THE EFFECTS OF CARBON DIOXIDE ON STORAGE FUNGI OF MAIZE[1)]

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ABSTRACT

The effects of CO_2 on storage fungi of maize was investigated together with aflatoxin production.

Stacks of stored maize were enclosed with PVC plastic and treated with CO_2 for varying length of storage from 10 to 120 days. The concentration of CO_2 used was 2.4 kg/ton. The control groups consisted of stacks of maize enclosed in plastic sheets but not treated with CO_2 and stacks not enclosed in plastic sheets. Twelve species of fungi were isolated from the stored maize using dilution method. Among these were *Aspergillus candidus*, *A. flavus*, *A. niger*, *A. penicilloides*, *A. tamarii*, *A. versicolor*, *A. wentii*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. repens*, *Mucor hiemalis*, and *Penicillium citrinum*.

The concentration of CO_2 applied had no significant effect on the total population of fungi and the population of each species of fungus, except *E. chevalieri*, which population was reduced. The total population of fungi increased significantly when the length of storage was increased.

The aflatoxin B_1 content of maize either enclosed in plastic sheets and treated with CO_2 (32.05 ppb) or just enclosed in plastic sheets (33.52 ppb) were lower than that of the unenclosed and untreated (98.78 ppb). The control showed that the aflatoxin content increased with the length of storage.

INTRODUCTION

In Indonesia maize is a secondary important crop after rice. During storage maize could be infested by insects, mites, microorganisms and rodents. Among microorganisms, fungi is the most important cause of deterioration of stored products (Christensen & Kaufmann, 1974).

Aspergillus and *Penicillium* are the common fungi on stored products. They can cause loss in weight, discolouration of seeds, heating and mustiness, and produce mycotoxins.

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Among the known mycotoxins, the most important from the viewpoint of direct health hazards to human and domestic animal are the aflatoxins, because they are carcinogenic. They are produced by *Aspergillus flavus* and *A. parasiticus*. Its production depends on the species or strain of the fungus, and on the ecological conditions for its development in particular food source, temperature and humidity (Butler, 1974; Christensen & Kaufmann, 1977; Neergaard, 1979).

The tropical climate of Indonesia provides favourable condition for fungal growth. They can develop on temperatures between 5-35 C and on relative humidities between 70-90% (Christensen, 1978).

Aflatoxin contamination in maize has been reported in Indonesia. Maize stored in BULOG warehouses in 1982 had been reported to be contaminated with aflatoxin at considerable concentrations (Shinta *et al.*, 1983). Furthermore, six samples of maize collected from farmers in Lampung Province, South Sumatra were contaminated by aflatoxin B_1 at a range between 2-83 ppb (Rahayu & Dharmaputra, 1988).

Fumigation is primarily used for insect control, but little is known on the effect of fumigants to the development of storage fungi and on aflatoxin production on maize. Among fumigants, CO_2 is the more often used for controlling insects during long-term storage of rice.

The objective of this study was to get information on the effects of CO_2 on storage fungi of maize. Aflatoxin production were also analyzed.

MATERIALS AND METHODS

Stacks of maize and fumigation of CO_2

Stacks of maize were enclosed in PVC plastic (0.3 mm thick) and treated with CO_2 for 10, 30, 60, 90 and 120 days. The concentration of CO_2 used was 2.4 kg/ton. The control groups consisted of stacks of maize enclosed in PVC plastic but not treated with CO_2 (vacuum condition) and stacks not enclosed in PVC plastic. Each treatment (including control) consisted of 2 stacks (2 replications). Each stack was randomly arranged and it consisted of 4 bags (50 kg of maize/bag).

Methods of sampling

Initial samples were taken before fumigation (0 day), and 10, 30, 60, 90, 120 days after fumigation. Initial samples were also taken from control groups. Samples were drawn from three points of each bag using a probe. These samples were mixed thoroughly to obtain the primary sample.

Obtaining working sample

To get working sample, the primary samples were divided using a sample divider. Then, the working samples were divided into two analysis samples, i.e. for fungal and aflatoxin analysis.

Fungal and aflatoxin analysis

Fungi of stored maize were isolated by dilution methods. The aflatoxin content (B_1) was determined by Thin Layer Chromatography method (Blaney *et al.*, 1984).

Identification of the fungi

Fungal identification was determined according to Raper & Fennel (1965), Barnett & Hunter (1972), Samson *et al.*, (1984), Klich & Pitt (1985) and Pitt & Hocking (1985).

The experiment was analyzed using Completely Randomized Factorial Design.

RESULTS AND DISCUSSION

Fungal analysis

Twelve species of fungi were isolated using dilution method, i.e. *Aspergillus candidus, A. flavus, A. niger, A. penicilloides, A. tamarii, A. versicolor, A. wentii, Cladosporium cladosporioides, Eurotium chevalieri, E. repens, Mucor hiemalis* and *Penicillium citrinum*.

Analysis of variance showed that on control (stacks unenclosed and untreated), CO_2 treatment (stacks enclosed and treated with CO_2), and control CO_2 (stacks enclosed and untreated with CO_2) gave significant differences on the population of *E. chevalieri* (Table 1).

Table 1. Analysis of variance on the effect of treatments and length of storage on population of each species of fungi (per gram of maize).

Fungi	Source of var.	Df	SS	MS	F-value
Aspergillus candidus	A	2	440248000000	220124000000	1.672
	B	5	647754000000	129551000000	0.984
	A x B	10	16170700000000	161707000000	1.229
	Error	18	23693000000000	131628000000	
A. flavus	A	2	858314000	429157000	0.197
	B	5	12070800000	2414160000	1.108
	A x B	10	19266100000	1926610000	0.884
	Error	18	39216900000	2178720000	
A. niger	A	2	19450600	9725278	0.763
	B	5	63134700	12626900	0.991
	A x B	10	126619000	12661900	0.994
	Error	18	229405000	12744700	
A. penicil-loides	A	2	10177700000	5088860000	0.653
	B	5	87062600000	17412500000	2.235
	A x B	10	10823400000	10823400000	1.389
	Error	18	14024300000	7791270000	

Table 1. (Continuation)

Fungi	Source of var.	Df	SS	MS	F-value	
A. tamarii	A	2	22004300	11002200	0.653	
	B	5	60451000	12090200	0.717	
	A x B	10	155882000	15588200	0.925	
	Error	18	303428000	16857100		
A. versicolor	A	2	188554000	94276800	0.874	
	B	5	630557000	126111000	1.169	
	A x B	10	982465000	98246500	0.910	
	Error	18	1942530000	107918000		
A. wentii	A	2	1914967	957484	0.291	
	B	5	34340000	6867990	2.087	
	A x B	10	17009700	1700974	0.517	
	Error	18	59234800	3290823		
Cladosporium cladosporioides	A	2	32375700	16187800	1.579	
	B	5	107024000	21404900	2.088	
	A x B	10	89390800	8939085	0.872	
	Error	18	184521000	10251200		
Eurotium chevalieri	A	2	183706000	918527000	4.798	*
	B	5	301126000	682253000	4.286	
	A x B	10	312069000	312069000	1.877	
	Error	18	4499390000	2496320000		
E. repens	A	2	333930000	166965000	1.392	
	B	5	930768000	186154000	1.552	
	A x B	10	750212000	75021200	0.626	
	Error	18	2158380000	119910000		
Mucor hiemalis	A	2	1227222	613611	1.000	
	B	5	3068056	613611	1.000	
	A x B	10	6136111	613611	1.000	
	Error	18	11045000	613611		
Penicillium citrinum	A	2	383797000	191899000	0.766	
	B	5	967798000	193560000	0.772	
	A x B	10	2476110000	247611000	0.988	
	Error	18	4512190000	250677000		
Total population	A	2	274320000000	137160000000	0.807	
	B	5	18368400000000	367368000000	2.162	
	A x B	10	15840900000000	158409666666	0.932	
	Error	18	30591400000000	169952000000		

A = Control, CO_2, Control CO_2
B = Length of storage
A x B = Interaction between treatment and length of storage
* = Significant different at 95% confidence level

Using Tukey's Multiple Comparison Test at 95% confidence level, the population of *E. chevalieri* in stacks treated with CO_2 was not significantly different with its population in stacks enclosed and untreated with CO_2, but significantly different in unenclosed and untreated stacks (Table 2).

Table 2. The effect of treatments on the population of *Eurotium chevalieri*.

Treatment	Mean of population (colonies/g)
Control	71324.733 b
CO_2	19454.671 a
Control CO_2	28370.167 a

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

The effect of CO_2 on aflatoxin production

Aflatoxins could be produced by certain strains of *A. flavus* and *A. parasiticus* (Diener & Davis, 1969).

Analysis of variance showed that on control, CO_2 treatment and control CO_2 gave very significant difference on aflatoxin B_1 production, while length of storage and interaction between treatments and length of storage gave significant difference on aflatoxin B_1 production (Table 3).

Using Tukey's Multiple Comparison Test at 95% confidence level, the aflatoxin content in CO_2 treatment (32.053 ppb) was not significantly different with its content in control CO_2 (33.520 ppb), but significantly different with its content (98.775 ppb) in control (Table 4). CO_2 could reduce aflatoxin production.

Table 3. Analysis of variance on the effect of treatments and length of storage on aflatoxin production.

Source of var.	Df	SS	MS	F-value
A	2	34849.207	17424.603	8.875 **
B	5	35715.189	7143.038	3.638 *
A x B	10	57224.492	5722.449	2.915 *
Error	18	35338.571	1963.254	

A = Control, CO_2, Control CO_2
B = Length of storage
A x B = Interaction between treatments and length of storage
* = Significantly different at 95% confidence level
** = Significantly different at 99% confidence level

On CO_2 treatment and control CO_2 the stacks were enclosed with plastic. It means that O_2 content was reduced and the metabolism of *A. flavus* was decreased and its ability to produce aflatoxins was also decreased. According to Garraway & Evans (1984), there was close relationship between vegetative growth of the fungus and aflatoxin production.

CO_2 at concentrations of 60-80% could inhibit the growth of *A. flavus*, while CO_2 at 100% inhibit aflatoxin production (Landers *et al.*, 1967).

The effect of interaction between control and length of storage was significantly different in the 90th day of storage, while in CO_2 treatment and control CO_2 during the storage was not significantly different (Table 4). The highest aflatoxin content was found on the 90th day of storage (112.185 ppb) (Table 4, Figure 1). The total population of fungi increased starting on the 60th day of storage (492683,333 colony/g) (Table 5). It was assumed that there was competition between *A. flavus* and the other species of fungi.

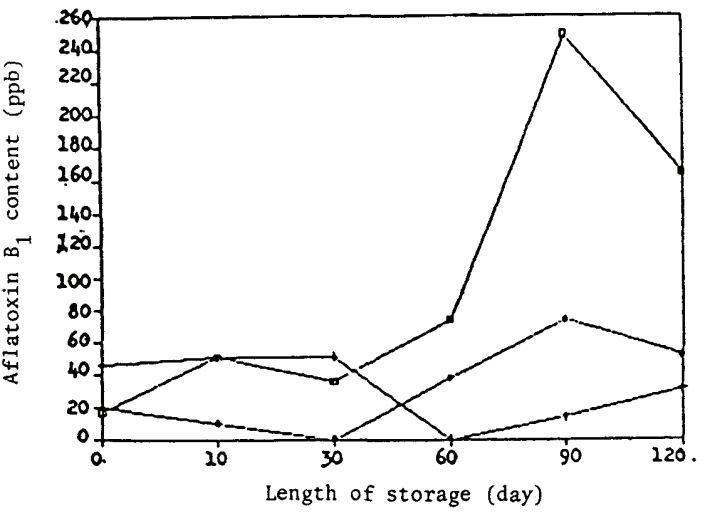
Table 4. The effect of treatments, length of storage and interaction between treatment and length of storage on aflatoxin production.

Effect	Mean aflatoxin B_1 production (ppb)
Treatment	
Control	98.775 b
CO_2	32.053 a
Control CO_2	33.520 a
Length of storage (day)	
0	29.864 a
10	37.378 ab
30	29.215 a
60	37.598 ab
90	112.185 b
120	82.633 ab
Interaction between treatment and length of storage (day)	
Control	
0	16.760 a
10	51.265 a
30	36.140 a
60	74.540 ab
90	248.745 b
120	165.200 ab
CO_2	
0	46.126 a
10	50.705 a
30	51.505 a
60	0.000 a
90	13.405 a
120	30.485 a

Table 4. (Continuation).

Effect	Mean aflatoxin B_1 production (ppb)
Control CO_2	
0	20.077 a
10	10.165 a
30	0.000 a
60	38.255 a
90	74.405 a
120	52.215 a

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



□ = control; + = CO_2; • = control CO_2

Figure 1. Relation between aflatoxin B_1 content and length of storage in each treatment.

Table 5. The effect of length of storage on total population of fungi.

Length of storage (day)	Total population of fungi (colony/g)
0	26722.233 a
10	352283.333 a
30	54216.667 a
60	492683.333 a
90	531333.333 a
120	597466.667 a

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

Figure 2 shows that *A. flavus* population was not correlated with aflatoxin content. If the population was high, the aflatoxin content was not always high. The existence of aflatoxin producing fungi in a commodity does not always indicate the presence of aflatoxin, because aflatoxin production depends on the species or strain of the fungus, environmental factors and interaction with other microorganisms (Butler, 1974; Neergaard, 1979; Lacey *et al.*, 1980). Aflatoxin could be produced only in some seeds (Dollear, 1969) and its distribution was not homogenous (WHO, 1979).

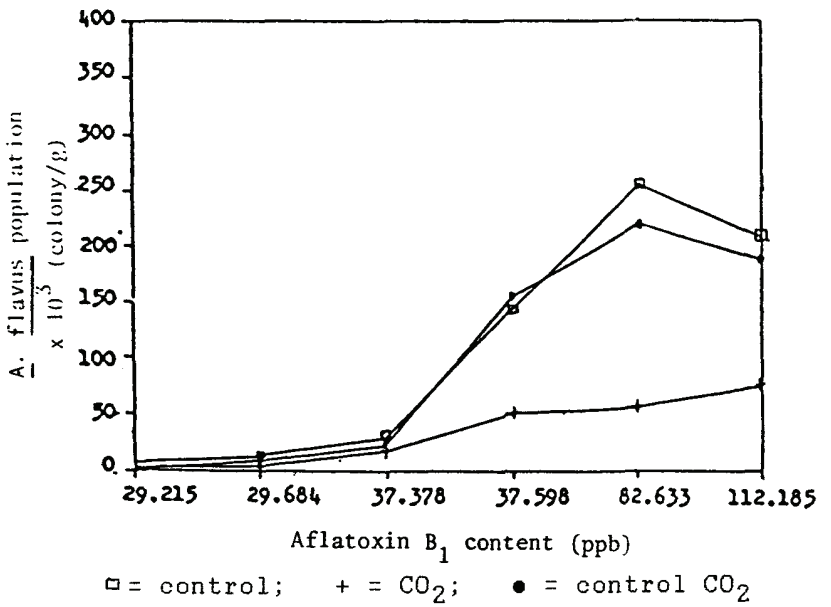


Figure 2. Relation between *Aspergillus flavus* population and aflatoxin B_1 content on each treatment.

CONCLUSION

The concentration of CO_2 applied had no significant effect on the total population of fungi and the population of each species of fungus, except on *E. chevalieri*, where its population was reduced.

The concentration of CO_2 applied could reduce aflatoxin production.

Plastic enclosure on stacks could inhibit fungal growth, because O_2 content decreased, and fungal metabolism was also decreased.

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TOXICITE DU DIOXYDE DE CARBONE SUR LES CHAMPIGNONS DES STOCKS DE MAIS

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RESUME

La toxicité du dioxyde de carbone sur les champignons du maïs a été étudiée en même temps que la production d'aflatoxines, le degré d'humidité et la qualité des semences. Des sacs de maïs en stock ont été placés dans des poches en plastique et traités au dioxyde de carbone pendant une durée allant de 10 jours à 1 ou 4 mois. La concentration en CO_2 utilisée était de 2,4 Kg/t. Le lot témoin était constitué de sacs de maïs placés dans les mêmes poches en plastique. Quinze espèces de champignons ont été isolées du maïs stocké en utilisant la méthode des dilutions. Parmi celles-ci se trouvaient : *A. flavus, Eurotium sp.1, Eurotium sp.2, Eurotium sp.3, A. niger, A. penicilloides, A. tamarii, A. versicolor, A. wentii, Cladosporium cladosporioides, Fusarium sp., Mucor hiemalis, Penicillium citrinum* et *Rhizopus oryzae.* La concentration en dioxyde de carbone a eu un effet net sur le pourcentage de grains infestés par *A. tamarii, Mucor hiemalis* et *Rhizopus oryzae.* Le pourcentage de grains infestés par le mycélium s'est avéré plus faible dans les cas mis en sacs en plastique traités que dans ceux à l'air libre ou dans ceux en sacs non traités. La concentration en dioxyde de carbone n'a pas eu d'effet net ni sur la population totale, ni sur la fréquence d'apparition de chaque espèce, sauf sur *Eurotium sp.1.* La durée de stockage a eu un effet net sur la contamination totale des grains. Cette population totale s'est accrue avec l'accroissement de la période de temps. La teneur en aflatoxine B1 du maïs, soit enfermé dans des sacs en plastique et traité au dioxyde de carbone (32,05 ppb) ou simplement enfermé en sacs plastique (33,52 ppb) était inférieure à celle du maïs non enfermé ou non traité (98,78 ppb). Le témoin a montré que la teneur en aflatoxine augmentait avec la durée du stockage. Il n'y a pas eu de nettes différences de degré d'humidité des grains entre les trois traitements (y compris le témoin). Le dioxyde de carbone pourrait donc faire durer la qualité des semences, en particulier celles qui sont intactes lors de l'entrée en stock.