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ISOLATION OF MYCOTOXINS PRODUCED BY AN ANTAGONISTIC FUNGAL ORGANISM, *ARACHNIOTUS SPECIES*

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Mycotoxins produced by ***Arachniotus species*** *during the fermentation of rice polishings showed positive results against a common soil-borne plant pathogen* ***Fusarium oxysporum.*** *These toxins were isolated from the broth by centrifugation and then through column chromatography using polarity gradient solvent on silicagel and simple activated charcoal column eluting with sterilized distilled water.*

INTRODUCTION

A considerable amount of our agricultural produce is affected by various pathogens each year. Different pesticides are being used to control these pathogens. It has been reported that these chemical compounds do not decompose completey but they persist beyond the safe level at the time of consumption (Naghma, 1985). Besides the residual effects, these pesticides are very expensive. Pakistan has to spend a considerable amount of foreign exchange on their import. It was, therefore, necessary to study ways and means to tackle this problem using indigenous sources. Various organisms possess antagonistic behaviour against various plant pathogens. *Trichoderma harzianum* and *Arachniotus species* are already being used as antagonistic fungal organisms to control several soil-borne diseases (Akhtar, 1982). These organisms are known to control pathogenic fungi through the production of mycotoxins (Weindling, 1937). The most well known toxins produced by Trichoderma are gliotoxins and viridin (Bilai, 1963) but the toxins of Arachniotus are yet unreported in literature. Thus an attempt was made to culture A. species in shake media under predetermined conditions to produce mycotoxins during the fermentation of rice polishings. These toxins were then isolated by applying column chromatographic technique.

MATERIALS AND METHODS

The medium used to culture *Arachniotus species* had the following composition:

Rice polishings	50.0	g/L
Urea	3.128	g/L
$CaCl_2$	0.025	g/L
$MgSO_4.7H_2O$	0.025	g/L
KH_2PO_4	2.0	g/L

The fungus was grown in 250 ml Erlenmeyer flasks, containing 50 ml optimum growth medium. The flasks were plugged with cotton wool and autoclaved at 121°C for 15 minutes. The pH of the medium was adjusted to 4 with sterile 1M HCl. Spore inoculum one ml containing $10^7 - 10^8$ spores was added to each flask aseptically. Another set

of flasks was prepared as control (blank). All the flasks were kept on shaker with revolutions 150–200 per minute at 30°C temperature. Duplicate flasks were harvested after 0, 6, 9, 12, 15 and 18 days of incubation alongwith the control. The broth was separated from the biomass by filtration and tested for biological activity against *Fusarium oxysporum* by spore germination test.

Isolation of mycotoxins: Mycotoxins thus produced were isolated from the broth by applying Cole's technique (1977). The eluates were evaporated to dryness. The residue was dissolved in one ml sterilized distilled water and subjected to spore germination test. But this test was not found to be conclusive, therefore, another method of separation was adopted. The broth was passed through an activated charcoal bed (2 cm in height) using sterilized distilled water as eluant (Malik *et al.*, 1986). The clear transparent solution obtained after centrifugation was subjected to spore germination test which showed positive results.

RESULTS AND DISCUSSION

Incubation period for mycotoxin production: The production of mycotoxins under predetermined conditions at different intervals was studied. The results as shown in Table 1 revealed that after 6 days of incubation period maximum mycotoxins were produced as indicated by zero percent spore germination. Further increase in the incubation period (9 days) reduced the production of mycotoxins to one–tenth. This level of production remained constant even after 12, 15 and 18 days of incubation. Decrease in mycotoxin production reflects also reduced toxicity of the broth. This may be attributed to transformation of mycotoxins into other products. It was thus inferred that 6 days incubation period was optimum for mycotoxin production from *Arachniotus species* when cultured on rice polishings at pH 4 and 30°C temperature.

Column chromatography: Zero percent spore germination as exhibited by all the solvent residues indicated that Cole's technique of separation

Table 1. Spore germination at various incubation periods

Days of incubation	Treatments	Germination (%)
Control	S.D.W*. + *F.oxysporum*	100
0	Filtrate + *F.oxysporum*	100
6	–do–	zero
9	–do–	10
12	–do–	10
15	–do–	10
18	–do–	10

*S.D.W. indicates sterile distilled water.

was not conclusive for this test (Table 2). Traces of organic solvent remaining after drying may act as an inhibitory agent for the spore germination. Furthermore, the organic solvents tested may contain some impurities which interfered the test.

In the light of the above observations, the broth was passed through a charcoal bed under partial pressure using sterilized water as eluant. The eluate thus obtained showed zero percent spore germination indicating the presence of mycotoxins. Hence it was inferred that mycotoxins produced by *Arachniotus species* can be isolated from the fermented broth by passing it through an activated charcoal bed with distilled water as eluant. It offers a simple, efficient and economical method to isolate the mycotoxins from the fermented broth through fermentation of rice polishings under optimum conditions with *Arachniotus species.*

Table 2. Spore germination test against different solvent eluate residues, using silicagel-G as adsorbent

Treatments	Test organism	Germination (%)
Benzene	Fusarium oxysporum	Zero
Diethyl ether	–do–	–do–
Chloroform	–do–	–do–
Ethyl acetate	–do–	–do–
Acetone	–do–	–do–
Methanol	–do–	–do–
Sterilized distilled water	–do–	–do–

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