African Journal of Biotechnology Vol. 6 (6), pp. 718-719, 19 March 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Short Communication

Efficacy of *Euphorbia milli* and *Euphorbia pulcherrima* on aflatoxin producing fungi (*Aspergillus flavus* and *Aspergillus parasiticus*)

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Accepted 5 February, 2007

Efficacy of seven different concentrations (5, 10, 15, 20, 25, 30 and 35 mg/ml) of dry flower powder of *Euphorbia milli* and *Euphorbia pulcherrima* was tested on the growth of aflatoxin producing toxigenic strains of fungi' *Aspergillus flavus* and *Aspergillus parasiticus* in Sabouraud Dextrose Agar medium (SDA). Total (100%) inhibition of growth of both *A. flavus* and *A. parasiticus* was observed at 30 mg/ml concentration of *E. milli* dry flower powder. Total inhibition (100%) of growth of *A. flavus* was observed at 30 mg/ml concentration of *E. pulcherrima* and for *A. parasiticus*, it is 35 mg/ml. Bioassay with groundnut seeds soaked with different concentration (5 – 35 mg/ml) of flower extract proved that both fungi were incapable of infecting the seeds in the presence of 30 and 35 mg/ml of both *E. milli* and *E. pulcherrima*.

Key words: Aflatoxin, *Aspergillus flavus, Aspergillus parasiticus, Euphorbia milli*, *E. pulcherrima*.

INTRODUCTION

Many agricultural commodities are vulnerable to attack by a group of fungi that are able to produce toxic metabolites called mycotoxins. Among various mycotoxins, aflatoxins have assumed significance due to their deleterious effects on human beings, poultry and live stock. The effectiveness of the food-grade antioxidants butylated hydroxytoluene (bht), trihydroxybutyrophenone (thb), propyl paraben (pp) and butylated hydroxyanisole (bha) at 1, 10 and 20 mmol l^{-1} concentrations on germination, growth, and aflatoxin B1 (Afb1) production by *Aspergillus flavus* were determined (Passone et al., 2005). Aflatoxins are group of hepatocarcinogenic secondary metabolites produced by *A. flavus* and *Aspergillus parasiticus* (Lopez and Ramos, 2002). The extracts of several wild and medicinal plants have also been tested against aflatoxin producing fungi (Bilgrami, 1984).

Although hundreds of plant species have been tested for antimicrobial properties (Arora and Ohlon, 1997), the vast majorities have not yet been adequately evaluated (Balandrin et al., 1985). *Euphorbia milli* (Euphorbiaceae) is a small perennial prickly much branched shrub with showy crimson flowers and *Euphorbia pulcherrima* (Euphorbiaceae) is a plant with red bracts (Kumar and Prasad, 1992). The effect of flowers of these plants as fungitoxicant on aflatoxin producing fungi *A. flavus* and *A. parasiticus* has not yet been studied. The present study was undertaken to assess the fungitoxic role of dry flower powder of *E. milli* and *E. pulcherrima* against aflatoxin producing strains of *A. flavus* and *A. parasiticus*. Aqueous flower extracts were also used for the bioassay of groundnut against these fungi (Abubacker and Ramanathan, 2003).

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MATERIALS AND METHODS

The two plants; *E. milli* and *E. pulcherrima* were procured from the Nilgiris Mountains, Tamil Nadu, India. Flowers of each plant were collected and thoroughly washed with 2% sodium hypochlorite solution and subsequently with sterile distilled water. After sterilization, the flowers were dried and grounded to powder. Different concentration of dry flower powder; 100, 200, 300, 400,

Table 1. Effect of crude flower extracts of *Euphorbia milli* and *E. pulcherrima* on aflatoxin-producing fungi, *A. flavus* and *A. parasiticus*, at room temperature.

Plant	Fungus	Control (i) (5 mg/ml)	Control (ii) (5 mg/ml)	Concentration (mg/ml)						
				5	10	15	20	25	30	35
E. milli	*A. flavus*	+ + + +	-	+ + +	++	+	+	+	-	-
	A. parasiticus	+ + + +	-	+ + +	++	+	+	+	-	-
E. pulcherrima	*A. flavus*	+ + + +	-	+ + +	++	+	+	+	-	-
	A. parasiticus	+ + + +	-	+ + +	++	+	+	+	+	-

+ + + + = Normal growth; + + + = 25% of the inhibition; + + = 50% of the inhibition;
+ = 75% of the inhibition; and - = 100% of the inhibition.
Control (i) = Medium without flower extract.
Control (ii) = Medium with fluconozole fungicide.

500, 600 and 700 mg were mixed with 20 ml of SDA medium to constitute 5, 10, 15, 20, 25, 30 and 35 mg/ml, respectively. For bioassay, dry flower powders (*E. milli* and *E. pulcherrima*) of different strengths respectively 50, 100, 150, 200, 250, 300, and 350 mg/ml were mixed with 10 ml of sterile distilled water to constitute 5, 10, 15, 20, 25, 30, and 35 mg/ml, respectively. The groundnut seeds were coated (soaked for 30 min) with the flower extract of different concentration and inoculated with *A. flavus* and *A. parasiticus* fungi from SDA medium.

The toxigenic strains, *A. flavus* and *A. parasiticus*, used in this present experiment were successfully isolated from groundnut using direct plate method on SDA and the selective media – *A. flavus*/*parasiticus* Agar (AFPA) medium. Control (i) contained only 20 ml of SDA medium and control (ii) contained 100 mg fluconazole fungicide added to 20 ml of SDA medium (5 mg/ml). The agar plate containing 20 ml medium and flower extract was inoculated with 0.5 ml spore suspension prepared from 5 day old culture and incubated for 5 days at room temperature. The experiment was conducted in duplicates for each concentration of flower extract of *E. milli* and *E. pulcherrima*.

RESULTS AND DISCUSSION

Growth of isolated aflatoxin producing fungi *A. flavus* and *A. parasiticus* were inhibited by treatment with dry flower powder of *E. milli* and *E. pulcherrima* (Table 1). Total inhibition (100%) was noticed in *A. flavus* treated with 30 and 35 mg/ml of *E. milli* dry flower powder. Similarly total inhibition of (100%) was noticed in *A. parasiticus* treated with 30 and 35 mg/ml of *E. milli*. A similar trend (100% inhibition) was seen in control (ii) with fluconazole treatment. Total inhibition (100%) was also noticed in *A. flavus* when treated with 30 and 35 mg/ml of *E. pulcherrima*. But in the case of *A. parasiticus* total inhibition was observed only in the concentration of 35 mg/ml of *E. pulcherrima*. Also the control (ii) with fluconozole treated and 30 mg/ml of *E. milli* dry flower powder inhibited 100% of *A. flavus* and *A. parasiticus*. Control (ii) showed 100% inhibition. Bioassay with groundnut seeds coated with 5, 10, 15, 20, 25, 30, and 35 mg/ml of flower extracts of *E. milli* and *E. pulcherrima* confirmed their fungitoxic properties, especially at 30 and 35 mg/ml concentration.

Total inhibition of growth of *A. flavus* for *E. milli* dry flower powder was possibly due to the interference of the simple phenols and phenolic acids, quinones, flavones, flavonoids and flavanols. This kind of interference may be at the biosynthetic level. The level of inhibition differs between *A. flavus* and *A. parasiticus*. Earlier studies (Singh, 1983; Bhatnagar and McCormick, 1987) have suggested that growth and aflatoxin production by *A. flavus* and *A. parasiticus* are independent phenomena. The isolation and characterization of the compounds present in the *E. milli*, capable of inhibiting aflatoxin-producing fungus *A. flavus*, would be useful since success in this aspect could provide a means for the elimination or control (fungistatic) of aflatoxin contamination in food stuffs (Mislivec et al., 1998).

In conclusion, both *E. milli* and *E. pulcherrima* inhibited significantly the growth of *A. flavus* and *A. parasiticus*. Their extracts or dry flower powder might be useful in controlling aflatoxin contamination in food and feed as well in controlling aspergillosis, a large spectrum of disease caused by members of genus *Aspergillus*.

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