as low as possible, so that the hermetic storage would be effective enough to control the insects.

Sidik (2000) reported that lack of oxygen had a significant impact on the mortality on insects found in milled rice throughout the 16 months of storage. According to Sukprakarn *et al.* (2000) live insects in maize stored under airtight conditions (using Volcani cubes) for 3 and 9 months, were not observed.

Table 10. Population of live adult insects (insects/bag) on paddy and maize during storage.

Treatments		Duration of storage (days)								
		0			45			90		
		Rd.	*Sz*	*Tc*	*Rd.*	*Sz*	*Tc*	*Rd.*	*Sz*	*Tc*
Paddy:										
Control	1	10	10	10	10	54	83	58	22	52
	2	10	10	10	10	34	72	155	31	43
	3	10	10	10	12	35	81	220	51	115
Total		**30**	**30**	**30**	**32**	**123**	**236**	**433**	**104**	**210**
Average		**10**	**10**	**10**	**10.7**	**41**	**78.7**	**144.3**	**34.7**	**70**
Hermetic	1	10	10	10	0	0	0	21	34	30
	2	10	10	10	0	0	0	77	25	9
	3	10	10	10	0	0	0	32	12	12
Total		**30**	**30**	**30**	**0**	**0**	**0**	**130**	**71**	**51**
Average		**10**	**10**	**10**	**0**	**0**	**0**	**43.3**	**23.7**	**17**
Maize:										
Control	1	10	10	10	10	28	30	44	66	13
	2	10	10	10	10	31	23	55	20	8
	3	10	10	10	10	25	24	88	36	18
Total		**30**	**30**	**30**	**30**	**84**	**77**	**187**	**122**	**39**
Average		**10**	**10**	**10**	**10**	**28**	**25.7**	**62.3**	**40.7**	**13**
Hermetic	1	10	10	10	0	0	0	68	12	8
	2	10	10	10	0	0	0	15	20	3
	3	10	10	10	0	0	0	27	11	6
Total		**30**	**30**	**30**	0	**0**	**0**	**110**	**43**	**17**
Average		**10**	**10**	**10**	0	**0**	**0**	**36.7**	**14.3**	**5.7**

Note: Rd. = *R. dominica*; Sz = *S. zeamais*; Tc. = *T. castaneum*

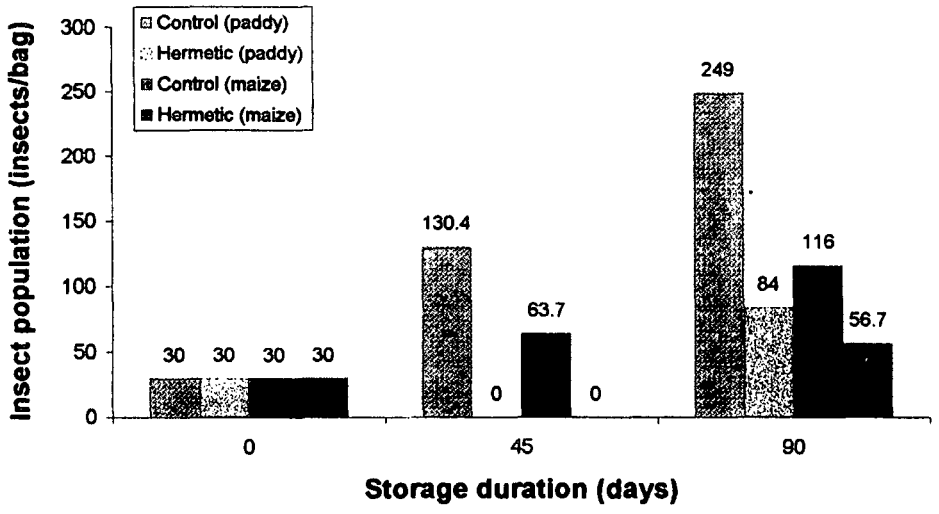


Figure 36. Population of live adult insects on paddy and maize packed under hermetic and normal conditions

- **Physical properties of paddy and maize**

The percentage of physical property of paddy, in terms of empty, yellow and chalky kernels packed either under hermetic or normal conditions increased after 90 days of storage (Figure 37).

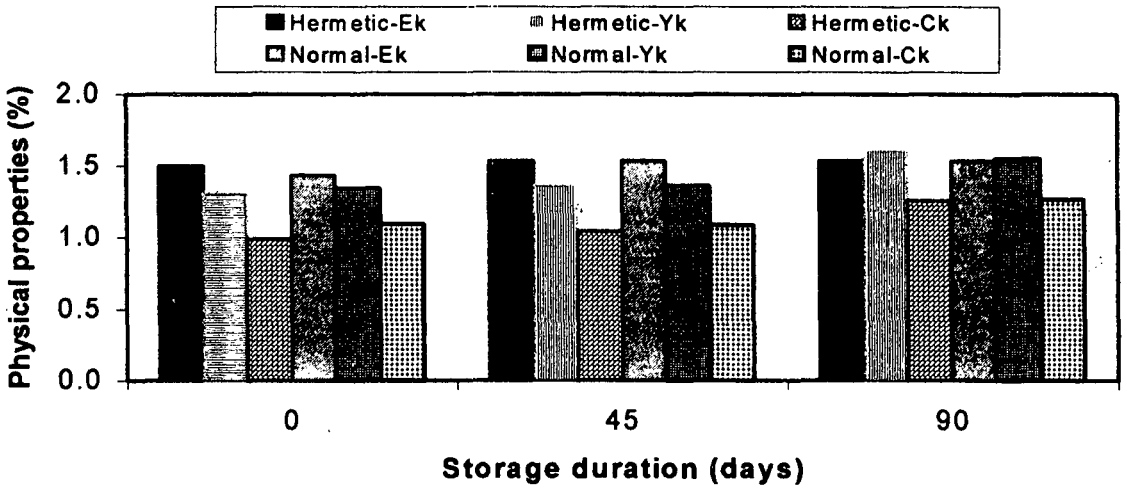


Figure 37. Physical properties of **paddy** during storage (Ek = Empty kernels; Yk = Yellow kernels; Ck = Chalky kernels)

In general, the percentage of physical property of maize, in terms of damaged, broken and wrinkle kernels packed either under hermetic or normal conditions increased after 90 days of storage (Figures 38 and 39). The increase of the percentage of physical properties was caused by the increasing of total population of insects (Figure 36). Dharmaputra *et al.* (1994) reported that there is correlation between population of *Sitophilus zeamais* and damaged kernels of maize.

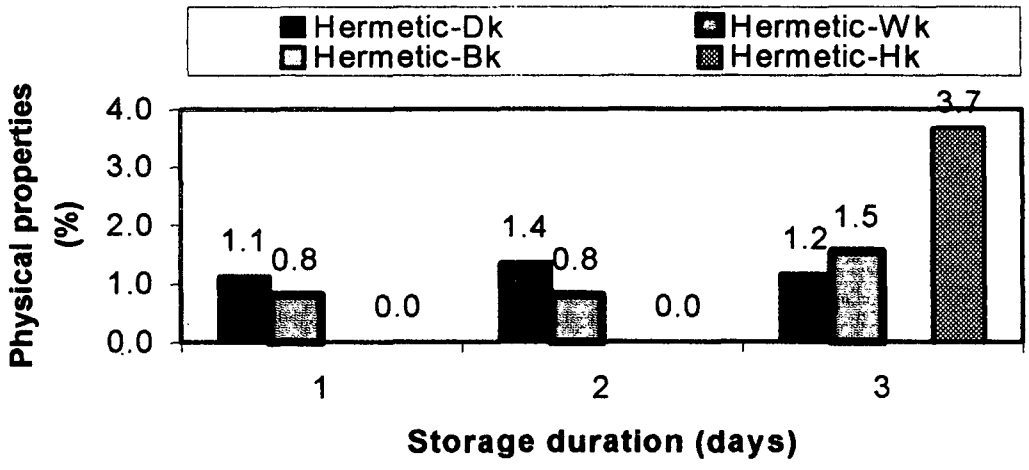


Figure 38. Physical properties of **maize** packed under hermetic condition during storage Dk = Damaged kernels; Wk = Wrinkle kernels; Bk = Broken kernels; Hk = Hole kernels)

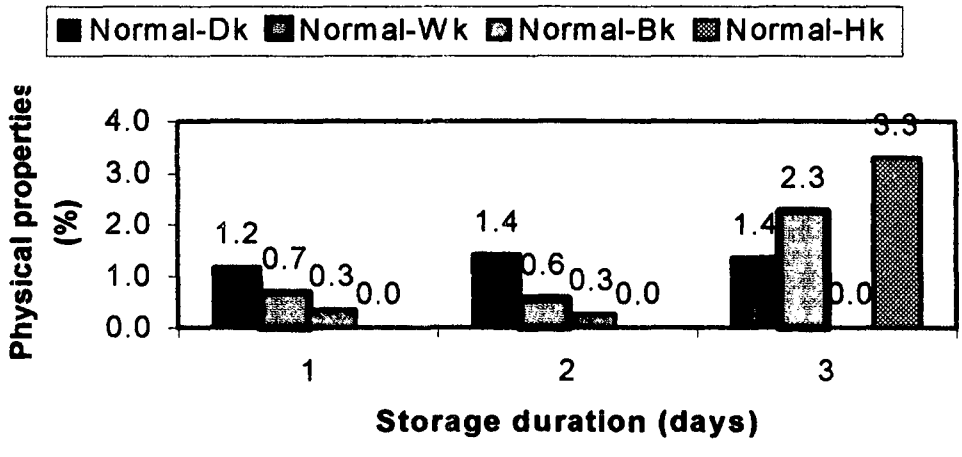


Figure 39. Physical properties of **maize** packed under normal condition during storage Dk = Damaged kernels; Wk = Wrinkle kernels; Bk = Broken kernels; Hk = Hole kernels)

c. Results of Susceptibility Test

• Susceptibility to phosphine

Results of susceptibility test on percentage of insect mortality of *R. dominica, S. zeamais* and *T. castaneum* at various phosphine concentration level is shown at Table 11.

Table 11. Mortality of three insects species at various phosphine concentration levels

Insects species	Concentrations (mg/l)						
	0.00	0.01	0.015	0.02	0.025	0.03	0.04
R. dominica	0.0	1.5	4.0	16.0	36.5	68.5	78.5
S. zeamais	0.0	66.5	74.5	76.5	90.5	97.5	100
T. castaneum	0.0	3.0	11.5	21.0	87.5	89.0	93.5

Probit analysis on mortality figures resulted in a, b, LC_{50} and LC_{95} values as stated in Table 12.

Table 12. Values of a, b, LC_{50} and LC_{95} for each test insects species

Insects species	a	b	LC_{50}	range	LC_{95}	range
R. dominica	14.17	5.90	0.028	0.025 – 0.032	0.053	0.043 – 0.08
S. zeamais	10.85	2.80	0.008	0.001 – 0.012	0.032	0.022 – 0.13
T. castaneum	17.16	7.29	0.022	0.016 – 0.028	0.036	0.028 – 0.11

Based on the results of susceptibily test, it could be concluded that *R. dominica* was the most resistant to phosphine, followed by *T. castaneum*, while *S. zeamais* was the most susceptible. This result confirmed the previous experiment conducted by Bulog and BIOTROP (1990), which found that susceptibility level to phosphine varied among insect species.

Comparing the LC_{50} and LC_{99} values of the tests on equivalent values of susceptible strains for the same species as reported by FAO (1975), it could be concluded that the species of insects used in this test were less susceptible than FAO values. Therefore, the three species had indicated a potency to be resistant to phosphine.

Table 13. LC_{50} and $LC_{99,9}$ susceptible strains of some post harvest insects species (FAO, 1975)

Insects species	LC_{50}	LC_{95}
R. dominica	0.008	0.028
S. zeamais	0.007	0.013
T. castaneum	0.009	0.028

- **Susceptibility to Fenitrothion**

Results of susceptibility test on the percentage of insect mortality of *R. dominica, S. zeamais* and *T. castaneum* after 6 and 24 hours at various fenitrothion concentration levels are shown in Table 14 and 15.

Table 14. Mortality percentage of three insects species at various fenitrothion concentration levels after 6 hours

Insects species	Concentrations (%)				
	0.00	0.0125	0.025	0.05	0.01
R. dominica	0.0	2.5	5.83	6.67	15.0
S. zeamais	0.0	50.0	65.0	83.3	95.0
T. castaneum	0.0	62.5	75.83	84.17	88.33

Table 15. Mortality percentage of three insects species at various fenitrothion concentration levels after 24 hours

Insects species	Concentrations (%)				
	0.00	0.0125	0.025	0.05	0.01
R. dominica	0.0	6.67	7.5	10.0	40.0
S. zeamais	0.0	100	100	100	100
T. castaneum	0.0	100	100	100	100

Probit analysis on mortality figures resulted in a, b, LC_{50} and LC_{95} values as stated in Table 16. The data from the test with *T. castaneum* and *S. zeamais* after exposure of 24 hours to fenitrothion were not analyzed by probit method because the number of dead insect reached 100 percent.

Table 16. Values of a, b, LC_{50} and LC_{95} for each test insects species after 6 hours

Insects species	A	b	LC_{50}	range	LC_{95}	range
R. dominica	4.88	0.96	1.32	0.3796 - 103.642	67.53	4.808 - 841462.25
S. zeamais	8.30	1.76	0.014	0.0101 - 0.0167	0.12	0.0847 – 0.1881
T. castaneum	7.24	0.99	0.006	0.0017 - 0.0094	0.25	0.0309 – 1.0065

Based on the results of the susceptibility test and probit analysis it could be concluded that *T. castaneum* was the most susceptible species to fenitrothion after 6 hours of treatment among the three species as indicated by their lowest LC_{50} , in contrast *R. dominica* was the most tolerant insect

Bulog recommended dosage rates of fenitrothion for surface spraying in stores (0.025 %), was still able to control *T. castaneum* and *S. zeamais*,

however, for *R. dominica* the dosage rates should be increased more than 0.025 %. The results indicated *that R. dominica* has a tendency to become resistant.

d. Data on methyl bromide consumption

There were two major certified companies for importing methyl bromide in Indonesia, namely, PT Asomindo Raya and PT Panca Ratna. Between two companies, PT Asomindo Raya was the biggest importer of methyl bromide in Indonesia. Between 1998 to 2000 company imported 70-96% of total methyl bromide demand in Indonesia (Table 17).

In 1998 total import of methyl bromide was 222.5 tons, and in 1999 increased about 14.2% to 254.0 tons. In 2000 the import of methyl bromide decreased around 25 % to 192.2 tons. There was no clear explanation on this decline, but it seems likely the price of methyl bromide and quantity of commodity were responsible for such a decrease.

Table 17. Import of methyl bromide (tons) in Indonesia between 1998-2000

No.	Importer	1998	1999	2000
1.	PT. Asomindo Raya	192.5	178.7	182.7*)
2.	PT. Panca Ratna	30.0	75.3	6.4
Total		222.5	254.0	189.1

*) *Estimated value based on data of previous survey conducted in 1998*

In current survey, companies data on the use of methyl bromide were obtained from 15 fumigation companies in Java and Lampung (Table 18). PT Sucofindo as fumigation company in Indonesia was still the largest user of methyl bromide. In 1998 to 2000, PT Sucofindo used 104 to 110 tons of methyl bromide per year which contributed about 71.3% to 74.4% of the total uses of MeBr in Indonesia. This result in line with the previous survey conducted in 1998.

The second largest fumigation company was PT Atlas Nusantara, which used methyl bromide about 7.2 tons in 1998, and in 2000 was 3.7 tons. The decreasing of methyl bromide uses possibly due to less tonnage of commodity fumigated in 2000.

Table 18. The quantity of methyl bromide (kg) used in Indonesia, 1998-2000

No.	Fumigation companies *)	1998	1999	2000
1.	DOLOG East Java (AR**)	4500	2000	4700
2.	PT Pan Asia SC(Surabaya)(PASC, JKT)	2223	2825	1871
3.	PT Mandiri Perlisa (AR)	78.5	1318	974
4.	PT Atlas Nusantara (Surabaya) (AR)	1284	2223	1575.5
5.	PT Panca Ratna (PR)	139	NA***)	1135
6.	PT Beckjorindo Paryaweksana (AR)	1200	1200	1200
7.	PT Caretama Inspindo Carya (AR)	15500	15500	15500
8.	PT Pan Asia SC (Lampung)	0	0	0
9.	DOLOG Lampung	0	0	0
10.	PT Sucofindo, Lampung (AR, PR)	4000	4000	4000
11.	PT Sucofindo, Jakarta (AR. PR)	110900	108250	104050
12.	PT Sumber Alam Bahagia (AR)	1750	1500	3000
13.	PT Rentokil Initial (AR)	NA	2100	3850
14.	PT. Bhanda Ghara Reksa (AR)	250	400	350
15.	PT Atlas Nusantara (Jakarta) (AR)	7200	5850	3725
Total		149074.5	147165.6	145930.5

*) Major companies in Java and Lampung
**) Companies of methyl bromide; AR= Asomindo Raya; PR=Panca Ratna
***) Not available

From this survey, there were differences between amount of methyl bromide imported and methyl bromide used. The rest of methyl bromide might be used for pre-shipment fumigation of export products and for quarantine measures in major seaports such as Makasar, Denpasar, Belawan Medan. In this respect, methyl bromide was used for pre-shipment of various export products, primarily agricultural products. In Indonesia, methyl bromide uses was not allowed to be used in sector such as agricultural production activities e.g. soil fumigation.

The use of methyl bromide in Indonesia was regulated by the Ministry of Agriculture through a ministrial decree. The Indonesian Pesticide Commission of the Ministry of Agriculture was responsible for implementing this regulation. Importers and fumigation company must have a license issued by the Pesticide Commission and they had to submit report on their activities to Pesticide Commission annually.

Table 19. Modalities of methyl bromide utilization

No.	Fumigation companies	Commodities	Dosages (g/m^3)	Technique of application	Target pests/diseases	Alternative to MeBr
1.	PT Pan Asia SC	Pallet	48	Gasing	Storage pests	Photoxin Magtoxin Celphos
		Rattan	48	N A		
		Wood	48	N A		
		Rice	28	Gasing		
		Paddy	21	N A		
2.	PT Mandiri Perlisa	Rice	28	Gasing	Storage pests	NA
		Paddy	21	Gasing		
3.	PT Atlas Nusantara Surabaya	Rice	28	Gasing	*Tribolium* sp. *S. oryzae* Rat	NA
		Paddy	21	Staple		
4.	PT Panca Ratna	Rice	28	Gasing	Storage pests	NA
		Paddy	21	Gasing		
5.	PT Beckjorindo Parya Weksana	Coffee	24-48	Tent fumigation	Storage pests	PH_3
		Black pepper	24-48	Space fumigation		
		Coprex	24-48	Space fumigation		
6.	Caretama Inspindo Carya	Coffee		Space fumigation	Storage pests	Phostoxin
7.	PT Sucofindo Lampung	Woods	24-48	Tent Fumigation	Storage pests	Phostoxin
		Coffee	24-48	Space Fumigation		
		Pepper	24-48	Space fumigation		
		Tapioca	24-48	Space fumigation		
		Corn	24-48	Space fumigation		
8.	PT Sucofindo Jakarta	Agricultural products	16-32	Tent fumigation	Storage pests	Phostoxin HCN
		Estate products	24-32	Space fumigation		
		Forestry products	48-64	Space fumigation		
		Industrial products	32-48	Space fumigation		
9.	PT. Sumber Alam Bahagia	Rattan	48	Space fumigation	Storage pests	PH_3
10.	PT. Rentokil	Building	24-48	Undersheet/space fumigation	Urban pests	PH_3
11.	PT Bhanda Ghara Reksa	Furniture	48	Gasing	*Tribolium* sp. Termite	NA
		Agricultural products	48	Gasing		
12.	PT Atlas Nusantara Jakarta	Rice	21	Fumigation	Storage pests	Phostoxin

CONCLUSIONS AND RECOMMENDATIONS

A. CONCLUSIONS

1. The alternative technology demonstrated both tested in Bulog storage complex in Tambun and Seameo Biotrop facilities in Bogor had given a better indication that all technologies tested were able to be used as alternative to methyl bromide. However, among the three technologies, Eco2Fume had a better result as compared to phosphine tablets and hermetic system, in terms of time of gas released, peak of concentration in relation to time, efficacy and quality maintenance of the stored commodities.
2. The presence of carbon dioxide in the pressurized phosphine (Eco2Fume) increased the penetration of phosphine gas into the stack gave a better results in increasing mortality rate, which means increasing efficiency. In addition, carbon dioxide reduced the possibility of fungi to develop in the tested commodities and also to reduce the production of aflatoxin.
3. Hermetic systems seemed to give a prospective method as alternative technology to methyl bromide, providing vacuum conditions and oxygen concentration be kept at recommended levels. Inability to ensure such conditions insects and fungi could develop and contribute significantly to grains deterioration.
4. Type of commodity fumigated had a significant effect on the absorption of phosphine gas during exposure period of fumigation. Paddy absorbed phosphine gas much greater than maize, and the rate of absorption seemed to increase as moisture contents of the stored commodity was higher than the recommended moisture contents for stored commodity (14%). In this respect, initial quality of the grain played a significant role in determining the absorption rate of phosphine during fumigation process.
5. The results of susceptibility tests of three species of insects to fenithrothion indicated that ***Rhyzopertha dominica*** was the least susceptible to this insecticide as compared to ***Tribolium castaneum*** or ***Sitophilus zeamais***. Therefore ***R. dominica*** tended to be resistant to fenithrothion. It was found out in the

susceptibility test of similar species to phosphine, that ***R. dominica*** also had a strong potency to be resistant to this fumigant

6. Small survey conducted to check the consumption of methyl bromide in Indonesia during 1998-2000, revealed that consumption of this fumigant declined from 149.1 tons in 1998 to 145.9 tons in 2000. The reduction on methyl bromide usage possibly due to decrease on the quantity of stored commodities and less export products. However, there is a possibility that increasing quantity of products and pre-shipment purposes in the coming years in line with improvement in agricultural sector, might increase the consumption of methyl bromide.

B. RECOMMENDATIONS

1. Recognising that the three alternatives technology demonstrated in this observation had given a prospective results, in terms of technical feasibility, operation and complexity in applying this system , it is recommended that further observation in operational scale be conducted. Such an activities should be enlarged to cover a wider area of operation, total quantity as well as methods of application.
2. Eco2Fume gas although gave a better results in terms of efficacy, effectiveness in releasing phosphine gas, inhibition of fungal development and minimizing the production of aflatoxin, than the other two systems (phosphine tablet and hermetic system), still need further observation to verify its operating costs and cost per ton of fumigation.
3. Considering the hermetic systems was not applied properly that gave only one month observation, detail study on the use of this system should be conducted. The use of specially designed 'chamber' that could provide a totally sealed and vacuum conditions is recommended in order to get a better results.
4. Further study on the resistance of dominant species of stored products in Indonesia to phosphine and fenithrothion should be carried-out, to validate the findings in this demonstration. Increasing number of insects species resistant to these two chemicals would affect the effort to increase efficiency in

maintaining grain quality, since increasing dosage rates correlated directly to cost per ton of treated commodities.

5. Routine survey on the importation and uses of methyl bromide in Indonesia for different purposes should be conducted widely. Data and information in this aspect would be of importance to justify finding new alternatives to methyl bromide.

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ANNEXES

Annex 1.

SCHEDULE OF ACTIVITIES OF DEMONSTRATION PROJECT
ON ALTERNATIVE TECHNOLOGY TO METHYL BROMIDA
BULOG-UNIDO PROJECT

TAMBUN GODOWN

2 0 0 1

No.	ACTIVITIES	April				May				June				July				August				September				October				November				December				January 02			
		week				week				week				week				week				week				week				week				week				week			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
I.	PREPARATION FOR FUMIGATION																																								
	a. Paddy and Corn Stacking																																								
	b. Fumigation Equipment																																								
	c. Installing Dispenser																																								
II.	FUMIGATION																																								
III.	AERATION																																								
IV.	BIO-ASSAY / INSECT CONTROL																																								
	a. Deploying the tube																																								
	b. Observation																																								
V.	a. CH3BR use (survey)																																								
	b. Survey Report																																								
VI.	SAMPLING																																								
	a. Bait-trap Deployment																																								
	b. Bait-trap Observation																																								
	c. Spear Sampling																																								
	d. Mycology Test																																								
	e. Quality Test																																								
	f. Alfatoxin test																																								
VII.	PROGRESS REPORT																																								
VIII.	FINAL REPORT																																								

Annex 2.

SCHEDULE OF ACTIVITIES OF DEMONSTRATION PROJECT ON ALTERNATIVE TECHNOLOGY TO METHYL BROMIDA BULOG-UNIDO PROJECT

BIOTROP GODOWN

2 0 0 1

No.	ACTIVITIES	April				May				June				July				August				September				October				November				December			
		week				week				week				week				week				week				week				week				week			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
I.	PREPARATION FOR FUMIGATION																																				
	a. Paddy and Corn Stacking		■	■						■																												
	b. Fumigation Equipment				■					■																												
	c. Installing Dispenser				■					■																												
	d. Put insect in plastic bag				■					■																												
II.	FUMIGATION				■					■																												
III.	AERATION				■																																
IV.	BIO-ASSAY / INSECT CONTROL																																				
	a. Deploying the tube				■				■				■				■																					
	b. Observation				■				■				■				■																					
V.	a. CH3BR use (survey)																																				
	b. Survey Report																																				
VI.	SAMPLING																																				
	a. Bait-trap Deployment			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■																			
	b. Bait-trap Observation			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■																			
	c. Spear Sampling			■				■				■				■																					
	d. Mycology Test			■																																	
	e. Quality Test			■				■	■			■	■			■	■						■														
	f. Alfatoxin test			■				■				■				■							■														
VII.	PROGRESS REPORT																			■	■																
VIII.	FINAL REPORT																						■	■	■	■	■	■	■	■	■	■	■	■			
																							■	■	■	■	■	■	■	■	■	■	■	■			

■ Schedule of hermetic storage laboratorium scale

Annex 3. Fungal species on **paddy** treated with phosphine and ECO2FUME during storage

Fumigant	No.	Fungal species		
		Duration of storage (days)		
		0	7	90
a. Phosphine	1	*A. candidus*	*A. candidus*	*A. candidus*
	2	*A. flavus*	*A. flavus*	*A. flavus*
	3	*A. fumigatus*	*A. fumigatus*	*A. niger*
	4	*A. niger*	*A. niger*	*A. tamarii*
	5	*A. tamarii*	*A. tamarii*	*Eupenicillium cinnamopurpureum*
	6	*Chaetophoma* sp.	*E. chevalieri*	*Eurotium repens*
	7	*Curvularia lunata*		*Peniciilium citrinum*
	8	*Fusarium semitectum*		*P. oxalicum*
b. ECO_2FUME	1	*Aspergillus candidus*	*A. candidus*	*A. candidus*
	2	*A. flavus*	*A. flavus*	*A. flavus*
	3	*A. fumigatus*	*A. fumigatus*	*A. fumigatus*
	4	*A. tamarii*	*A. niger*	*A. niger*
	5	*Chaetophoma* sp.	*A. tamarii*	*A. penicillioides*
	6	*Curvularia lunata*	*Chaetophoma* sp.	*A. tamarii*
	7	*C. pallescens*	*P. citrinum*	*Curvularia lunata*
	8	*Penicillium citrinum*		*Eurotium repens*
	9			*Penicillium islandicum*
	10			*P. oxalicum*
	11			*W. sebi*
	12			Species C*

* unidentified

Annex 4. Fungal species on **maize** treated wit phosphine and ECO_2FUME during storage

Fumigant	No.	Fungal species		
		Duration of storage (days)		
		0	7	90
a. Phosphine	1	*A. candidus*	*A. candidus*	*A. candidus*
	2	*A. flavus*	*A. flavus*	***A. flavus***
	3	*A. niger*	*A. fumigatus*	*A. fumigatus*
	4	*A. ochraceus*	*A. niger*	*A. niger*
	5	*A. tamarii*	*E. chevalieri*	*A. penicillioides*
	6	*E. chevalieri*	*E. repens*	*A. tamarii*
	7	*P. citrinum*	*S. raccemosum*	*E. chevalieri*
	8			*E. repens*
	9			*Mucor praini*
	10			*M. saturnius*
	11			*S. raccemosum*
b. ECO_2FUME	1	*Aspergillus candidus*	*A. candidus*	*A. candidus*
	2	*A. flavus*	*A. flavus*	*A. flavus*
	3	*A. fumigatus*	*A. fumigatus*	*A. fumigatus*
	4	*A. ochraceus*	*A. niger*	*A. niger*
	5	*A. niger*	*A. tamarii*	*A. penicillioides*
	6	*A. tamarii*	*E. chevalieri*	*E. chevalieri*
	7	*Eurotium chevalieri*	*E. repens*	*E. repens*
	8		*Syncephalastrum racemosum*	*Mucor praini*
				M. saturnius
				Penicillium islandicum
				S. racemosum

Annex 5. Fungal species on paddy treated with phosphine and ECO_2FUME during storage

Fumigant	No.	Fungal species Duration of storage (days) 0	7	45	90
a. Phosphine	1	*A. candidus*	*A. candidus*	*A. candidus*	*A. candidus*
	2	*A. flavus*	*A. flavus*	*A. flavus*	*A. flavus*
	3	*A. tamarii*	*A. fumigatus*	*A. fumigatus*	*A. fumigatus*
	4	*Curvularia lunata*	*A. niger*	*Chaetophoma* sp.	*A. niger*
	5	*E. repens*	*A. tamarii*	*C. lunata*	*A. penicillioides*
	6	*F. semitectum*	*C. lunata*	*E. chevalieri*	*A. tamarii*
	7	*P. citrinum*	*E. chevalieri*	*E. repens*	*C. lunata*
	8		*Pithomyces chartarum*	*Pithomyces chartarum*	*E. chevalieri*
	9		Species B*	*S. racemosum*	*E. repens*
	10		Species A*	*T. padwickii*	*P. citrinum*
	11				*P. islandicum*
	12				*Pithomyces chartarum*
	13				*T. padwickii*
	14				*W. sebi*
	15				Species C*
b.ECO_2FUME	*1*	*Aspergillus candidus*	*A. candidus*	*A. candidus*	*A. candidus*
	2	*A. flavus*	*A. flavus*	*A. flavus*	*A. flavus*
	3	*A. tamarii*	*A. fumigatus*	*A. fumigatus*	*A. penicillioides*
	4	*Curvularia lunata*	*A. niger*	*A. tamarii*	*A. tamarii*
	5	*Eurotium repens*	*A. tamarii*	*Cladosporium cladosporioides*	*Curvularia lunata*
	6	*Fusarium semitectum*	*A. tenuis*	*Curvularia lunata*	*C. pallescens*
	7	*Penicillium citrinum*	*Curvularia lunata*	*E. chevalieri*	*Endomyces fibuliger*
	8		*E. chevalieri*	*E. repens*	*Eurotium chevalieri*
	9		*E. repens*	*Mucor rouxii*	*E. repens*
	10		*F. semitectum*	*Penicillium citrinum*	*Penicillium citrinum*
	11		*P. simplicissimum*	*Pithomyces chartarum*	*P. islandicum*
	12		Species A *	*Syncephalastrum racemosum*	*P. simplicissimum*
	13			*Wallemia sebi*	*S. racemosum*
	14				*Trichoniella padwickii*
	15				*W. sebi*
	16				Species C*
	17				Species D*

* unidentified

Annex 6. Fungal species on **maize** treated wit phosphine and ECO_2FUME during storage

Fumigant	No.	Fungal species			
		Duration of storage (days)			
		0	7	45	90
a. Phosphine	1	*A. flavus*	*A. flavus*	*A. candidus*	*A. flavus*
	2	*A. tamarii*	*A. fumigatus*	*A. flavus*	*A. niger*
	3	*E. chevalieri*	*A. niger*	*A. tamarii*	*A. penicillioides*
	4	*E. repens*	*A. tamarii*	*Cladosporium cladosporioides*	*C. cladosporioides*
	5		*E. chevalieri*	*E. repens*	*E. chevalieri*
	6		*E. repens*	*S. raccemosum*	*E. repens*
	7		*S. raccemosum*	*W. sebi*	*S. raccemosum*
	8		*W. sebi*		*W. sebi*
b. ECO_2FUME	1	*Aspergillus candidus*	*A. flavus*	*A. flavus*	*A. flavus*
	2	*A. flavus*	*A. niger*	*A. niger*	*A. niger*
	3	*A. tamarii*	*A. tamarii*	*A. tamarii*	*A. penicillioides*
	4	*Eurotium repens*	*E. chevalieri*	*E. repens*	*A. tamarii*
	5	*Penicillium citrinum*	*E. repens*	*Penicillium citrinum*	*E. chevalieri*
	6		*S. racemosum*	*S. racemosum*	*E. repens*
	7		*W. sebi*	*W. sebi*	*S. racemosum*
	8				*W. sebi*

Annex 7 . Fungal species on **paddy** packed under hermetic and normal conditions during storage

Condition	No.	Fungal species		
		Duration of storage (days)		
		0	45	90
Hermetic	1	*Aspergillus flavus*	*A. clavatus*	*A. flavus*
	2	A. fumigatus	*A. flavus*	*A. fumigatus*
	3	*A. niger*	*A. fumigatus*	*A. penicillioides*
	4	*A. tamarii*	*A. niger*	*A. niger*
	5	*Cladosporium cladosporioides*	*A. tamarii*	*A. tamarii*
	6	*Fusarium moniliforme*	*A. penicillioides*	*C. cladosporioides*
	7	*F. oxysporum*	*C. cladosporioides*	*Curvularia lunata*
	8	*F. semitectum*	*Endomyces fibuliger*	*Eurotium chevalieri*
	9	*Paecylomyces lilacinus*	*Eurotium chevalieri*	*E. repens*
	10	*Penicillium corylophilum*	*E. repens*	*Phoma* sp.
	11	***Phoma shorgina***	*F. moniliforme*	*Synchepalastrum racemosum*
	12	*Trichocladium* sp.	*F. proliferatum*	SG1*
	13	Species B*	*F. semitectum*	SG2*
	14	Species R*	*Penicillium cammemberti*	
	15		***P. citrinum***	
	16		*Phoma shorgina*	
	17		Species G1*	
	18		Species G2*	
	19		Species G3*	
	20		Species G4*	
	21		Species G5*	
	22		***Species G7****	
	23		***Species G9****	
	24		***Species G16****	
	25		***Species G20****	

* unidentified

Annex 7. (continued)

Condition	No.	Fungal species		
		Duration of storage (days)		
		0	45	90
Normal	1	*Acremonium strictum*	Aspergillus flavus	*A. clavatus*
	2	*Aspergillus flavus*	*A. niger*	*A. flavus*
	3	*A. fumigatus*	*Cladosporium cladosporioides*	*A. fumigatus*
	4	*A. niger*	*Curvularia pallescens*	***A. niger***
	5	*Chaetophoma* sp.	*Endomyces fibuliger*	*A. penicillioides*
	6	*Cladosporium cladosporioides*	*Eurotium chevalieri*	*A. versicolor*
	7	*Curvularia lunata*	*E. repens*	*Cladosporium cladosporioides*
	8	*Fusarium moniliforme*	*F. moniliforme*	*Curvularia lunata*
	9	*F. oxysporum*	*F. semitectum*	*Endomyces fibuliger*
	10	*F. semitectum*	*Penicillium cammemberti*	*Eurotium chevalieri*
	11	*Nigrospora oryzae*	***P. citrinum***	*F. moniliforme*
	12	*Penicillium corylophilum*	*P. islandicum*	*F. semitectum*
	13	***Phoma shorgina***	*Phoma shorgina*	*Mucor circinelloides*
	14	***Plenodomus*** sp.	*Wallemia sebi*	*Phoma* sp.
	15	***Trichocladium*** sp.	Species G1*	*Synchepalastrum racemosum*
	16		Species G2*	*Wallemia sebi*
	17		Species G3*	Species SG1*
	18		Species G4*	
	19		Species G5*	
	20		***Species G7****	
	21		Species G10*	

* unidentified

Annex 8. Fungal species on **maize** packed under hermetic and normal conditions during storage

Condition	No.	Fungal species		
		Duration of storage (days)		
		0	45	90
Hermetic	1	*Aspergillus flavus*	*Acremonium strictum*	*Acremonium strictum*
	2	*A. niger*	*Aspergillus clavatus*	*Aspergillus flavus*
	3	*A. tamarii*	*A. flavus*	*A. niger*
	4	*A. wentii*	*A. niger*	*A. tamarii*
	5	*Endomyces fibuliger*	*A. tamarii*	***A. terreus***
	6	*Fusarium moniliforme*	*Endomyces fibuliger*	*A. wentii*
	7	*Penicillium citrinum*	*F. moniliforme*	*Mucor* sp.
	8	*Pithomyces chartarum*	*F. semitectum*	*Penicillim citrinum*
	9		*Nigrospora oryzae*	
	10		*Penicillium citrinum*	
Normal	1	*Aspergillus flavus*	*Acremonium strictum*	*Aspergillus flavus*
	2	*A. niger*	*Aspergillus flavus*	*A. fumigatus*
	3	*A. tamarii*	*A. niger*	*A. niger*
	4	*A. terreus*	*A. tamarii*	*A. tamarii*
	5	*Cladosporium cladosporioides*	*A. versicolor*	*A. penicillioides*
	6	*Endomyces fibuliger*	*Endomyces fibuliger*	*A. wentii*
	7	*Fusarium moniliforme*	*Fusarium moniliforme*	*Fusarium moniliforme*
	8	*Penicillium citrinum*	*Penicillim citrinum*	*Mucor* sp.
	9		Species J1*	*Penicillim citrinum*
	10			***Synchepalastrum racemosum***

* unidentified

Annex 9. Characteristics of PVC plastic and polyethylene films*

No.	Tested parameter	Reference standard	Result
1.	PVC plastic		
	- thickness	ASTM 0645 M-%	0.0141 ± 0.00016 cm
	- Water Vapor Transmission rate (WVTR)	ISO 2528-74	10.81 ± 0.884 g/m^2/24 h t = 24.5 °C RH = 73%
	- Gas Transmission Rate:	ISO 2556-74 (E)	
	- O_2		59.68 cc/m^2/24 h
	- CO_2		144.13 cc/m^2/24 h
2.	Polyethylene films		
	- thickness	ASTM 0645 M-%	0.0262 ± 0.00016 cm
	- Water Vapor Transmission rate (WVTR)	ISO 2528-74	8.5217 ± 0.7891 g/m^2/24 h t = 24.5 °C RH = 73%
	- Gas Transmission Rate:	ISO 2556-74 (E)	
	- O_2		62.24 cc/m^2/24 jam
	- CO_2		84.02 cc/m^2/24 jam

* Analyzed by the Institute for Research and Development of Chemical Industry, Jakarta

Annex 10.

QUESIONAIRE OF SURVEY
ON THE COMSUMPTION OF METHYL BROMIDE IN INDONESIA

1. Name of company : .....
   Address : .....
   .....
   Kind of service : .....
   Commodities : .....

2. Utilization Methyl Bromide

Year	Amount of methyl bromide used	Commodities	Dosage	Type of application	Target of organisms
1998					
1999					
2000					

3. Name of providers services methyl bromide products : .....
   Address : .....

4. Other control technique used as alternative to methyl bromide:
   .....
   .....

5. If methyl bromide is banned, what alternative technology will be used?

   .....

   .....

6. Name of person in charge of methyl bromide procurement and use :

   a. Procurement : .....

   b. Application : .....

7. Effectively of methyl bromide fumigation observed :

   .....

   .....

8. Benefit and lost of methyl bromide used :

   .....

   .....

9. Claim from users :

   a. .....

   b. .....

Distribution Utilization of Methyl Bromide

Name of company : .....

Address : .....

Kind of Commodities	1998		1999		2000	
	Commodities (ton)	CH_3Br (kg)	Commodities (ton)	CH_3Br (kg)	Commodities (ton)	CH_3Br (kg)
Preshipment						
Storage						

QUESIONAIRE FOR IMPORTER

1. Name of company : .....

   Address : .....

   .....

   .....

2. Amount of methyl bromide imported

Year	1998	1999	2000
Amount (ton)			

3. The name of company exporting methyl bromide :

   a. .....

   b. .....

   c. .....

   d. .....

   e. .....

4. Name of company supplied :

   a. .....

   b. .....

   c. .....

   d. .....

   e. .....

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