Effect of extracts from nine plant species found in Africa on the mycelial growth of *Aspergillus flavus* Link

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Abstract

Farmer surveys conducted in 1991 and 1992 revealed that some farmers combat storage moulds using locally available plants. Extracts and combinations of extracts of nine different plant species that are used as traditional medicines and aromatic spices in Nigeria were tested for the effect in vitro on the growth of *Aspergillus flavus*. Aqueous extracts from the combination of dried fruits of *Xylopia aethiopica* and dried seeds of *Piper guineense* completely inhibited the growth of the fungus. Dried roots of the Zingiberaceae and fresh leaves of the Labiateae showed a range of fungistasis. Significant fungistatic effects were seen for all but two of the plant species tested, though some fungal inhibition was short lived. Testing to identify efficacious plant species from various agroecological zones is continuing.

Introduction

Aspergillus flavus Link and the aflatoxins it produces are recognised as dangerous storage contaminants of *Zea mays* L. through the world (Zuber et al. 1987). Control technologies including host plant resistance, chemical protectants and detoxicants, and storage system management have been tried with varying degrees of success (Zuber et al. 1987). Interviews with farmers in Nigeria and Benin Republic, West Africa reveal that farmers are concerned about protecting their harvested grain, and one method that some use is to layer the storage structure with leaves of common edible plants such as *Verbina amygdalina* Delile. (Udoh et al., unpublished data).

Fungitoxic, fungistatic, or fungicidal activity of plant extracts on plant pathogens has been widely reported (Audhesh and Satapathy 1977; Naidu and Jon 1981; Divivedi and Dubey 1986; Tripathi et al. 1986; Awuah 1989; Shetly et al. 1989). Natural products from many plants are known to be microbial suppressants (Annapurna et al. 1983; Rama et al. 1988). Volatile plant substances have long been recognised for medicinal and other useful purposes (Dalziel 1937; Divivedi and Dubey 1986; Abbiw 1990).

Extracts from plants commonly found in tropical Africa have been tested for *in vitro* fungistatic effects. Awuah (1989) found that extracts from *Ocimum gratissimum* Linn. (Labiateae) led to a 24.6% reduction *in vitro* radial growth of *Rhizopus sp.* and to a 60% reduction of *Ustilaginoidea* virens. In the same work, extracts from *Xylopia aethiopica* Dunal. (Anonaceae) were strongly fungitoxic against *Rhizopus sp.* and *Ustilago maydis* but ineffective against *U. virens*.

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In Nigeria in 1991, it was noticed that leaf mulch from certain shrubs in alley cropping trials was exerting allelopathic effects on the crops (Hauser 1993). It was seen that *Acioa barteri (Dactyladenia barteri)* Welw. (Chrysobalanaceae), *Cassia siamea* Lam. (Leguminoseae), *Flemingia macrophylla* Baker (Leguminoseae), and *Gmelina arborea* Roxb. (Verbenaceae) leaf mulches did not support any fungal growth, while other mulches were acting as fertile media.

Many plants are recognised in West Africa for medicinial uses or are aromatic and used as spices (Abbiw 1990). The fruits of *X. aethiopica* are used as a cough medicine, as a carminative and, to some extent, as a purgative. *Piper guineense* Schum. and Thonn. (Piperaceae) is used as a carminative, especially for griping conditions. In eastern Nigeria there is an age-old practice to mix dried seeds of *X. aethiopica* and *P. guineense* in soups made for mursing mothers as it is believed to have germicidal activities in the womb (abbiw 1990).

A series of experiments testing leaf litter and aqueous extracts of these plants against various plant pathogens was undertaken. Thus, some commonly used spices and botanical medicines, as well as plants being promoted for alley cropping, were tested for their effects on growth of *Aspergillus flavus* in vitro.

Materials and Methods

Fresh leaf extracts from plants used in alley crops

In vitro studies were conducted with aqueous extracts from *A. barteri, F. macrophylla, C. siamea*, and *G. arborea* leaves that had been air-dried for one week. The aqueous extracts were prepared by soaking 50 g of finely ground plant leaf in 250 mL of distilled water for 1 hour. The mixtures were strained through cheese cloth and 106 µm sieves. A 150 mL aligust of the filtrate was added to 50 mL liquified potato dextrose agar (PDA). The media were autoclaved at 121°C for 15 minutes and then poured into 9 cm diameter petri plates. After solidification, five plates of each leaf extract agar medium were inoculated by placing 3 mm diameter discs, cut from the margins of actively growing colonies of *A. flavus* on PDA, in the centre of each plate. The plates were sealed with parafilm and incubated at 28±1°C. Plates of PDA without leaf extract served as controls. Fungal colony diameters were measured after 1 week of growth and expressed in centimetres. In all trials, the colony diameter of the fungal pathogens were recorded on the 8th day of incubation. Analysis of variance and mean separation tests were conducted on the fungal growth on the different media.

Leaf extracts stored for a week in refrigeration

In a second test, 50 g of air-dried leaves of each of the above species were soaked in 250 mL of distilled water and kept in the referigerator for 24 hours. The contents were strained through cheese cloth and then stored in the refrigerator for 1 week. After refrigeration, 150 mL of each extract was mixed

with 50 mL of distilled water to which 4 g technical agar had been added. The media were autoclaved and poured into plates, with five replications per plant material. The later states were carried out as in experiment 1.

Extracts of dried and fresh aromatic and medicinal plants

Dried parts (seeds, fruits, bulbs) of six plant species and fresh leaves of two species were used in this study. The dried portions were from: *Xylopia aethiopia; Piper guineense; Monodora myristica* Dunal. & Holl. (Anonaeceae); *Tetrapleura tetraptera* (Schum. & Thonn.) Taub (Leguminoseae); *Aframomum* sp. Schum. (Zingiberaceae); and *Zingiber officinale* Rosx. & Holl (Zingiberaceae). Fresh leaves from *Ocimum gratissimum* and *O. basilicum* Linn. (Labiateae) were also used. Fifty grams of each plant sample were macerated in 150 mL of distilled water in a blender. A combination of *X. aethiopica* (25 g) and *P. guineense* (26 g) was also macerated in 150 mL of distilled water to form a mixture. The extracts were strained through a cheese cloth and then made up to 200 mL. The plates were prepared, inoculated, and fungal growth was measured as in the first experiment.

Results

Fresh leaf extracts from plants used in alley crops

The leaf extract of *A. barteri* in PDA significantly ($p<0.05$) inhibited the growth of *A. flavus* as the colony only reached 55% the size of the PDA control (Table 1). On *C. siamea* and *F. macrophylla*, radial growth of *A. flavus* was significantly reduced by 21.21 and 15.74%, respectively, relative to the PDA control. *G. arborea* had no effect on *A. flavus*.

Leaf extracts stored for a week in refrigeration

A slower rate of colony development was observed on the technical agar medium (WA) as compared with the PDA medium in the other experiments. On WA the average across all treatments was 2.85 cm growth after 1 week in culture. There were no significant differences among the colony sizes when the aqueous leaf extracts had been refrigerated for a week before mixing with media and inoculating. Mycelial growth of *A. flavus* was 2.65 cm on the medium containing *Acioa barteri* extract, but the inhibition was not significantly different from the other media or the control (Table 2).

Extracts of dried and fresh aromatic and medicinal plants

Of all plant extracts tested, only the mixture of *X. aethiopica* (25 g) and *P. guineense* (25 g) completely inhibited *A. flavus* growth (Table 3). The other extracts exerted varying degrees of fungistasis. Mycelial growth was less than 50% of the control when the medium contained extracts of seeds of *Aframomum sp.* and *Z. officinale*. Extracts of *O. gratissimum* were also effective against *A. flavus*, inhibiting colony growth by 50%, relative to the control. Extracts from *M. myristica* (29% reduction) and *P. guineense* (27.9% inhibition), and *O. basilicum* (16.8%) were suppressive compared with the control. *X. aethiopica* and *T. tetraptera*, on the other hand, stimulated growth of *A. flavus* in culture (Table 3).

Discussion

The data show clearly that certain plant extracts control growth of *Aspergillus flavus in vitro*. On extracts from 12 plant species and one mixture, the impact on *A. flavus* growth ranged from complete fungistasis to growth promotion. The combination of *X. aethiopica* and *P. guineense* resulted in an apparent synergistic suppression of *A. flavus* growth, as neither of the plant extracts alone produced a strong fungistatic effect.

In general, fresh leaf extracts were more effective than those that had been stored chilled. This indicates that the active compounds are subject to degradation if left in water. The influence of autoclaving on the fungistatic properties of the extracts is unknown. The initial information generated by these *in vitro* experiments opens the way for further research into what may lead to some very effective solutions to the problem of aflatoxin contamination of stored maize.

The use of botanicals to protect stored grain in traditional systems in West Africa is not a new concept, but neither is it a

Table 1. Effect of some leaf extracts on the mycelial growth (colony diameter) of *Aspergillus flavus* after 1 week in culture

Source of extract	Plant part	Cm	% of control
Acioa barteri	fresh leaves	3.22	55.32 a
Cassia siamea	fresh leaves	4.58	78.72 b
Flemingia macrophylla	fresh leaves	4.90	84.26 c
Gmelina arborea	fresh leaves	5.74	98.63 e
Control	PDA	5.82	100.00 e

Means followed by the same letter are not significantly different (LSD, p=0.05). PDA = Potato Dextrose Agar

Table 2. Effect of some leaf extracts on the mycelial growth (colony diameter) of *Aspergillus flavus* after 1 week in culture

Source of extract	Plant part	Cm	% of control
Acioa barteri	fresh leaves	2.65	87.45 a
Cassia siamea	fresh leaves	2.78	91.75 a
Gmelina arborea	fresh leaves	2.78	91.75 a
Flemingia macrophylla	fresh leaves	3.00	99.00 a
Control	WA	3.03	100.00 a

Means followed by the same letter are not significantly different (LSD, p=0.05). WA = Technical Agar in water.

Table 3. Effect of some plant extracts on the mycelial growth (colony diameter) of *Aspergillus flavus* after 1 week in culture

Source of extract	Plant part	Cm	% of control
Xylopia aethiopica + Piper guineense	dry fruits + dry seeds	0.00	0.00 a
Aframomum sp.	dry seeds	1.90	33.15 b
Zingiber officinale	dry bulbs	2.70	47.12 c
Ocimum gratissimum	fresh leaves	2.90	50.61 c
Monodora myristica	dry seeds	4.07	71.03 d
Piper guineense	fresh leaves	4.13	71.55 d
Ocimum basilicum	fresh leaves	4.77	83.24 e
Control	PDA	5.73	100.00 f
Xylopia aethiopica	dry fruits	5.87	102.44 f
Tetrapleura tetraptera	dry fruits	7.90	137.87 g

Means followed by the same letter are not significantly different (LSD, p=0.05). PDA = Potato Dextrose Agar

widespread practice. For it to become an accepted practice among peasant farmers, several criteria would have to be met. First, the plant species would have to be easily available, either growing abundantly naturally, or easy to cultivate. Second, it would have to be noticeable effective and simple to use. From the development point of view, before any type of implementation at the farm level can be attempted, potential plant products must be assessed for inherent toxicity, mode of action, and the durability of the fungistatic effect. For example, *C. siamea* has been reported to be poisonous to pigs when used as fodder (Abbiw 1990); so obviously this plant is not very promising as a protectant for stored grain. *A. barteri* and *F. macrophylla* will need to be tested for toxicity. *Aframomium* sp., and some other commonly grown spices, represent a few West African plants that have antifungal properties, and are likely to be safe to use.

The least laborious utilisation of botanicals in traditional storage systems would be to layer fresh leaves between stacks of maize cobs in the bin. Another option would be to grind seeks, bark or dried leaf material to use as a dust or a wettable powder. Extraction with water and spraying is more laborious, but still a technology accessible to small scale farmers.

In spite of the *in vitro* effectiveness of extracts from some plants, further experiments on their ability to control the spread of *A. flavus* in storage must be conducted before definite statements on their usefulness can be made. Plants from various agroecosystems are being screened for inhibition of *A. flavus*.

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