قسم النبات.
كلية العلوم - جامعة أسوان.
رئيسي القسم: د. أ.د. أحمد صطيفي أحمد.

مدى سمية وأنواع السمنة الفطرية المنتجة بواسطة الفطرات المعزولة من الجاموس المصابة بالالتهابات الرئوية

اسحاق القاضي، محمد سهيل الدين، إبراهيم عبد الله، عادل الباش

دراسة استقصائية عن مدى سمية وأنواع السمنة المنتجة بواسطة 34 حالة معزولة من الفطرات تم عزلها من 185 حالة من الجاموس المصاب بالالتهابات الرئوية، تبين أن حوالي 60٪ من الفطرات الخاضعة تعتبر سامة. كان جنس إسبريجيللucs هو أكثر الأجناس المختارة سمية، حيث تبين أن حوالي 75٪ من المعزولات التابعة لهذا الجنس تعتبر من الفطرات السامة وذلك باستخدام طريقة الميكس نسيج الفطر السامة جداً.

البحث لا ينتهى أن أنواع الفطرات البحرية المعروفة باسم "ارتينيا سالينا" ليست سامة 50٪ من معزولات جنس الميكرور والبنسيلوم. بالكشف عن أنواع السمنة المنتجة بواسطة الفطرات المختارة، وذلك باستخدام طريقة التحليل الكروماتوغرافي على رفائق السليكا وجد أن 14 حالة معزولة من المعزولات المختارة لها القدرة على انتاج سموم فطرية معروفة. تم تعريف السمنة المنتجة وبيانها كالتالي:

- سموم الأفلاتوكسين، ووجد أنها تنتج بواسطة أربعة معزولات من فطرة إسبريجيللucs فايلس، ومعزولة واحدة من فطرة إسبريجيللucs براستيكس.
- سم الفطرية المعروف باسم حمض الكوجيك حيث انتاجه معزولتين من فطرة إسبريجيللucs فويميجاتس.
- أما سم المسترين فقد ثبت انتاجه بمعزولتين من فطرة بنسيلوم نانون.
- بينما سم استريجالاموسيستين ثبت انتاجه بواسطة معزولتين من فطرة إسبريجيللucs نيد بولا تيل وثلاثة معزولات من فطرة إسبريجيللucs تيرس.
Dept. of Botany,
Faculty of Science & Vet. Medicine, Assiut University,
Head of Dept. Prof. Dr. A.M. Ahmed & Prof. Dr. I.S. Abdallah.

TOXIGENICITY AND TOXINS PRODUCED BY FUNGI ISOLATED FROM
CLINICALLY POSITIVE PNEUMONIC CASES OF BUFFALO CALVES
(With One Table)

By
I.A. EL-KADY; M.B. MAZEN; L.S.A. ABD ALLAH and A.H. ELYAS
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SUMMARY

34 different cultures of fungi isolated from 185 clinically positive
pneumonic cases of buffalo calves were tested for both toxicity
and toxins production. About 60% of the isolates proved to be toxic
to brine shrimp. Aspergillus was the most toxigenic to brine shrimp,
57% of its isolates were toxic to this test organism comparable
with 50% in case of both Mucor and Penicillium. Thin layer chroma-
tographic analysis showed that 14 isolates produced known mycotoxins.
Toxins identified are aflatoxin B₁, B₂, G₁ and G₂ produced by four
isolates of A. flavus and one isolate of A. parasiticus, kojic acid
by two isolates of A. fumigatus, citrinin by two isolates of P. notatum
and sterigmatocystin by two isolates of A. nidulans and three isolates
of A. terreus.

INTRODUCTION

RAMAZZINI (1705) the father of occupational medicine accurately described diseases
of workers inhaling "foul and mischievous powder" from handling food, fodder and fiber crops.
In recent decades such pulmonary disease have been causally related to fungi. The term "toxo-
mycosis" has been applied to diseases produced by inhalation of fungal spores, mycelia, or
decaying matter upon which fungi are growing. SAMSONOV (1960) classified diseases resulting
from the absorption of fungal toxin through the mucous membranes of the respiratory tracts
as toxomyces. KOVATS and BUGYI (1968) extended the term to include the alveolar reactions
which are called hypersensitivity pneumonitis or extrinsic allergic alveolitis (PEPYS, 1969). The
mechanism of these diseases appear to include the toxic effects of fungal products and host
defensive immune responses.

A few studies have attempted to assess the effect of mycotoxins in pulmonary disease.
HESSLELINE et al. (1966) and GOLDBLATT (1966), reported that acut inhalation exposure to
aflatoxin has destructive affects upon the exposed cells of the respiratory tract and provide
the first experimental evidence of health hazard of inhaled mycotoxin. The carcinogenic effect
of aflatoxin on respiratory tract has been investigated by DICKENS et al. (1966). Pulmonary
alveolar cell hyperplasia (adenomatosis) and diffuse interstitial pneumonia in cattle have been
attributed to toxin produced in feeds infected with moulds.

In a previous study (MAZEN et al, 1982) the mycoflora of 185 clinically positive pneumonic
cases of buffalo calves was reported. This study was undertaken to assess the toxicity and
mycotoxin-producing potentialities of the previously isolated fungi.
Cultivation:

Inocula of 1 ml. of spore suspension form 2-week old cultures maintained on Czapek's medium were transferred to 250 ml. Erlenmeyer flasks, each containing 50 ml. of Czapek's medium, in which glucose (10 gm/L) replaced sucrose, and supplemented by 1 gm/L of each of yeast extract and peptone. Flasks were incubated as surface cultures at 28°C for two weeks.

Extraction of Mycotoxins From Fungal Cultures:

At the end of incubation period, the contents of each flask (medium + mycelium) were homogenized with 100 ml. of chloroform for 5 min in a high speed blender (16.000 r.p.m.). The extraction procedure was repeated three times. The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulphate, filtered then concentrated to near dryness.

Thin Layer Chromatographic Analysis:

The chloroform extracts were analyzed for the presence of known mycotoxins using thin layer chromatographic plates according to the method previously used (EL-KADY and ABDEL HAFIZ, 1981). Standard mycotoxin references used included aflatoxin B1, B2, G1, G2, M1 and M2, patulin, versicolorin, sterigmatocystin, ochratoxin A, kojic acid and penicillic acid. Thin layer plates were developed in toluene-ethyl acetate-formic acid (6:3:1, v/v/v) and chloroform methanol (97:3, v/v) and treated according to the method of SCOTT et al. (1970).

Brine Shrimp Test:

The method described by KORPINEN (1974) was used. Brine shrimp (Artemia salina) "eggs" were hatched in artificial sea water (5-7 per cent salt) at 28°C. Two to three teaspoonfuls of eggs were inoculated into one liter of water. Air was conducted into the water in small bubbles through a tube. Three days after the emergence of first nauplius larvae, the hatched larvae were used as test animals. 0.02 ml. of the chloroform extract were applied to 6 mm. diameter filter paper disc of Whatman No. 1. After chloroform had completely evaporated, the disc were placed into a test tube, and an estimated 40-100 Artemia salina larvae in 3 ml. salt water were transferred into the tube. The tubes were kept at 28°C. The results were read after 2 days of incubation. Control tubes with 0.02 ml. of chloroform were always included in the experiments. The affected Artemia larvae were immobilized and sank to the bottom. Mortality of the larvae over the control mortality was regarded as toxicity. The titration of every preparation was repeated 3-4 times.

RESULTS and DISCUSSION

34 different isolates belonging to four genera and ten species isolated from 185 positive pneumonic cases of buffalo calves were tested for both toxicity and toxins production. Results of brine shrimp bioassay (Table 1), indicates that nearly 60% of all the isolates were toxic to brine shrimp (induced more than 50% mortality), 16 isolates from Aspergillus, one from Mucor, and two of Penicillium. The results also reveal that the genus Aspergillus was the most toxigenic to brine shrimp since 57% of its isolates were toxic to this test organism comparable with 50% in case of Mucor and Penicillium. The two isolates of Rhizopus proved to be non toxic under our experimental conditions.

Thin layer chromatographic analysis of the culture extracts of the different fungal isolates tested (Table 1), showed that 14 out of 19 toxic isolates produced known mycotoxins. Toxins
identified were aflatoxins B₁, B₂, G₁ and/or G₂, produced by four isolates of A. flavus and one isolate of A. parasiticus; kójic acid by two isolates of A. fumigatus; citrinin by two isolates of Penicillium notatum and sterigmatocystin by two isolates of A. nidulans and three isolates of A. terreus. Toxins produced by five isolates out of 19 toxic isolates could not be detected owing to the lack of authentic toxin references.

Detection of seven different toxic metabolites produced by about 60% of the tested isolates strengthen our initial concern that a potential hazard due to the presence of toxigenic moulds in the examined pneumonic cases. Few studies, have attempted to assess the role of mycotoxins in aspergillosis. The earliest suggestion that human pulmonary disease is produced by mycotoxins is in reports of invasive aspergillosis. GOWING and HAMLIN (1960) found extensive tissue necrosis around the invading mycelia suggesting that toxic substances, were produced by Aspergillus growing in tissue. Enhancement of mycelial growth was attributed to tissue destruction by fungal products. Aflatoxin inhaled as aerosols damage avian and mammalian air way cells. High doses produce hemorrhage, impair pulmonary clearance and cause cells to exfoliate, (HESSELTINE et al. 1966 and GOLDBLATT, 1969). As reported by Edwards, and AL-ZUBAIDY, (1977), aflatoxin results in immunosuppression with increased susceptibility to bacterial, viral, fungal and parasitic diseases. In general, young animals of any species are more susceptible to the acute toxic effects of aflatoxins than are older animals of the same species. This support our previous results (MAZEN et al. 1982), in which it had been shown that about 38%, 24%, 21% and 16% of the positive pneumonic cases were recorded in animals of different ages ranging from 1-3 month, 3-6, 6-9, and more than 10 months, respectively.

REFERENCES


Table (1)
Fungi isolated from different positive pneumatic cases, toxicity to brine shrimp and mycotoxins produced

<table>
<thead>
<tr>
<th>No. of isolate</th>
<th>Fungi isolated from different cases</th>
<th>Toxicity to Brine shrimp (% mortality)</th>
<th>Toxins produced*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A. flavus</td>
<td>100</td>
<td>B1, B2, G1, G2</td>
</tr>
<tr>
<td>2</td>
<td>A. flavus</td>
<td>100</td>
<td>B1, B2</td>
</tr>
<tr>
<td>3</td>
<td>A. flavus</td>
<td>100</td>
<td>B1, B2</td>
</tr>
<tr>
<td>4</td>
<td>A. flavus</td>
<td>100</td>
<td>N.D.</td>
</tr>
<tr>
<td>5</td>
<td>A. flavus var. columnaris</td>
<td>100</td>
<td>B1, G2</td>
</tr>
<tr>
<td>6</td>
<td>A. fumigatus</td>
<td>100</td>
<td>U.T.F.</td>
</tr>
<tr>
<td>7</td>
<td>A. fumigatus</td>
<td>30</td>
<td>N.D.</td>
</tr>
<tr>
<td>8</td>
<td>A. fumigatus</td>
<td>100</td>
<td>Kojic acid</td>
</tr>
<tr>
<td>9</td>
<td>A. fumigatus</td>
<td>90</td>
<td>Kojic acid</td>
</tr>
<tr>
<td>10</td>
<td>A. nidulans</td>
<td>10</td>
<td>N.D.</td>
</tr>
<tr>
<td>11</td>
<td>A. nidulans</td>
<td>100</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>22</td>
<td>A. parasiticus</td>
<td>100</td>
<td>B1, B2</td>
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</tr>
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<td>Citrinin</td>
</tr>
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</tr>
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</tr>
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</tr>
<tr>
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<td>70</td>
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</tr>
<tr>
<td>34</td>
<td>Mucor racemosus</td>
<td>30</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

* B1 = aflatoxin B1, B2 = aflatoxin B2, G1 = aflatoxin G1, G2 = aflatoxin G2
N.D. = Not Detected
U.T.F. = Unidentified toxic factor