

**Figure 1.** Freshly isolated protoplasts of *Sesamum indicum* L. var. T-13.

different culture media at a density of 1 ml suspension per 2 ml of liquid media.

Freshly isolated protoplasts were colourless spherical and of various sizes with a distinct nucleus and cytoplasmic strands (Figure 1).

In the present investigation, hypocotyl segments were found to be the best source of rapid isolation of protoplasts. Earlier workers have also found hypocotyls to be a good source of stable protoplasts well suited for growth, fusion and regeneration<sup>2-5</sup>. Roy and De<sup>6</sup> also found hypocotyls to be the most ideal material for protoplast isolation with regard to rapid division and high plating efficiency.

Crop improvement using genetic engineering requires the execution of several procedures which are difficult to accomplish in the required sequence in any economic species. Plant protoplasts would undoubtedly play an important role in the rapidly emerging plant biotechnology for crop improvement especially in the case

of oilseeds which are known to possess limited genetic variability. Hence further work on the culture of the isolated protoplasts of *Sesamum indicum* would be interesting and rewarding.

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## Isolation and characterization of an aflatoxin-inhibiting metabolite from *A. niger*

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**The factor from *Aspergillus niger* that inhibits aflatoxin production by *A. flavus* was purified from culture broth and identified as oxalic acid. The inhibition was verified by using authentic samples of oxalic acid.**

THE interaction of *A. niger* with *A. flavus* against production of aflatoxin has been reported by several workers<sup>1-3</sup>. Horn and Wicklow<sup>3</sup> observed that lowering of pH is one of the factors suppressing aflatoxin production. They also speculated on the production of an antimetabolite by *A. niger* causing additional inhibition. The isolation, purification and characterization of the aflatoxin-inhibiting metabolite secreted by *A. niger* in liquid synthetic medium are described here.

*A. flavus* (F<sub>2</sub>) and *A. niger* cultures were isolated in this laboratory, and maintained on Czapek-Dox agar and potato dextrose agar slants respectively at 6-8°C. Spores were obtained by subculturing on the agar slants and incubating at 28°C for 5 days. To prepare the crude metabolite, 1 ml distilled water suspension of the *A. niger* culture ( $0.7 \times 10^6$  no./ml) was dispersed into sterile Czapek-Dox-casein<sup>4</sup> medium and incubated at 28°C for seven days under stationary culture conditions. The mycelial mat was filtered off through a cheese cloth. The filtrate was concentrated under reduced

pressure at 60°C and treated with activated charcoal. To the cool and clear filtrate was added distilled ethanol up to 70% and kept in cold for about 2 h to allow the white precipitate to settle. It was removed by filtration. The aqueous alcohol phase was concentrated again under reduced pressure at 45°C. Crystalline white precipitate separated after allowing it to stand in cold for several hours. White precipitate was collected by filtration, dissolved in sufficient amount of 1N HCl, and subjected to Dowex-50 ion exchange column chromatography. Eluants used were 80% ethyl alcohol, and 0.4 N, 0.8 N, 2 N and 4 N of  $\text{NH}_4\text{OH}$  solution in the same order. Of these fractions, solid separated from ethyl alcohol fraction was recrystallised from acetone. The crystalline solid thus obtained was identical to an authentic sample of oxalic acid in its physical and chemical properties namely, solubility, chromatographic mobility, crystalline structure, melting point (102°C-monohydrate) and equivalent weight (63).

Fifty mg of this solid and the other fractions from Dowex-50 column were tested for aflatoxin-inhibiting activity by adding each into the medium<sup>4</sup> inoculated with toxigenic *A. flavus*, and incubated for seven days at 28°C. The culture broths were extracted with methanol and chloroform for aflatoxins. Aflatoxins were estimated by visual TLC method<sup>5</sup>. Results indicated that the ethyl alcohol fraction exhibited maximum antagonistic potential curtailing aflatoxin biosynthesis to the extent of 85%.

After identifying the inhibitor as oxalic acid, an authentic sample (BDH India, GR grade) of oxalic acid was tested for its inhibitory activity by supplementing various levels (50 to 1000 ppm) to the culture medium and incubating with *A. flavus* for production of aflatoxin under conditions previously mentioned. Extraction and estimation procedure for aflatoxin was the same as described earlier. Figure 1 indicates that there was no lowering of pH at the end of seven days as a result of addition of oxalic acid, which implies that decrease in production of aflatoxin by oxalic acid is not by lowering the pH. The figure also reveals that

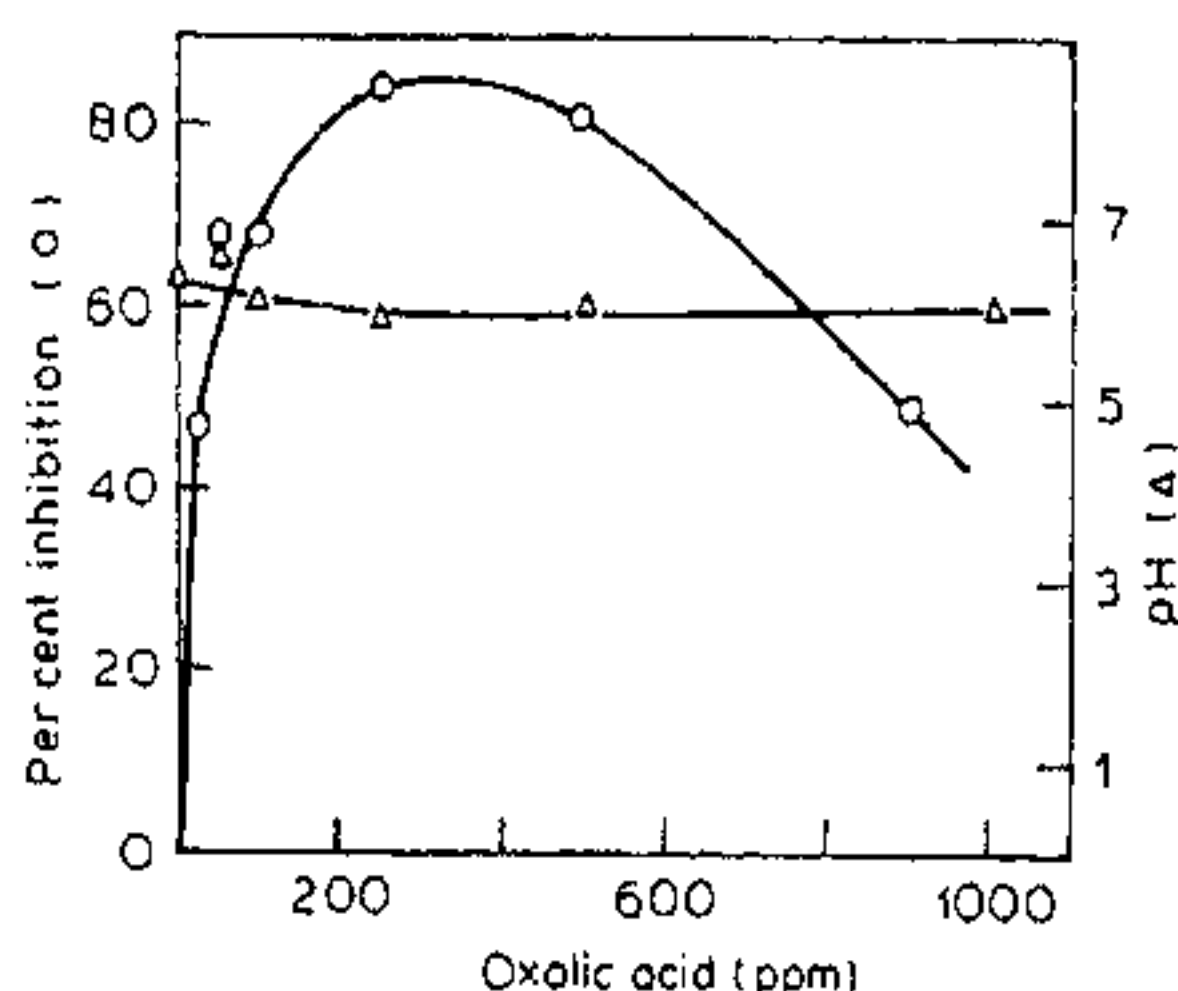


Figure 1. Inhibition of aflatoxin production by *A. flavus* in presence of oxalic acid in Czapek-Dox liquid medium in seven days at 28°C.

maximum inhibition occurred at 250 ppm level of oxalic acid. The mycelial mats collected (as described earlier) on preweighed folded filter paper circles were dried at 90°C to a constant weight. The weights recorded were very close (0.9 g) to each other indicating that the decrease in aflatoxin production is not due the inhibition of the growth of aflatoxin-producing organism.

The study unravels that oxalic acid is one of the major inhibitory factors produced by *A. niger* to antagonize biosynthesis of aflatoxin by *A. flavus* in liquid synthetic medium. The inhibition is not due to the lowering of pH. Although production of oxalic acid was reported in 1956 itself by Hayashi *et al.*<sup>6</sup>, it has only now been recognized as a factor inhibiting aflatoxin synthesis.

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## Difluoromethylornithine sensitivity of ornithine decarboxylase from *Acanthamoeba culbertsoni* and *Giardia lamblia*

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*Acanthamoeba culbertsoni* showed remarkable resistance to  $\alpha$ -difluoromethylornithine (DFMO), while multiplication of *Giardia lamblia* is inhibited by this compound. Ornithine decarboxylase (ODC) activity has been detected in both these parasites. DFMO inhibits ODC of *A. culbertsoni* and *G. lamblia* to a similar extent in the cell-free extracts as well as whole cells. The refractoriness of ODC to DFMO or the impermeability of cells to the inhibitor do not seem to be involved in DFMO resistance of *A. culbertsoni*.

DL- $\alpha$ -Difluoromethylornithine (DFMO, eflornithine) is a