Mycoflora and mycotoxin-producing fungi of air-dust particles from Egypt

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Abstract

Using the dilution-plate method, 27 genera and 64 species were collected from 20 air-dust samples on glucose – (24 genera and 57 species) and cellulose – (21 genera and 45 species) Czapek’s agar at 28 °C. There are basic similarities between the mycoflora of air-dust on the two media and the most prevalent species were Aspergillus niger, A. flavus, A. ochraceus, A. terreus, A. versicolor, Penicillium chrysogenum, P. funiculosum, Alternaria alternata, Cladosporium herbarum, Fusarium oxysporum, Rhizopus stolonifer and Trichoderma viride. Chaetomium globosum, Stachybotrys chartarum, Humicola grisea and Arthrobotrys oligospora were common only on cellulose agar plates.

Extracts of mycelium from 25 isolates were tested with brine shrimp (Artemia salina); of these 23 displayed varying degrees of toxicity. Thin layer chromatographic analysis of 12 isolates of Aspergillus flavus revealed that 4 strains were producing detectable aflatoxin. Zearalenone production was noted for 3 out of 5 strains of Fusarium oxysporum and 2 out of 5 strains of F. solani.

Introduction

Spores and other particles of actinomycetes, bacteria and fungi are always present in atmospheric, air-dust and grain-dust particles (4, 5, 8, 11, 12, 19, 20, 21, 24, 25, 26, 30, 35, 41, 42). These spores and particles are associated with human and animals diseases such as allergy, poisoning and infection (9, 23, 27, 37, 38, 39).

In Egypt, a few investigations were carried out on the mycoflora in the air at different governorates (6, 29, 31, 33), but none of these studies were focused on air-dust particles.

In this study, composition, density and frequency of occurrence of mycoflora, as well as mycotoxin-producing fungi in air-dust particles from Upper Egypt were estimated. This and previous studies (7, 29, 31, 33) may help in finding suitable means to control some human diseases in Egypt such as chronic bronchitis, emphysema, asthma and allergies.

Material and methods

Twenty samples, 1/4 kg each, of air-dust (downfall dust) were collected during April and May 1984, from the dust that settled on the roofs of Faculty of Science and selected houses in Minya, Assiut, Sohag and Quena Governorates which lie in Upper Egypt (5 samples from each Governorate). Samples were sifted through a mesh screen (opening diam. 63 μm) to remove large dust particles, and all samples were stored at 2-4 °C.

Mycoflora analysis of air-dust particles

The dilution-plate method was used for the estimation of mycoflora in the air-dust. The two agar media used were: glucose – (10 g/L) and cellulose – (19 g/L) Czapek’s agar to which rose bengal (1/15000) was added as a bacteriostatic agent (44). Twelve plates were used for each dust sample (6
plates for each medium). Plates were incubated at 28 °C for 1-2 weeks and the developing fungi were identified and counted and the numbers were calculated per 1 mg air-dust particles. The colonies of slow-growing fungi which were about to be overgrown, as well as mycelial fragments of some colonies, were transferred to Czapek’s agar +0.05% yeast extract or to malt extract agar.

Cultivation for toxin production

Inocula of 1 ml of spore suspension from 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 g/L) replaced sucrose, and supplemented by 1 g/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 the incubation period, the contents of each flask (medium + mycelium) were homogenized with 100 ml of chloroform for 5 min in a high speed blender (16000 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, and 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 by El-Kady and Abdel-Hafez (13). Different standard mycotoxin references were used. Thin layer plates were developed in toluene-ethylacetate-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. (43).

Brine shrimp test

The method described by Korpinen (22) was used. Brine shrimp (Artemia salina) ‘eggs’ were hatched in artificial sea water (5-7% salt) at 28 °C. Two to three tea-spoonfuls of eggs were inoculated into one liter of water. Three days after the emergence of first nauplius larvae, the hatched larvae were used as test animals. An aliquot (0.02 ml) of the chloroform extract of mycelium was applied to 6 mm diameter filter paper disc of Whatman No. 1. After the chloroform had completely evaporated, the disc was placed into a test tube, and an estimated 40-100 Artemia salina larvae in 3 ml salt water were transferred into the tube, and incubated at 28 °C. The results were read after 24 hrs 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.

Results and discussion

A. Fungi recovered on glucose-Czapek’s agar

The total count of fungi in air-dust particles ranged between 1.26 and 23.66 colonies/mg dry dust, but in the majority of samples it was less than 5.2 colonies/mg dust. Abdel-Hafez & Shoriet (5) found that the total count of fungi in air-dust from Taif city at Saudi Arabia, fluctuated between 2.2 and 18.4 colonies/mg dry dust.

Twenty-four genera and 57 species were collected from 20 air-dust samples on glucose-Czapek’s agar at 28 °C (Tables 1 & 2). Most of the preceding fungi were also reported from atmosphere and soils in Egypt (1, 6, 7, 29, 31, 32, 33, 34). The most prevalent genera were Aspergillus, Penicillium, Alternaria and Cladosporium followed by Fusarium, Rhizopus and Trichoderma. Palmgren et al. (36) observed that Aspergillus, Penicillium and Fusarium were prevalent in grain dust from New Orleans area elevators. The most common fungi in corn dust in southern Georgia were species of yeasts, Aspergillus, Penicillium, Cladosporium, Alternaria, Helminthosporium and Fusarium (42).

Aspergillus was the most common genus and occurred in 100% of the samples comprising 61.3% of total fungi. It was represented by 16 species of which A. niger, A. flavus, A. ochraceus, and A. ter-
