LABORATORY EVALUATION OF PLANT EXTRACTS FOR THE CONTROL OF ASPERGILLUS GROWTH AND AFLATOXIN FORMATION

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ABSTRACT

Plant species that were reported to have fungicidal properties on other fungal genera and have of medicinal value was collected and extracted using water. Crude water extracts of 6 out of 97 plant species screened for their fungicidal properties in vitro showed inhibitory activity against Aspergillus flavus and A. parasiticus.

The crude extracts of garlic (Allium sativum) was found to be the most effective against Aspergillus flavus and A. parasiticus. In their decreasing order the following plant extract: Kalachuchi (Plumiera acuminata) > Bulak-manok (Ageratum conyzoides) > Sampa-sampalukan (Phyllanthus nirure L.) while Talisay (Terminalia catappa L.) > Kakawati (Gliricidia sepium Jacq.) were quite effective on Aspergillus flavus and A. parasiticus, respectively. Also, aflatoxin formation was not detected on potato dextrose broth with the above mentioned crude extracts inoculated with aflatoxin-forming organisms.

INTRODUCTION

Aflatoxin is highly toxic and carcinogenic fungal metabolite synthesized mainly by Aspergillus flavus and A. parasiticus. A number of circumstantial evidence have linked aflatoxin to liver cancer in humans. A study conducted in the Philippines in 1975. "(ref. 1)" indicate that the incidence of primary liver cancer by region is directly related to the per capita consumption of corn. Corn is one of the commodity in the country that has been found to contain highly toxic levels of aflatoxin under natural conditions "(ref. 2)"

Past and current studies on aflatoxin in the Philippines have been mostly on surveys, monitoring contaminated commodities and a very limited studies on chemical control measure on corn were made. Nothing is being done regarding the use of plant extracts with fungicidal activity to control this problem. Extracts of various indigenous and readily available plant materials may be very promising and advantageous in terms of cost, practicability, safety, and availability.

This study was conducted primarily to (1) undertake collection of Philippine plants with possible fungicidal activity, (2) extract materials from the different parts of the plant (roots, stems, leaves, and flowers, rhizomes, cloves and fruit peelings) for preliminary toxicity tests, (3) screen and investigate the efficacy of crude plant extracts against Aspergillus flavus and A. parasiticus growth, sporulation, spore germination and aflatoxin formation.

PROCEDURES/METHODOLOGY

Preparation of the crude plant extracts. Plant species that were reported to have fungicidal properties on other fungal genera and have of medicinal value was collected, extracted using water following the standard microbiological technique.

Effect of crude plant extracts on the growth of *Aspergillus flavus* and *A. parasiticus*. Extracts from various plant species were screened for their activity against *A. flavus* and *A. parasiticus*. Agar plates were prepared by pouring to each plate 3 ml of the crude plant extracts and 18 ml of the PDA precooled to 40°C. The plates were carefully rotated to insure even distribution of the extracts in each plate.

An agar disc of the test organisms were placed at the center of the agar plate. For the control, the PDA plates without the plant extracts were inoculated with the same test organisms. For comparison 2 ppm Benlate (fungicide found to be very effective for *Aspergillus* spp.) was included in the test.

The plates were incubated at room temperature and the growth and sporulation were observed and measured 7 days after inoculation. Three trials were made with five replicates per trial.

Effect of various plant extracts on conidial germination of *Aspergillus* spp.

To test the effect of various crude plant extracts on spore germination, fungal spores from seven-day-old culture were spread on thin agar blocks infused with crude plant extract. Each agar block was mounted on glass slide and incubated in a covered petri dish lined with moist filter paper. The percentage of spore germination in each treatment was determined after 30 hrs.

Aflatoxin analysis of *Aspergillus* isolates. To determine the effect of various plant extracts on aflatoxin production by the isolate under identical conditions of growth, a loopful of dry spores from a 2-week old culture of the isolate on PDA was inoculated to each of the two 250-ml Erlenmeyer flasks, each containing 50 ml of potato dextrose broth "(ref. 3)" and 8.3 ml of each plant extract at 1:1 concentration and were incubated at 28C for 8 days. An aflatoxin-producing isolates was included as a control. The aflatoxin contents were determined for each isolate by the method of Wilson et al. "(ref. 4)"

RESULTS AND DISCUSSION

Results showed that 6 out of 97 plant species screened have inhibitory activity on the growth, sporulation and spore germination of *A. flavus* and *A. parasiticus*. (Tables 1, 2, 3, 4).

Table 1. Potential plant extracts against A. flavus.

Treatment	Mean colony diameter (mm) ^{1/}
Garlic	0.0 a ^{2/}
Kalachuchi	35.3 b
Bulak-manok	40.2 b
Sampa-sampalukan	40.9 b
Dried Neem leaves	34.4 b
Benlate	0.0 b
Control	72.1 c

^{1/} Average of 3 trials with 5 replicates per trial.

^{2/} In each column, values followed by the same letter do not differ significantly ($P=0.05$) according to Duncan's Multiple Range Test.

Table 2. Potential plant extracts against A. parasiticus,

Treatment	Mean colony diameter (mm) ^{1/}
Garlic	0.0 a ^{2/}
Kakawati	46.1 ab
Kalachuchi	35.6 ab
Neem leaves	36.5 ab
Benlate	0.0 a
Control	76.8 b

^{1/} Average of 3 trials with 5 replicates per trial

^{2/} Means in a column followed by a common letter are not significantly different at the 5% probability level by DMRT.

Table 3. Effect of various plant extracts on conidial germination of Aspergillus flavus.

Treatment	Percent Germination ^{1/}
Garlic	0.000 a ^{2/}
Benlate	5.000 a
Sampa-sampalukan	97.200 c
Bulak-manok	97.233 c
Kalachuchi	73.33 b
Control	78.900 b

^{1/} Average of 3 trials. Ten microscopic fields/treatment/trial were observed.

^{2/} Means in a column followed by a common letter are not significantly different at the 5% probability level by DMRT.

Table 4. Effect of various plant extracts on conidial germination of Aspergillus parasiticus.

Treatment	Percent Germination ^{1/}
Garlic	0.000 a ^{2/}
Benlate	5.000 b
Kalachuchi	85.000 c
Kakawati	100.000 d
Control	95.567 d

^{1/} Average of 3 trials. Ten microscopic fields/treatment/trial were observed.

^{2/} Means in a column followed by a common letter are not significantly different at the 5% probability level by DMRT.

Garlic (Allium sativum) extract was found to be the most effective on both Aspergillus species.

Results showed that no detectable aflatoxin were observed on potato dextrose broth (PDB) with garlic extract inoculated with A. flavus and A. parasiticus while the control of each organism yielded 70 and 50 ppb B₁, respectively.

Data showed that other plant extracts were more effective in inhibiting aflatoxin formation than suppressing Aspergillus flavus and A. parasiticus growth, sporulation and conidial germination.

The results confirm the knowledge that substances possessing antifungal properties are not uncommon among higher plants. Many species of higher plants possess active substances to which phytopathogenic fungi are sensitive. These antifungal properties may constitute the inherent resistance factors of some plants to fungal attack.

The present findings is not yet sufficient to draw concrete conclusions since only in vitro tests have been done. These findings would, however, still be useful since it was already found out which of these plant extracts have the potential to control the growth of those storage fungi although nothing was reported about their antifungal properties in literature.

It is recommended that plant extracts found effective in in vitro studies be further evaluated using in vivo tests. The isolation and characterization of the active components should also be done.

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