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A bioassay technique for the detection of aflatoxin by using *Chlorella pyrenoidosa*

G. Uma Muralimohan and C. N. Reddy
Department of Botany, Gulbarga University, Gulbarga 585 106, India

The chlorophylytic activity of aflatoxin on *Chlorella pyrenoidosa* is clearly evident and the degree of toxicity of aflatoxin is found to vary in proportion to the amount of the toxin present. This bioassay technique is found to be quite simple and sensitive, and can be used to detect the aflatoxin content produced by the aflatoxigenic fungi either in culture or in the substrate on which it grows.

SEVERAL biological methods such as brine–shrimp bioassay, hen's egg embryo assay and tissue culture bioassays have been suggested[1] for the estimation of aflatoxins. However, they are not much in use as they are not as sensitive as TLC and are mostly used as confirmatory tests. Schoental and White[2] have reported that 1–10 µg aflatoxin/ml is an inhibitor of chlorophyll synthesis in the leaves of cress (*Lepidium sativum*) and suggested that this effect could be elaborated into a simple test for aflatoxin detection in suspected material. Slowatizky *et al.*[3] have also reported the inhibition of chlorophyll synthesis due to aflatoxins, resulting in virescence and albinism in maize leaves. Since chlorophylytic toxin activity of many pathogenic fungi and bacteria has been tested on *Chlorella*[4], an attempt is made in this paper to develop an easy method of identification of aflatoxigenic activity of fungi that produce aflatoxins, by using *Chlorella*.

Two *Aspergillus flavus* isolates from garlic (*Allium sativum*) and chillies (*Capsicum fruitense*) contaminated with aflatoxins[5] were grown on Czapek's broth supplemented with caesin to give 0.5 g nitrogen/litre at pH 4.5, as stationary cultures at room temperature (26 ± 2°C). After 7 days of growth, the culture filtrates were obtained through seitz filter. In a separatory funnel 50 ml of cell-free culture filtrate was extracted with double the volume of chloroform. The chloroform extracts were collected and concentrated to dryness by heating on a hot-water bath. The residues were dissolved in 5 ml of chloroform and treated as culture extracts; 100 µl of each of these extracts was loaded on TLC plate for chromatographic separation. The quantitative estimations of aflatoxins were done by comparing the intensity of the fluorescent spots of the sample with the corresponding spots of the standards[6, 7]. The remaining extract was subjected to evaporation again and the residue was further dissolved in 5 ml of sterile distilled water and treated as water wash, and used to determine the degree of aflatoxin toxicity by employing the modified method of Warren and Winstead[4]. Two ml of *C. pyrenoidosa* liquid culture was taken in three test tubes (8 mm diameter) and in two of these test tubes 100 µl of water wash having aflatoxin (from both isolates) was added separately. The third one, without any extract, was treated as a control. All the test tubes were sealed with aluminium foil and incubated at room temperature (26 ± 2°C). After 24 h the test tubes were observed under UV light for chlorophylytic activity.

It is evident from Table 1 that both the isolates of *A. flavus* are found to be potentially aflatoxigenic and produced AfB1 and B2 in cultures. Garlic isolate produced more toxin (112.5 µg/ml) than the isolate from chillies (75.0 µg/ml) in culture. Both the isolates produced AfB1 and AfB2 and the amount of AfB1 was found to be more than AfB2 (Table 1). A similar degree of toxin elaboration by these two isolates was observed in their respective commodities[5]. The chlorophylytic nature of aflatoxin from these two cultures on *C. pyrenoidosa* was more clear when observed under long-wave UV light. The degree of toxicity was found to vary in proportion to the quantity of the toxin present in the test sample, as could be seen from the emission of strong violet brown colour by *Chlorella* in control and dull green or greenish colour in test sample, with its intensity depending on the amount of aflatoxin. This change in the intensity of colour depending upon toxin, as evidenced in the present test provides a clear clue to the detection of aflatoxin and can be used even to determine the amount of aflatoxin present in an unknown sample by comparison with a standard index of known concentrations.

Table 1. Amount of aflatoxin produced in culture filtrates of *A flavus* isolates

Source of *A flavus* isolate	Amount of aflatoxin µg/ml				
	B1	B2	G1	G2	Total
Garlic	75 0	37 0	0 0	0 0	112 5
Chillies	50 0	25 0	0 0	0 0	75 0

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Synergistic action of different strains of *Bacillus thuringiensis* against cotton leaf worm *Spodoptera littoralis* (Boisduval)

L. Nadarajan* and D. Martouret

Station de recherché de lutte biologique, INRA, LaMinere, France
*Present address: Regional Agricultural Research Station, Kerala Agricultural University, Pattambi 679 306, India

The insecticidal activity of the crystal toxin of different strains of bacteria, *Bacillus thuringiensis* was tested against the cotton leaf worm, *Spodoptera littoralis* (Boisduval). According to the LC_{50} values against the neonate larvae, the order of toxicity was *B. thuringiensis* var. *entomocidus* > *aizawai* > *berliner* > *kurstaki*. The strains *aizawai* and *entomocidus* when combined showed only additive action whereas *entomocidus–berliner* and *aizawai–kurstaki* combinations showed potentiation suggesting a synergistic action.

LITTLE work is conducted to study the biological activity of mixtures of different strains of *Bacillus thuringiensis*. Hence an attempt is made in this study to see the joint action of different strains of *B. thuringiensis*. The test insect used was cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae).

Culture of *S. littoralis*, maintained for several generations in the laboratory at La Minere (INRA Research Station), France, was used for conducting the experiment. The insect was reared in 20–25°C and 75% RH on an artificial diet. Adult moths laid the eggs on filter paper folds and they were sterilized in the fumes of formalin for 20 min and kept at 25°C for eclosion. In about four days the larvae hatched out and the first instar unfed larvae were used. Different strains of *B. thuringiensis* in the crystal (endotoxin) form were supplied by Pasteur Institute, Paris, France (courtesy Miss La Cadet).

The stock solutions for different strains were prepared as follows. Vials having crystal suspension were agitated for 10–15 min in a vertical shaker and diluted with sterile water. A serial dilution procedure was followed. The concentrations of stock solutions are shown in Table 1. Control was only sterile water with 0.5 ml of wetting agent. Spraying was done using Burgerjon tower[1] on 1 cm diameter circular bits of cabbage leaves, which were airdried and used to feed 20 neonate larvae. The containers were kept at 25°C and 75% RH.

All the treatments were sprayed at a time and the mortality rate at LC_{25}, LC_{50} and LC_{90} was plotted. The

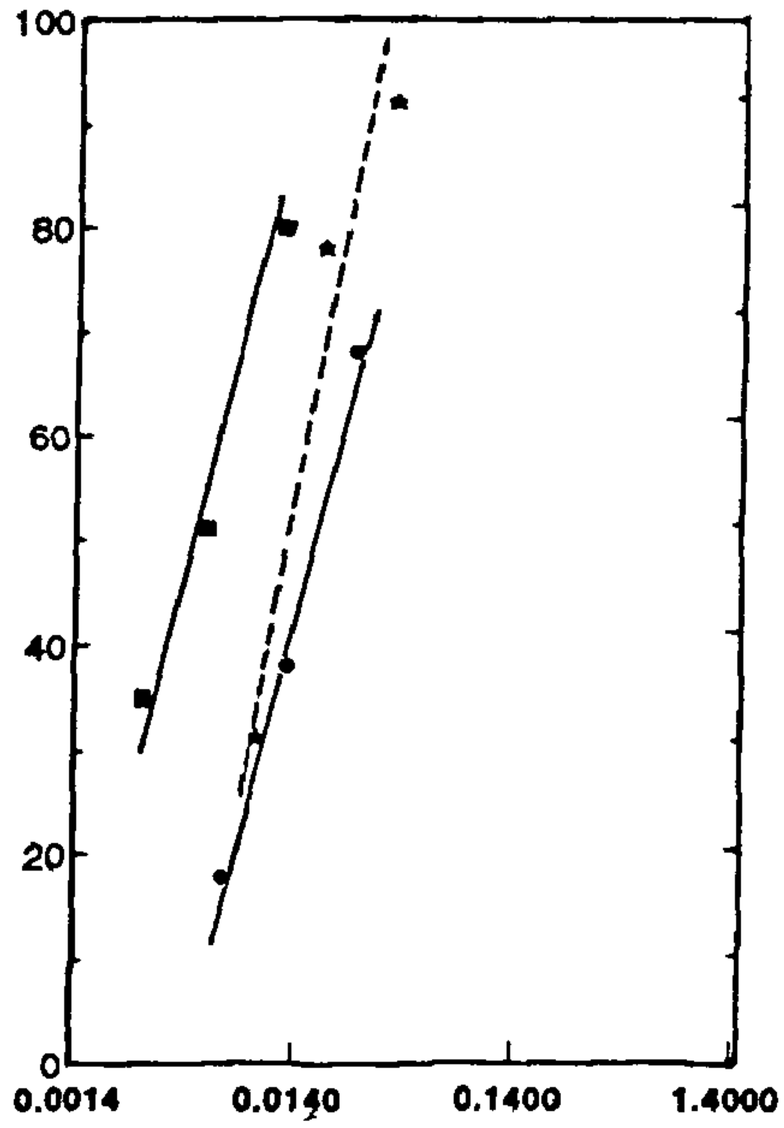

Figure 1. Toxicity of *entomocidus*, *aizawai* and their combination.

Table 1. Toxicity of different strains of *B. thuringiensis* to cotton leafworm *S. littoralis* (Boisduval)

Strains	Code no	Stock soln (LC_{50} (72 h) (% concentration))		No of assays*
B. thuringiensis var. *entomocidus*	605	0 33	0.0055 ± 0 001	6
aizawai	635	0 44	0 011 ± 0 002	9
berliner	663	0 34	0 124 ± 0 074	8
kurstaki	672/564	0 35	0 218 ± 0 121	5

*each assay is with four replications of 20 observations each