THE EFFECT OF CARBON DIOXIDE ON ASPERGILLUS FLAVUS AND EUROTIUM REPENS

(A STUDY DONE AT MYCOLOGY LABOROTARY IN BIOTROP, BOGOR, INDONESIA).

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ABSTRACT

The effects of different concentrations of carbon dioxide (20 40, 60 and 80%) on:
(1) mycelial growth and conidiation of A. flavus;
(2) mycelial growth, conidiation and cleistothecia formation of E. repens
were studied. The experiment was conducted in 3.3 liters jars (gas tight) at room temperature, using 3 replicates for each treatment.

Potato Dextrose Agar was used as the medium to study the effects of CO_2 on mycelial growth, conidiation and cleistothecia formation of A. flavus while Czapek Yeast Extract 20% Sucrose Agar 9CYE 20S) was used as a medium to study the effects of CO_2 on mycelial growth, conidiation and cleistothecia formation of E.repens.

The results showed that CO_2 affected mycelial growth, sporulation, spore germination and cleistothecia formation significantly. $C0_2$ at 20% concentration started to inhibit the three parameters in A. flavus while at the same $C0_2$ concentration, cleistothecia formation was totally inhibited in E. repens. The parameters decreased with the increase of $C0_2$ concentration.

INTRODUCTION

Maize, rice, peanut and soyabean are four main leading crops in the Southern Asia. These crops constitute a major source of foods and of raw mateials for industrial processing. Maize is used for food for man and domestic animals, and for processing for starch, oil and other materials. Peanut, fourth important after rice, maize and soyabean, is vital ingredient of diet. Its main use is for the manufacture of oil.

Maize and peanut are both exceedingly durable and highly perishable and are subjected to losses during storage. They are highly susceptible to invasion and damage by insects, mites and fungi. Conditions of storage promote the development of insects, mites and fungi making these stored products (grains and seeds) highly susceptible to invasion and damage by these deteriorating organismes. Extensive spoilage results within a few days to a few weeks. The fungi are of a prime importance since they have been recognized as a major cause of deterioration of stored products resulting in loss of quality and quantity (Christensen and Kaufman, 1974).

Fungi growing in these stored grains cause losses of which the major types are:

(1) germinability decreases;
(2) discoloration of part (germ or embryo, or all of the seed or kernel);
(3) heating and mustiness;
(4) various biochemical changes;
(5) mycotoxins production, for example aflatoxin which if consumed, maybe hazardous to human and domestic animals health due to carcinogenic effect. It may result in severe and sometimes fatal liver damage;
(6) weight loss.

According to Pitt and Hocking (1985), Aspergillus and Penicillium are fungi causing changes mentioned above. The genus Aspergillus alone comprises of more species (such as A. glaucus, A. candidus, A. flavus and A. parasiticus produce aflatoxin (Butler, 1974). The genus Penicillium contains a large number of species infesting stored products such as P. citrinum, P. cyclopium and P. rubrum. Several species of Fusarium produce different kinds of toxins and some of them are extremely potent. It is known the Fusarium is a common cause of blight and decay in many kinds of plants, including seeds and fruits. F. tricinctum or F. sporotrichoides produces a highly lethal toxin which is also hazardous to people and domestic animals. While Eurotium repens can cause spoilage of stored products in an environment with low water activity (Pitt and Hocking, 1985).

The mere presence ofstrains of fungus that produce toxin in all of these products does not guarantee the production of aflatoxins or other mycotoxins, since special conditions are required to produce appreciable or measurable amounts of toxin. Nevertheless, special conditions are also required to promote growth of mycelium, sprorulation and germination of the species.

Fumigation, using carbon dioxide as a primary fumigant, is being applied widely to long-term products in order to control insects but less work has been done to control the development of storage fungi by using the same fumigant. Dharmaputra and co-workers (1990) reported that based on the study of 3 isolates of A. flavus, mycelial growth, sporulation, spore germination and aflatoxin production decreases with the increase of carbon dioxide concentration.

Therefore, the main objective of this study is to know the effeect of different concentration of carbon dioxide on:

(1) mycelial growth and conidiation of A. flavus;
(2) mycelial growth, conidiation and cleistothecia formation of E. repens.

MATERIALS AND METHOD

CONTROL OF STORAGE FUNGI USING CARBON DIOXIDE.

A. flavus and E. repens were inoculated by using reproductive structures from slant agar on petri dishes containing Aspergillus Differential Agar (ADM) Czapek Yeast Extract Agar (CYA) respectively. They were inoculated at 25°C for 7 days. A. flavus at 5mm in diameter was transfered from CYA to Czapek Yuast Extract 20% Sucrose Agar (CYE 20S) and they were incubated for 20 days at room temperature. After 2 days, the petri dishes containing 2-day old fungi were replaced with sterile filter paper. The diameter of colonies were measured before treatment, and then they were placed inside the experimental jar and the petri dishes were supported by plastic screen and wire. The jar was covered and secured by plasticine. Two plastic tubes were inserted into the jar for introducing carbon dioxide. The air was removed from the jar with a vacuum pump. Carbon dioxide was introduced into the jar at the concentration of 20, 40, 60 and 80% and for the control, air was used. The experiment was run with three replications with each treatment and carbon dioxide concentration was measured by using $C0_2$-meter and the jars were incubated for 7 days. The effect of CO_2 on mycelial growth and reproductive structures formation.

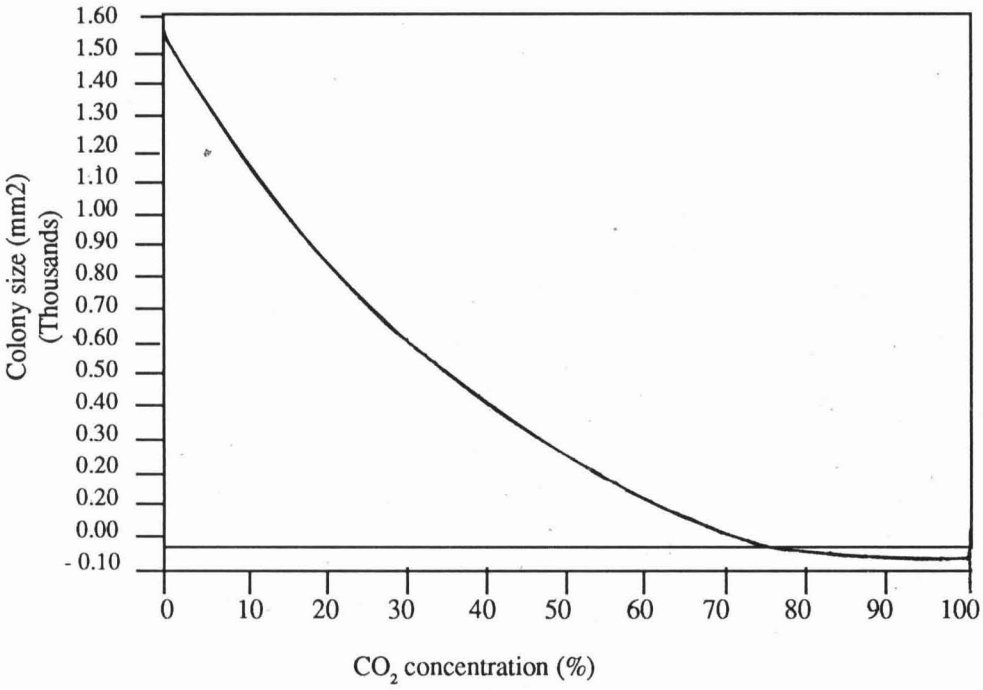
The mycelial growth was observed by measuring the diameter of the colonies after treatment. The change in colony size was calculated and the data were analyzed by using Factorial Randomized Design.

Table 1. Change of colony size after treatment (mm^2)

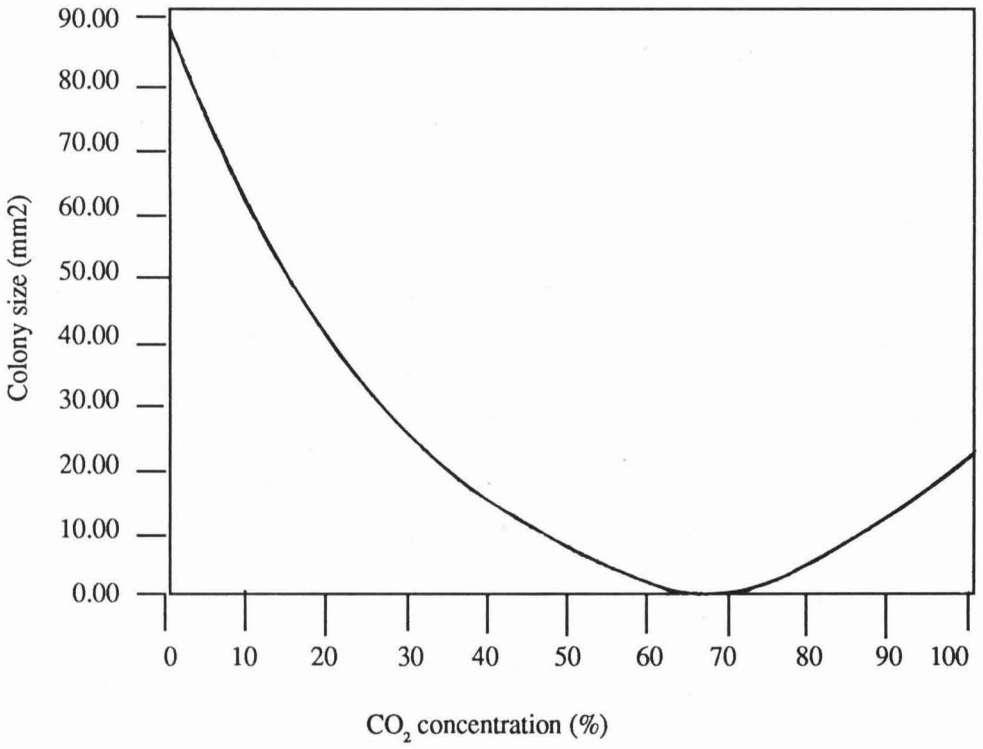
Treatment		Change of colony size
Aspergilluus flavus	0%	1512.37 e
	20%	1259.95 d
	40%	288.37 c
	60%	121.00 b
	80%	30.44 ab
Eurotium repens	0%	91.54 ab
	20%	77.07 ab
	40%	17.09 ab
	60%	8.06 ab
	80%	0.00 ab
DMRT	99%	99.45

Numbers followed by the same letter do not differ significantly.

Response of A. flavus towards different concentration of carbon dioxide



Response of E. repens towards different concentration of carbon dioxide.



RESULTS AND DISCUSSIONS

Diener and Davis (1969) stated that concentration of carbon dioxide and oxygen needed by the fungi for their growth, sporulation and spore germination vary with the species and the strains of the fungi.

Based on the analysis of variance, there was significant differences among the isolates of A. flavus and E. repens and interaction between the isolates and CO_2 concentrations, while CO_2 concentrations gave very significant difference on the mycelial growrh (Table 1). This shows that the growth of mycelium was inhibited when given different CO_2 concentration.

The effect of increasing CO_2 concentration to the A. flavus and E. repens isolates caused a decrease in the surface area of the colony of the fungi. A very significantdecrease in the surface area of the colony was shown in the isolate of A. flavus compared to the E. repens. (Table 1).

From this study, it was also observed that CO2 has only inhibitory effect. This can be demonstrated by transferring the fungi that had been fumigated with CO2 to a normal environment that will promote the growth of the fungi. The fungi were still able to grow indicating that they are still viable.

Based on the visual inspection, sporulation of the A. flavus isolate was inhibited at 20%, 40%, 60% and 80% CO_2 concentration. The higher the concentration of CO_2, the lesser is the sporulation observed due to its inhibitory effect (Tavle 1).

Table 2. The effect of different concentration of carbon dioxide on conidiation (A. flavus) and cleistothecia formation (E. repens)

Concentration of CO_2	A. flavus	E. repens
Control 0%	+++	+++
20%	++	-
40%	+	-
60%	+	-
80%	+	-

Note : + : presence of conidia and cleistothecia
- : absence of conidia and cleistothecia.

Landers et. al. (1967) reported that an increase in the CO_2 concentration inhibit the mycelial growth. The CO_2 fumigation was also found to give inhibitory effect on the cleistothecia formation as shown in Table 2. There was an overgrowth of cleistothecia in the control fungi, while at 20%-80% CO_2 concentration, cleistothecia formation was also totally inhibited. According to Diener and Davis (1969), CO_2 and O_2 tolerant concentration needed for vegetative growth, sporulation and germinability of the spores are different for every fungi species and strain. The results represented in Table 2 indicated that mycelial growth in A. flavus isolate was inhibited at 60% CO_2 concentration while at 20% CO_2 concentration, cleistothecia formation in E. repens was inhibited.

CONCLUSIONS

CO_2 concentration significantly influenced mycelial growth sporulation and spore germination of A. flavus and E. repens isolates.

CO_2 at 20% concentration started to inhibit mycelial growth sporulation and spore germination of the A. flavus isolate. The three parameters decreased with the increase of CO_2 concentration.

At 20% CO_2 concentration, cleistothecia formation was totally inhibited in the isolate of E. repens.

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