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مدى سمية وأنواع السموم الفطرية المنتجة بواسطة الفطريات المعزولة من الجاموس المصاب بالالتهابات الرئوية

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دراسة استقصائية عن مدى سمية وأنواع السموم المنتجة بواسطة ٣٤ حالة معزولة من الفطريات تم عزلها من ١٨٥ حالة من الجاموس المصاب بالالتهاب الرئوى، ثبت أن حوالي ٦٠٪ من الفطريات المختبرة تعتبر سامة. كان جنس الاسبرجيللس هو أكثر الاجناس المختبرة سمية، حيث تبين أن حوالي ٥٧٪ من المعزولات التابعة لهذا الجنس تعتبر من الفطريات السامة وذلك باستخدام طريقة البيض حديث الفقس لاحد أنواع القشريات البحرية المعروفة باسم "ارتيميا سالينا". ثبت سمية ٥٠٪ من معزولات جنس الميكوكر والبنسليوم. بالكشف عن أنواع السموم المنتجة بواسطة الفطريات المختبرة، وذلك باستخدام طريقة التحليل الكروماتوجرافي على رقائق السليكا وجد أن ١٤ حالة معزولة من المعزولات المختبرة لها القدرة على انتاج سموم فطرية معروفة. تم تعريف السموم المنتجة وبيانها كالتالي:

- سموم الافلاتوكسين، ووجد أنها تنتج بواسطة أربعة معزولات من فطرة اسبرجيللس فلافس، ومعزولة واحدة من فطرة اسبرجيللس باراستيكس.
- السم الفطرى المعروف باسم حمض الكوجيك ثبت انتاجه بمعزولتين من فطرة اسبرجيللس فيوميجاتس.
- أما سم السترنين فقد ثبت انتاجه بمعزولتين من فطرة بنسليوم نوتاتم.
- بينما سم استريجماتوسيستين ثبت انتاجه بواسطة معزولتين من فطرة اسبرجيللس نيد يولانس وثلاثة معزولات من فطرة اسبرجيللس تيرس.

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TOXIGENICITY AND TOXINS PRODUCED BY FUNGI ISOLATED FROM CLINICALY POSITIVE PNEUMONIC CASES OF BUFFALO CALVES

(With One Table)

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SUMMARY

34 different cultures of fungi isolated from 185 clinicaly 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 chromatographic analysis showed that 14 isolates produced known mycotoxins. Toxins identified are aflatoxin B_1, B_2, G_1 and G_2 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 decads such pulmonary disease have been causally related to fungi. The term "toxomycosis" 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 toxine through the mucous membranes of the respiratory tracts as toxomycoses. KOVATS and BUGYI (1968) extended the term to include the alveolar reactions which are called hypersensitivity pneumonitis or extrinis 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 asses the effect of mycotoxins in plumonary disease. HESSELTINE 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 clinicaly positive pneumonic cases of buffaloe calves was reported. This study was undertaken to assess the toxicity and mycotoxin-producing potentialities of the previously isolated fungi.

MATERIAL and METHODS

Cultivation:

Inocula of 1 ml. of spore suspension form 2-week old cultures maintained on Czapek's medium were transferred to 250 ml. Erlenemeyer flaske, 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 HAFEZ, 1981). Standard mycotoxin references used included aflatoxin B_1, B_2, G_1, G_2, M_1 and M , 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 immobolized 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 geners 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 B_1, B_2, G_1 and/or G_2, 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 Penicillium notatum and sterigmatocystin by two isolats 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 asses 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 suggesing 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 mamalian 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 susceptibal 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.

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Table (1)

Fungi isolated from different positive pneumonic cases, toxicity to brine shrimp and mycotoxins produced

No. of isolate	Fungi isolated from different cases	Toxicity to Brine shrimp (% mortality)	Toxins produced*
	Aspergillus:		
1	A. flavus	100	B_1, B_2, G_1, G_2
2	A. flavus	100	B_1, B_2
3	A. flavus	100	B_1, B_2
4	A. flavus	40	N.D.
5	A. flavus var. columnaris	100	B_1, G_2
6	A. fumigatus	100	U.T.F.
7	A. fumigatus	30	N.D.
8	A. fumigatus	100	Kojic acid
9	A. fumigatus	90	Kojic acid
10	A. nidulans	10	N.D.
11	A. nidulans	100	U.T.F.
12	A. nidulans	100	Sterigmatocystin
13	A. nidulans	100	Sterigmatocystin
14	A. nidulans	30	N.D.
15	A. nidulans	10	N.D.
16	A. niger	10	N.D.
17	A. niger	80	U.T.F.
18	A. niger	20	N.D.
19	A. niger	10	N.D.
20	A. niger	100	U.T.F.
21	A. niger	10	N.D.
23	A. parasiticua	100	B_1, B_2
24	A. terreus	100	Sterigmatocystin
25	A. terreus	100	Sterigmatocystin
26	A. terreus	100	Sterigmatocystin
27	Penicillium notatum	90	Citrinin
28	Penicillium notatum	10	N.D.
29	Penicillium notatum	100	Citrinin
30	Penicillium notatum	30	N.D.
31	Rhizopus stolonifer	0	N.D.
32	Rhizopus stolonifer	10	N.D.
33	Mucor racemosus	70	U.T.F.
34	Mucor racemosus	30	N.D.

* B_1 = aflatoxin B_1, B_2 = aflatoxin B_2, G_1 = aflatoxin G_1, G_2 = aflatoxin G_2
N.D. = Not Detected
U.T.F. = Unidentified toxic factor