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EFFECT OF CONTROLLED ATMOSPHERES AND FUMIGANTS ON STORAGE FUNGI — A REVIEW OF RESEARCH ACTIVITIES AT SEAMEO BIOTROP

O.S. DHARMAPUTRA
SEAMEO BIOTROP, P.O. Box 116, Bogor, Indonesia,
and the Faculty of Mathematics and Natural Sciences,
Bogor Agricultural University, Jl, Raya Pajajaran, Bogor, Indonesia

ABSTRACT

Controlled atmospheres and fumigation are primary techniques of insect control, but little research has been done on the effects of carbon dioxide (CO_2) and fumigants on the development of fungi in stored products. Research has been carried out on the effects on storage fungi of CO_2 in maize, of phosphine (PH_3) in milled rice, maize and soybean meal, and of methyl bromide (MB) in milled rice and soybeans. The effects of CO_2 and PH_3 on the mycelial growth of *Aspergillus flavus* in pure culture, and on aflatoxin production in stored commodities, have also been studied. These studies yielded the information detailed below.

CO_2 had no significant effect on either the total fungal population or the population of individual fungal species infecting maize stored under warehouse conditions excepting *Eurotium chevalieri*, whose population was reduced during storage. PH_3 reduced the population of *A. wentii* but increased the population of *E. chevalieri* on maize after fumigation. PH_3 fumigation of soybean meal reduced fungal population; however, its effect was not persistent. Although fungal population was reduced immediately after fumigation, it then increased again after a certain period in storage. PH_3 retarded the growth of *A. penicillioides*, a predominant fungal species of milled rice stored in jute and polypropylene bags. MB reduced both the total fungal population and the individual populations of *E. chevalieri* and *E. rubrum* in milled rice 2 d after fumigation. However, populations increased again 45 d after fumigation. The fumigant also reduced both the total fungal population and the individual populations of *A. sydowii* and *E. chevalieri* on soybeans 2 d after fumigation. A mixture of 20% CO_2 and 0.5 mg/L PH_3 began to inhibit mycelial growth of *A. flavus* in pure culture. At 80% CO_2 and 3.5 mg/L PH_3, mycelial growth was almost totally inhibited.

INTRODUCTION

During storage, products may be infested by insects, mites, microorganisms and rodents. Among microorganisms, fungi are the most important cause of the deterioration of stored products.

In the tropics, *Eurotium* species and other *Aspergilli* are dominant, and *Penicillium* species play only a minor role (Pitt and Hocking, 1991). They can cause weight loss, seed discoloration, heating, mustiness and the production of mycotoxins, the most important of which are aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*.

Controlled atmospheres and fumigation are primary techniques of insect control, but little is known about the effects of carbon dioxide (CO_2) and fumigants on either the development of fungi or aflatoxin production in stored products. CO_2 is more often used for controlling insects during long-term storage of milled rice; the fumigants phosphine (PH_3) and methyl bromide (MB) are effective for controlling insects during long-term storage of products.

This paper describes the research results on the effects of CO_2, PH_3 and MB on the development of fungi in milled rice, maize, soybeans and soybean meal. The effects of CO_2 and PH_3 on aflatoxin production are also described.

REVIEW OF PUBLISHED STUDIES

The effects of CO_2 on mycelial growth and aflatoxin production of *A. flavus* in pure culture

Dharmaputra *et al.* (1990a) studied the effects of CO_2 concentrations of 20, 40, 60 and 80% on mycelial growth and aflatoxin B_1 production of three *A. flavus* isolates (BIG-16, BIG-17 and BIG-18). As a control, these fungal isolates were maintained in air. The CO_2 concentrations significantly affected both mycelial growth and aflatoxin production of the isolates of *A. flavus*. CO_2 at 20% started to inhibit the two parameters (Figs. 1 and 2), and all parameters decreased with increased CO_2 concentration. At 80% CO_2 mycelial growth of isolate BIG-17 was almost totally inhibited (Fig. 1). Aflatoxin production of the three isolates was also reduced with increased CO_2 concentration (Fig. 2).

The effects of PH_3 on mycelial growth and aflatoxin production of *A. flavus* in pure culture

An investigation was carried out by Dharmaputra *et al.* (1991) on the effects of PH_3 on both mycelial growth and aflatoxin B_1 production of two *A. flavus* isolates (BIG-17 and BIG-18). The concentrations of PH_3 used were 0.5, 1.5, 2.5 and 3.5 mg/L. As a control, these fungal isolates were maintained in air.

The PH_3 concentrations significantly affected both the mycelial growth and aflatoxin production of the isolates of *A. flavus*. Isolate BIG-17 was more sensitive than isolate BIG-18 (Fig. 3). Mycelial growth decreased with increasing PH_3 concentrations. Inhibition of mycelial growth commenced at 0.5 mg/L, and isolate BIG-17 was almost totally inhibited at 3.5 mg/L (Fig. 3).

Aflatoxin production decreased with increasing PH_3 concentration (Fig. 4). Although the two isolates were still able to produce aflatoxin after treatment with 3.5 mg/L, the amounts were low.

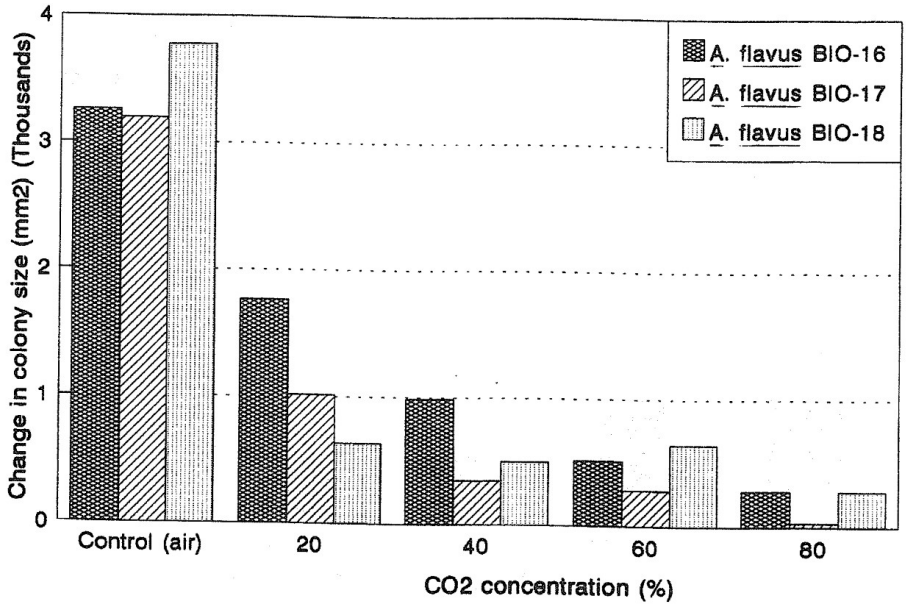

Fig. 1. Mycelial growth of *A. flavus* BIO-16, BIO-17 and BIO-18 after treatment with various concentrations of CO_2 for 7 d on potato dextrose agar (Dharmaputra *et al.*, 1990a).

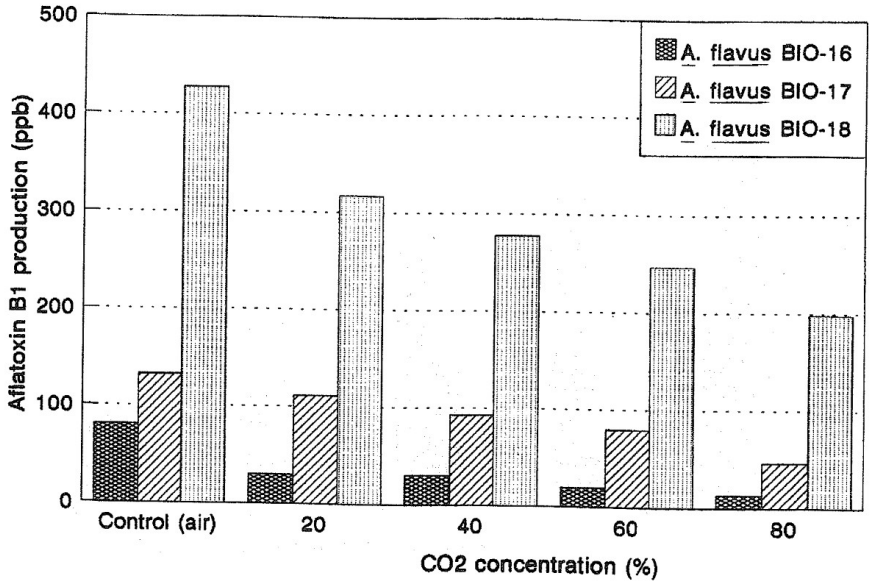

Fig. 2. Aflatoxin B_1 production of *A. flavus* BIO-16, BIO-17 and BIO-18 after treatment with various concentrations of CO_2 for 10 d on 10% coconut extract medium (Dharmaputra *et al.*, 1990a).

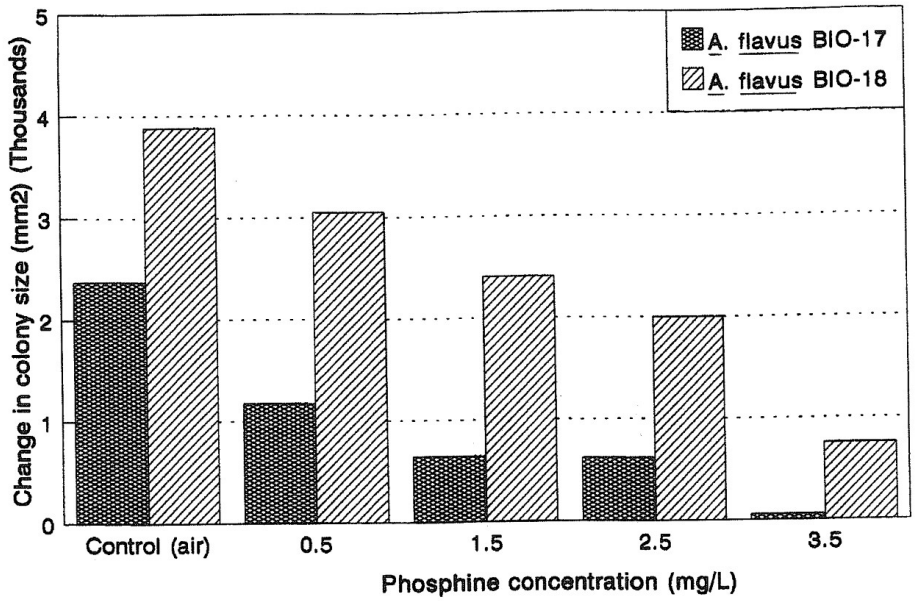

Fig. 3. Mycelial growth of *A. flavus* BIO-17 and BIO-18 after treatment with various concentrations of PH_3 for 5 d on potato dextrose agar (Dharmaputra *et al.*, 1991a).

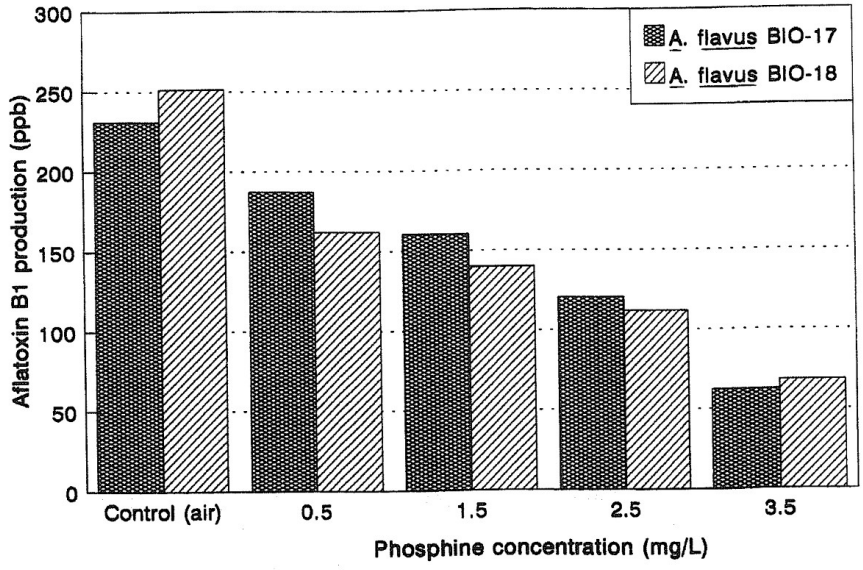

Fig. 4. Aflatoxin B_1 production of *A. flavus* BIO-17 and BIO-18 after treatment with various concentrations of PH_3 for 5 d on 10% coconut extract medium (Dharmaputra *et al.*, 1991a).

The effects of CO_2 on the development of fungi and aflatoxin production in stored maize

Dharmaputra *et al.* (1990b) studied the effects of CO_2 on the development of fungi and aflatoxin production in stored maize. Stacks of stored maize were sealed with PVC sheeting and treated with CO_2 for storage periods varying from 10 to 120 d. The concentration of CO_2 used was 2.4 kg/t. The control groups consisted of both stacks of maize sealed in plastic sheets, but not treated with CO_2, and stacks not sealed in plastic sheets. Twelve species of fungi were isolated from the stored maize using the dilution method. They were *A. candidus*, *A. flavus*, *A. niger*, *A. penicillioides*, *A. tamarii*, *A. versicolor*, *A. wentii*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. repens*, *Mucor hiemalis* and *Penicillium citrinum*.

The concentration of applied CO_2 had no significant effect on either the total population of fungi or the individual population of each fungal species, with the exception of *E. chevalieri*, whose population was reduced (Fig. 5). The total population of fungi increased significantly with the prolongation of storage duration.

The aflatoxin B_1 content of maize, whether in plastic sheets and treated with CO_2 or only in plastic sheets, was lower than that of the unsheeted and untreated stacks (Fig. 6). The control showed that the aflatoxin content increased with the increasing duration of storage.

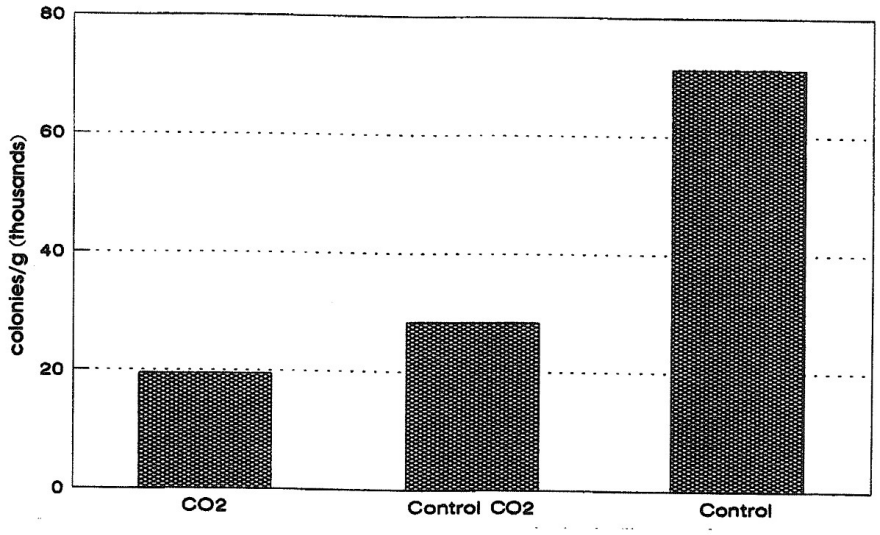

Fig. 5. Population of *Eurotium chevalieri* on maize treated with CO_2 and control (Dharmaputra *et al.*, 1990b). Control CO_2 = stacks of maize sealed in PVC sheeting but not treated with CO_2; control = stacks not sealed.

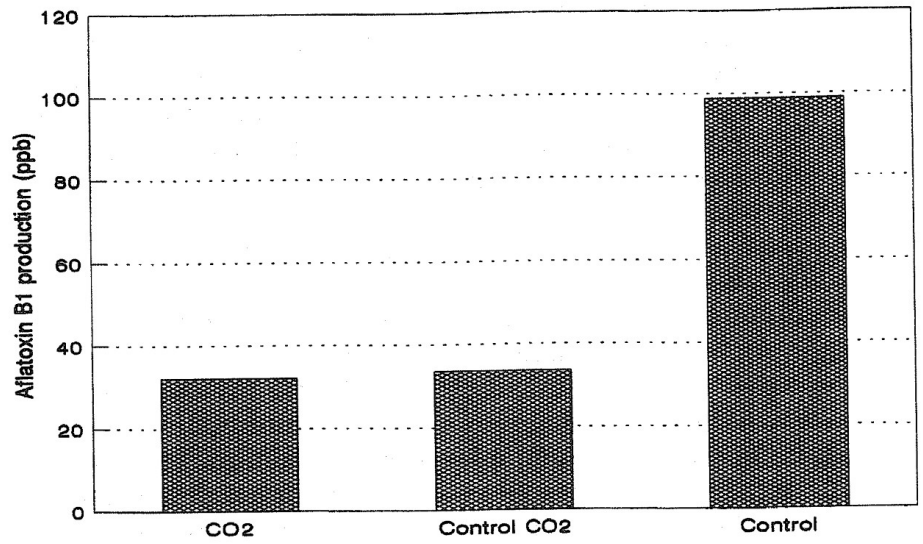

Fig. 6. Aflatoxin B_1 production on maize treated with CO_2 and control (Dharmaputra *et al.*, 1990b). Control CO_2 = stacks of maize sealed in PVC sheeting but not treated with CO_2; control = stacks not sealed.

The effects of PH_3 and bag type on the development of fungi in stored milled rice

An investigation was carried out by Dharmaputra *et al.* (1997) on the effects of PH_3 and bag type on the development of storage fungi in milled rice. The milled rice was stored in either jute or open-weave polypropylene bags for 49 weeks. The rice was fumigated with PH_3 at a dosage of 10 tablets/stack or 2 tablets/t for an exposure period of 5 d. Fumigation was repeated at 3-month intervals throughout the experiment.

The fungal population on rice was determined before each fumigation (at the beginning of storage and then at 11, 23, 35 and 47 weeks of storage) and immediately after fumigation (at 1, 13, 25, 37 and 49 weeks of storage).

During the 49 weeks of storage, 21 and 22 species of fungi were isolated from milled rice packed in jute and polypropylene bags, respectively. The predominant fungus in both bag types was *A. penicillioides*, which increased sharply in the first week; thereafter, the population remained relatively constant, both before and after fumigation (Fig. 7).

On milled rice packed in jute bags both the total fungal count and that of *A. penicillioides* were lower than on milled rice packed in polypropylene bags. The populations increased until the eleventh week of storage; thereafter, populations declined slowly with lengthening storage periods.

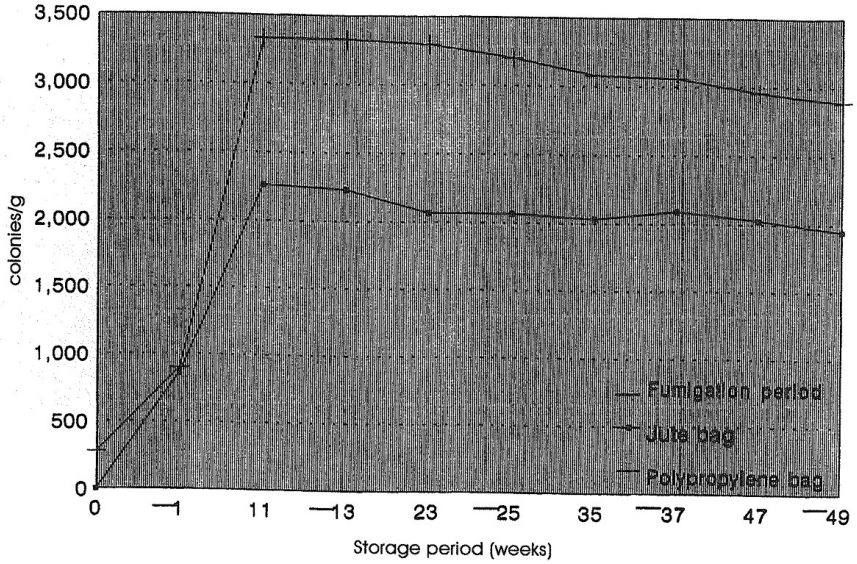

Fig. 7. Population of *A. penicilloides* on milled rice packed in jute or polypropylene bags both before and after fumigation with PH_3 (Dharmaputra *et al.*, 1996).

The effects of PH_3 on the development of fungi and aflatoxin production in stored maize

Dharmaputra *et al.* (1992a) studied the effects of PH_3 on both the development of fungi and aflatoxin production in stored maize. Stacks of stored maize were treated with 2 g/t PH_3 for 5 d. The control consisted of stacks of maize not treated with PH_3.

Using the dilution method, 11 species of fungi were isolated: *A. candidus*, *A. flavus*, *A. niger*, *A. tamarii*, *A. versicolor*, *A. wentii*, *C. cladosporioides*, *E. chevalieri*, *E. repens*, *M. hiemalis* and *P. citrinum*. PH_3 at the stated concentration reduced the population of *A. wentii* but increased that of *E. chevalieri* (Fig. 8). There was no significant difference between the stacks treated with PH_3 and the control in the aflatoxin B_1 content of maize (Table 1).

The effects of PH_3 on the development of fungi and aflatoxin production in stored soybean meal

Investigations were carried out by Dharmaputra *et al.* (1992b, 1993) on the effects of PH_3 on both the development of fungi and aflatoxin production in stored soybean meal. Soybean meal was stored in polypropylene bags for 190 d. Four stacks were treated with PH_3 (2.1 g/t) for 5 d, once at the beginning of storage and again after 95 d. Four untreated stacks served as control.

Using the dilution method, 17 species of fungi were isolated from the stored soybean

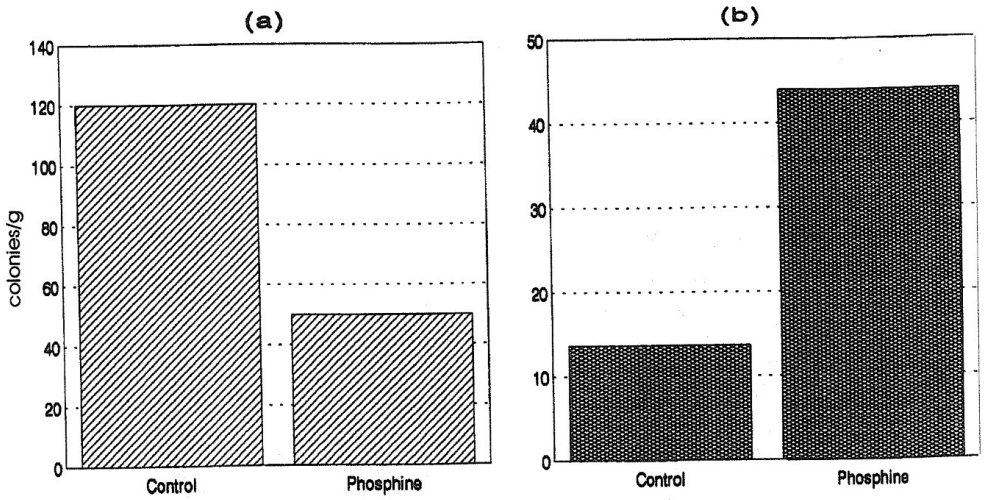

Fig. 8. Population of *A. wentii* (a) and *Eurotium chevalieri* (b) on maize treated with PH_3 and on control (Dharmaputra *et al.*, 1992a).

TABLE 1

The effects of treatment with PH_3 and fumigation period, and their interaction, on aflatoxin production on maize (Dharmaputra *et al.*, 1992a)

Effect	Aflatoxin B_1 production (ppb)
Treatment	
Control	38.87 a
Phosphine	37.78 a
Fumigation period	
0 d	24.43 a
5 d	26.22 a
Interaction between treatment and fumigation period	
Control, 0 d	36.76 a
Control, 5 d	39.98 a
Phosphine, 0 d	32.10 a
Phosphine, 5 d	43.46 a

Numbers followed by the same letter do not differ significantly according to Tukey's Multiple Comparison Test at 95% confidence level.

meal. The predominant species found in both treated and untreated stacks were *A. sydowii*, *E. chevalieri* and *Wallemia sebi*. There were fluctuations in the total populations of fungi during storage, both in the stacks treated with PH_3 and the untreated ones. The total populations decreased after treatment but then increased during storage (Fig. 9).

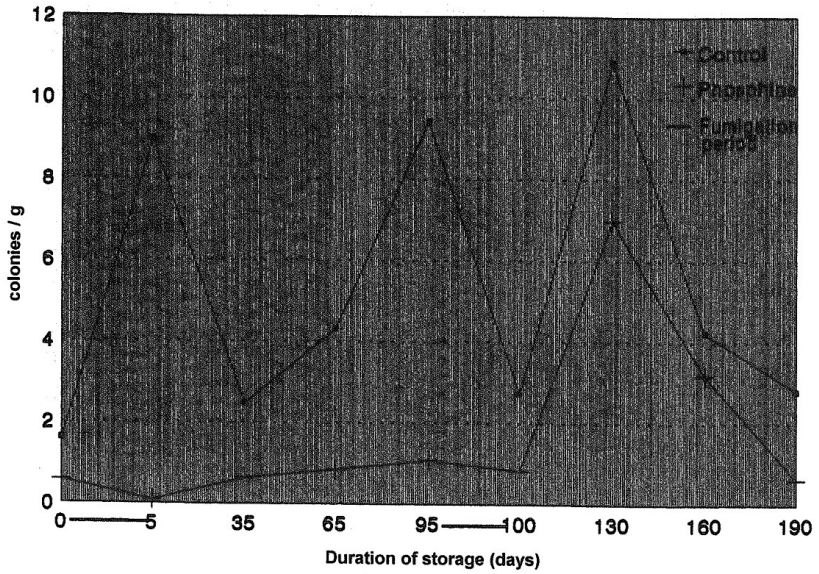

Fig. 9. Total population of fungi on soybean meal treated with PH_3 and on control (Dharmaputra *et al.*, 1992b, 1993).

There was a significant difference in aflatoxin B_1 production between the treated and the untreated samples (Table 2). Aflatoxin B_1 production increased during prolonged storage in both treated and untreated soybean meal (Fig. 10). These results indicate that PH_3 somehow inhibits aflatoxin B_1 production.

TABLE 2
Aflatoxin B_1 content on soybean meal treated with PH_3 and on control (Dharmaputra *et al.*, 1992b)

Duration of storage (d)	Control (ppb)	PH_3 (ppb)	F-value
0[a]	5.82	0.00	–
5	14.49	9.42	0.46
35	18.35	9.92	14.77*
65	18.42	11.21	23.50**
95[b]	22.42	12.67	10,255.91**
100	24.94	14.06	32,828.57**
130	27.85	14.84	497.96**
160	30.25	15.28	1,625.72**
190	31.12	23.55	10.33*

a = 1st PH_3 treatment; b = 2nd PH_3 treatment; * = significant difference at 95% confidence level; ** = significant difference at 99% confidence level.

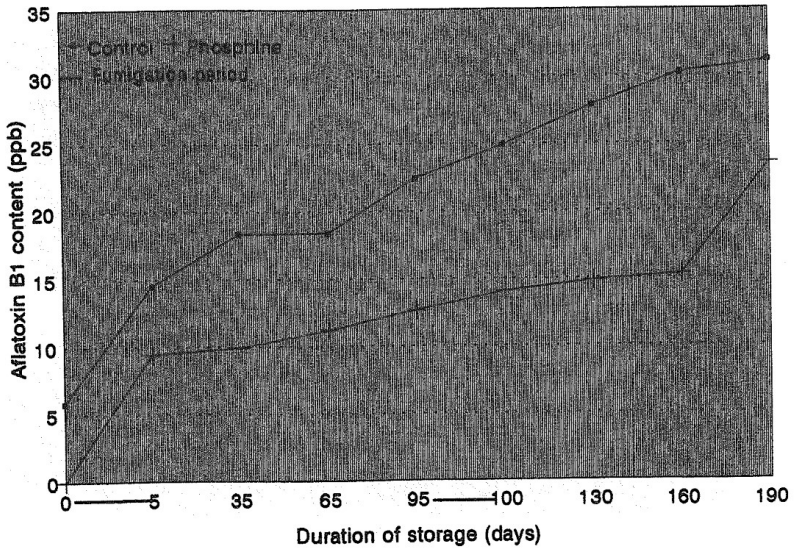

Fig. 10. Aflatoxin B_1 content on soybean meal treated with PH_3 and on control (Dharmaputra *et al.*, 1992b).

The effects of MB on fungi of stored milled rice and soybeans

Dharmaputra and Retnowati (1993) studied the effects of MB on fungal populations of stored milled rice and soybeans. Milled rice and soybeans were treated with 21 g/t MB for 24 h.

Both the total fungal population and the individual populations of *E. chevalieri* and *E. rubrum* in milled rice showed a decrease 2 d after fumigation but again increased 45 d after fumigation (Table 3). *A. niger* and *A. wentii* were not isolated either 2 d or 45 d after fumigation. The population of *A. flavus* decreased 2 d and again 45 d after fumigation, whereas populations of *A. versicolor* and *C. cladosporioides* increased. In soybeans, both the total fungal population and the individual populations of *A. sydowii* and *E. chevalieri* showed decreases 2 d after fumigation (Table 4).

CONCLUSIONS

In pure culture, CO_2 and PH_3 inhibited both mycelial growth and aflatoxin production of *A. flavus*. CO_2 at 80% had no significant effect on the total fungal population of maize but did reduce populations of certain individual species. The concentration of CO_2 which was applied reduced aflatoxin production.

PH_3 at 2 g/t reduced the population of certain fungal species and increased that of others; however, at the concentration applied, there was no significant difference in the treated and untreated samples in aflatoxin content of maize.

TABLE 3
Population of each fungal species on milled rice before and then 2 and 45 d after fumigation with methyl bromide (Dharmaputra and Retnowati, 1993)

Fungi	Fungal population (colonies/g)[1]		
	Before fumigation	2 d after fumigation	45 d after fumigation
A. flavus	19.2 a	1.4 b	0.7 b
A. niger	10.9 c	0.0 d	0.0 d
A. versicolor	0.0 e	2.4 e	9.3 f
A. wentii	6.5 g	0.0 h	0.0 h
C. cladosporioides	2.6 i	3.6 i	24.3 j
E. chevalieri	18.9 k	0.9 l	4.8 m
E. rubrum	6.2 n	0.0 o	0.3 o
Total[2]	100.1 p	42.4 q	195.1 r

[1]Numbers followed by the same letter do not differ significantly according to Tukey's Multiple Comparison Test at 95% confidence level.
[2]Total population of isolated fungi.

TABLE 4
Population of each fungal species on soybean before fumigation and 2 d after fumigation with methyl bromide (Dharmaputra and Retnowati, 1993)

Sampling time	Fungal population (colonies/g)[1]		
	Aspergillus sydowii	*Eurotium chevalieri*	Total[2]
Before fumigation	38.3 a	60.7 a	101.8 a
Two days after fumigation	10.7 b	1.0 b	24.0 b

[1]Numbers followed by the same letter do not differ significantly according to Tukey's Multiple Comparison Test at 95% confidence level.
[2]Total population of isolated fungi.

In soybean meal, PH_3 at 2.1 g/t reduced the total fungal population, but its effect was not persistent. It seems that PH_3 at this dosage level inhibits aflatoxin production.

On milled rice, 2 tablets/t of PH_3 retarded fungal growth. On milled rice and soybeans, 21 g/t of MB reduced populations of some fungal species.

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REFERENCES

Dharmaputra, O.S., Tjitrosomo, H.S.S., Wardhani, T.S. and Halid, H. (1990a) The effects of carbon dioxide on some biological aspects of *Aspergillus flavus*. In: *Proc. 13 ASEAN Seminar on Grain Postharvest Technology* (Edited by Naewbanij, J.O.), Darussalam, Brunei, 4–7 September 1990, 280–293.

Dharmaputra, O.S., Tjitrosomo, H.S.S., Aryani, F. and Sidik, M. (1990b) The effects of carbon dioxide on storage fungi of maize. In: *Proc. 5th Int. Working Conf. on Stored-Product Protection* (Edited by Fleurat-Lessard, F. and Ducom, P.), Bordeaux, France, 9–14 September 1990, 291–301.

Dharmaputra, O.S., Tjitrosomo, H.S.S., Sidik, M. and Umaly, R.C. (1991) The effects of phosphine on some biological aspects of *Aspergillus flavus*. In: *Fungi and mycotoxins in stored products* (Proc. of an Int. Conf.) (Edited by Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I.), Bangkok, Thailand, 23–26 April 1991, 244–248.

Dharmaputra, O.S., Tjitrosomo, H.S.S., Sidik, M. and Halid, H. (1992a) The effects of phosphine on storage fungi of maize. In: *BIOTROP Special Publication No. 45: Proc. Symp. on Pests of Stored Products* (Edited by Sidik, M., Rejesus, B.M., Sosromarsono, S., Champ, B.R., van S. Graver, J., Umaly, R.C. and Dharmaputra, O.S.), Bogor, Indonesia, 29–31 January 1991, 107–119.

Dharmaputra, O.S., Retnowati, I., Sidik, M. and Halid, H. (1992b) The effects of phosphine and length of storage on fungi, aflatoxin and protein content of soybean meal. In: *Proc. 15th ASEAN Seminar on Grain Postharvest Technology* (Edited by Naewbanij, J.O.), Singapore, 8–11 September 1992, 125–136.

Dharmaputra, O.S., Susilo, H., Sidik, M. and Halid, H. (1993) Effect of phosphine and duration of storage on fungal population and some chemical components of soybean meal. In: *Proc. Int. Conf. on Controlled Atmospheres and Fumigation in Grain Storages* (Edited by Navarro, S. and Donahaye, E.), Winnipeg, Canada, 11–13 June 1992, Caspit Press Ltd., Jerusalem, 191–201.

Dharmaputra, O.S. and Retnowati, I. (1993) The effects of methyl bromide on fungi of stored milled rice and soybean. Paper presented at the *6th National Congress of the Indonesian Mycrobiology Association*, Surabaya, 2–4 December 1993 (in Indonesian).

Dharmaputra, O.S., Putri, A.S.R. and Sidik, M. (1997) The effects of phosphine and bag type on storage fungi of milled rice. In: *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products* (Edited by Donahaye, E.J., Navarro, S. and Varnava, A.), Nicosia, Cyprus, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, 00–00.

Pitt, J.I. and Hocking, A.D. (1991) Significance of fungi in Stored Products. In: *Fungi and Mycotoxins in Stored Products (Proc. of an Int. Conf.)* (Edited by Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I.), Bangkok, Thailand, 23–26 April 1991, 16–21.