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Bioassay of Mycotoxins from Toxigenic Fungi Isolated from Food Stuff.

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ABSTRACT

Mycotoxins are toxic secondary metabolites generated by fungi and there are a lot of physical and bioassay methods performed to study the mycotoxins. Seventeen isolates of fungi belong to four fungal genera which isolated from foodstuffs were tested in this study. The isolates were grown on Czapek's agar medium, and extracts were prepared with 96% methanol and these extracts were examined to detect the biotoxicity using the cell density of *Chlorella vulgaris* after 24, 48 and 72 h. The fungal extracts of *Aspergillus terreus* 3NRC*, Mucor circinelloides* 4NRC*, Penicillium nigricans* 5NRC*, Fusarium moniliforme* 6NRC*, Aspergillus niger* 7 NRC*, Aspergillus candidus* 10 NRC*, Penicillium* sp. 11NRC, *Penicillium chrysogenum* 13NRC*, Penicillium expansum* 14NRC*, Penicillium digitatum* 15NRC and *Aspergillus parasiticus* 17NRC were inhibited the growth of *C. vulgaris* dramatically after 24, 48 and 72 h., the crude extracts of *Aspergillus flavus* 1NRC, *Aspergillus aculeatus* 2NRC, *Penicillium nigricans* 8NRC, *Penicillium duclauxii* 9NRC, *Aspergillus niger* 12NRC and 16NRC showed no significant inhibition of *C. vulgaris* at different time. The mycotoxin bioassay using of *Chlorella vulgaris* growth was considered as a rapid, easy and effective tool to detect mycotoxins.

Keywords: Bioassay; mycotoxin; fungi; *Chlorella vulgaris*.

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INTRODUCTION

Filamentous fungi bring out mycotoxins via its mycelia, it is well known that these fungi introduce one or great amount of these toxic secondary metabolites that have no biochemical significance in the growth of these fungi further more development [1].

A single species of fungi might generate you quit offering on that one or a few mycotoxins, also distinct mycotoxins might be handled by separate contagious species. Mycotoxins are an incredibly various assemblage of biological compounds of secondary fungal metabolites with low molecular weight (> 700 Da).

In spite of the fact that there are over 300 mycotoxins that have been isolated and chemically described [2], research has centered on forms creating noteworthy damages on people also their ranch or friend animals. Aflatoxins (*Aspergillus flavus* toxins) are those the vast majority concentrated group of mycotoxins which generated by various species of the genus *Aspergillus*. There are more than 20 isolated Aflatoxins (AF) derivatives formed by several fungal species. For instance, *Aspergillus flavus* produces AFB1 and AFB2 while *A. parasiticus* generates AFB1, AFB2, AFG1, furthermore AFG2 [3]. Ochratoxins are metabolites for the pair *Aspergillus* and also *Fusarium* species [4]. Ochratoxin A (OTA) is those a large portion poisonous compound from this group.

Trichothecenes are mostly handled toward a various *Fusarium* species (e. g. *F. sporotrichioides*, *F. graminearum*, *F. poae*, and *F. culmorum*), also might make generated by different genera for example, *Myrothecium* [5]. Zearalenone it will be a metabolite principally connected with different *Fusarium* species (i.e. *F. culmorum*, *F. graminearum*, and *F. sporotrichioides*) [6]. Fumonisins (B1 and B2) are known as cancer-promoting metabolites of *F. proliferatum* and *F. verticillioides* [7]. Moniliformin generated by various *Fusarium* species (mainly *F. proliferatum*) also is generally discovered on the corn kernel.

It could make exchanged on following era harvests furthermore survive for quite some time in the soil [8]. When these toxins would be ingested, breathed in or consumed through those skin, they foundation brought down performance, ailment or demise to man alternately animals what's more birds [9]. These toxins represent a large number upon billions of dollars yearly in misfortunes around the world done human health, animal health, furthermore affecting agricultural products [10,11].

Biological assay may be those determinations of the relative quality of a substance by analyzing its impact for a test living being with that of a standard preparation. It was progressively suitable to mycotoxins detection as a forerunner to chemical analysis. In recent toxicology, European Council asked for decrease in the amount from guaranteeing vertebrates used inside toxicology testing and their partly supplanting with invertebrate animals, plants or significantly organ, tissue, or cell cultures [12].

Algae are particularly suitableness for biotests due to their affectability to natural contamination What's more their plenitude in maritime systems. In addition, they bring no roots as higher plants furthermore reflect just the properties of the surrounding water instead of those of the soil higher plants that are established. Also, algal biotests are easy, also permit watching various generations [13].

The agar diffusion technique of *Chlorella vulgaris, Ustilago maydize* and *Trichoderma viride* used by Bean et al. [14] revealed that *Chlorella* was the extremely delicate organism against macrocyclic trichothecenes generated by various *Myrothecium* species tested. *Bacillus subtilis* and *C. vulgaris* were demonstrated to be particularly sensitive to mycotoxins [15]. The first target of this study was to identify mycotoxins producer fungi which isolated from food stuff. The second target was the using of *Chlorella vulgaris* as a bioassay tool to detect mycotoxins.

MATERIAL AND METHOD

Collection of samples.

Seventeen different isolates of filamentous fungi were collected from different six sources of food stuffs. They packed directly into sterilized plastic bags and were kept in refrigerator until use.

Isolation of fungi.

Food stuffs samples were subjected to series of washing with sterile distilled water. They were dried between sterile filter paper, cut into equal segments (about 1 cm each). Five segments were placed on the surface of the agar medium in each plate which supplemented with Rose-Bengal and chloramphenicol as bacteriostatic agents, then the plates were incubated at 28 °C for 7 days, and the developed colonies were isolated and grown again for three times until purification; the purified colonies were identified.

Identification of the fungal species.

Identification of the isolated fungi during the investigation was carried out using the morphological characteristics and microscopic features were examined by optical light microscope (10×90). The identification is according to the following references; Ainsworth [16]; Booth [17,18], for *Fusarium* species, Klich and Pitt [19], for *Aspergillus* species; Zycha [20], for Mucorales group and Ramirez [21] and Pitt [22,23], for *Penicillium* species.

Fungal crude extracts.

Each isolate was grown on Czapek's agar medium, incubated at 28°C for 10 days. After the incubation period, the fungal growth with agar media were cut into small pieces, transferred to a 250 ml Erlenmeyer flask containing 50 ml 96% methanol. The contents were shaken on a rotary shaker (120 rpm, 24 h) and filtered through filter paper (Whatman No.1). The residue was then washed twice with 96% methanol (25 ml each). The methanol extracts were combined, dried over anhydrous sodium sulphate, and then left to evaporate to near dryness under vacuum [14].

Thin layer chromatographic (TLC) determination of mycotoxins.

For qualitative determination of mycotoxin produced by different fungal isolates tested, TLC technique adopted by El-kady and Moubasher [24] was employed.

Biological assay procedure.

Chlorella vulgaris

Beijrinek medium was used for growth of *C. vulgaris* [25]. Algal cultures were grown at a temperature of 28 ±1°C in a light growth chamber (Forma Scientific, USA). The inoculums were maintained to be 0.123 O.D. 750 nm in all the cultures throughout the study period. Three replicates of 10 µl of the clean crude extract tubes and 6 ml of *Chlorella* culture was added to each tube under a septic condition, optical density (O.D. 750 nm**)** of cultures was measured at required time intervals (after 24, 48 and 72 h) using spectrophotometer (Thermo scientific, evolution 160 UV-Vis, double beam spectrophotometer, USA).

RESULT AND DISCUSSION

Isolated fungi.

Seventeen different isolates of filamentous fungi were collected from different six sources of food stuffs as shown in Figure (1). The growth of fungi was affected by essential parameters which are plant and animal tissues that are inherent part. The fungal isolates which isolated from various types of food stuffs have saprophytic nature and belonged to the class ascomycetes and class deuteromycetes and zygomycetes. Each of *Aspergillus* species and *Penicillium* species has significance presence in our research (six species per each one) and this result was in consonance with [26-28]. *Fusarium* species and *Mucor* species were also found [29].

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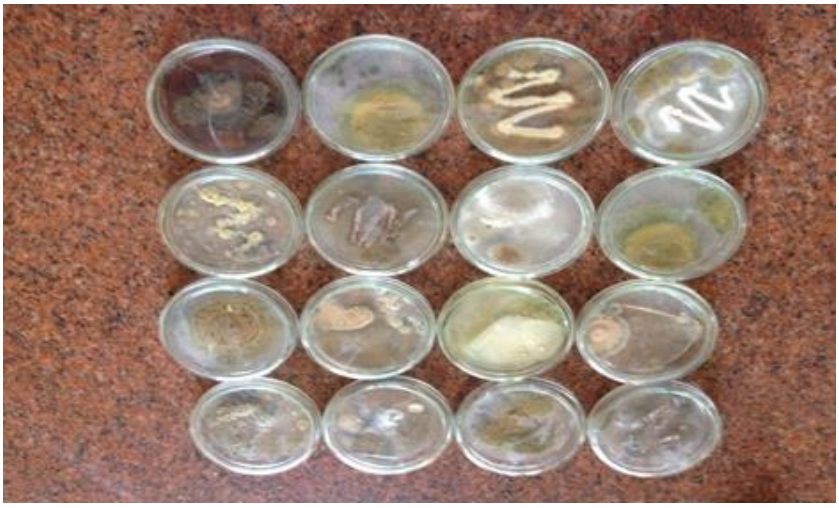

Figure 1. The different isolated filamentous fungi.

Screening for toxin producing fungal species.

The screening profile (Table 1) shows the ability of different fungal strains to produce toxin from 17 filamentous fungi were screened, all isolates have toxigenic activity except six isolates failed to produce any mycotoxin as manifested by The TLC analysis of the 17 crude extracts.

Table 1: screening for toxigenic activity production by different fungal strains.

Code number	Scientific Name	Toxigenic activity
1 NRC	*Aspergillus flavus*	-ve
2NRC	*Aspergillus aculeatus*	-ve
3NRC	*Aspergillus terreus*	+ve
4NRC	*Mucor circinelloides*	+ve
5NRC	*Penicillium nigricans*	+ve
6NRC	*Fusarium moniliforme*	+ve
7NRC	*Aspergillus niger*	+ve
8NRC	*Penicillium nigricans*	-ve
9NRC	*Penicillium duclauxii*.	-ve
10NRC	*Aspergillus candidus*	+ve
11NRC	*Penicillium* sp.	+ve
12NRC	*Aspergillus niger*	-ve
13NRC	*Penicillium chrysogenum*	+ve
14NRC	*Penicillium expansum*	+ve
15NRC	*Penicillium digitatum*	+ve
16NRC	*Aspergillus niger*	-ve
17NRC	*Aspergillus parasiticus*	+ve

Biological assay of mycotoxin.

The fungal extracts of *Aspergillus terreus* 3NRC*, Mucor circinelloides* 4NRC*, Penicillium nigricans* 5NRC*, Fusarium moniliforme* 6NRC*, Aspergillus niger* 7 NRC*, Aspergillus candidus* 10 NRC*, Penicillium* sp. 11NRC, *Penicillium chrysogenum* 13NRC*, Penicillium expansum* 14NRC*, Penicillium digitatum* 15NRC and *Aspergillus parasiticus* 17NRC were inhibited the growth of *C. vulgaris* dramatically after 24, 48 and 72 h. of which were the fungal isolates affected the growth of *C. vulgaris* as compared to the control (Figure 2) but the big shift was happened after 48 hr for every isolates with variable degrees. The lowest effect of *C. vulgaris* growth recorded using each of *Penicillium nigricans* 5NRC*, Fusarium moniliforme* 6NRC and *Aspergillus parasiticus* 17NRC. And this may be due to different reasons such as mycotoxin is less toxic and enhanced some enzymes activity as reduction of toxicity of *Penicillium nigricans* mycotoxin has been well explained by higher activity of glucose-6-phosphate dehydrogenase which is greatly specific for generation of NADPH [30]. The concentration of mycotoxin might also have been an effect on the results [31].

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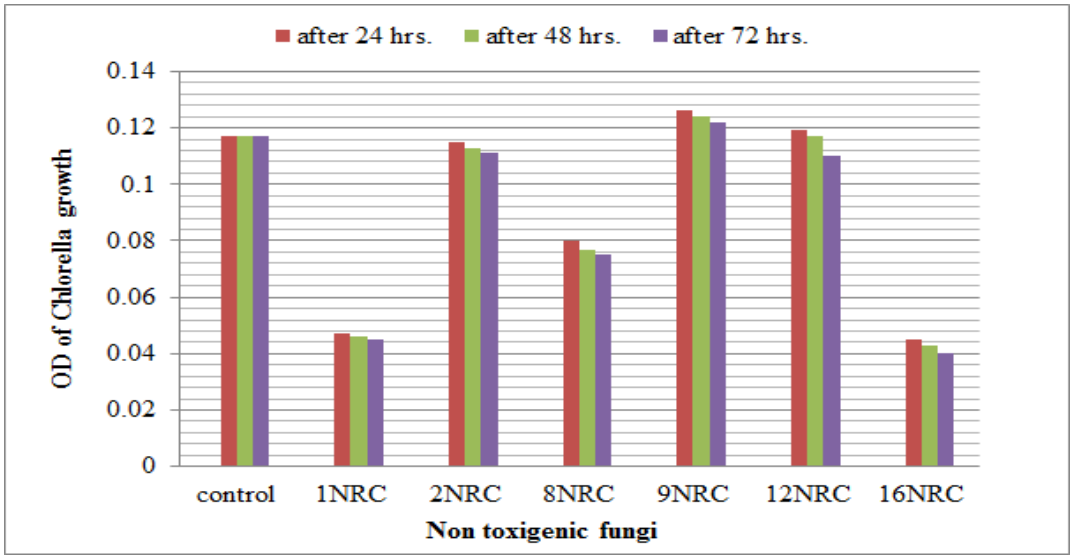

Figure 2. The effect of toxigenic fungi extracts on *Chlorella vulgaris* growth.

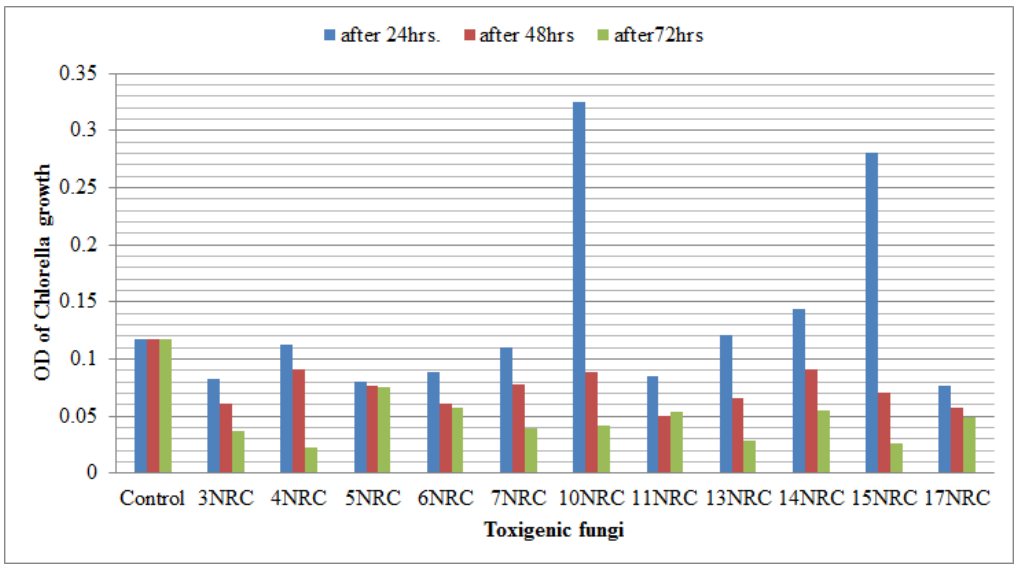

Figure 3. The effect of nontoxigenic fungi extracts on *Chlorella vulgaris* growth.

Otherwise, the crude extracts of *Aspergillus flavus* 1NRC, *Aspergillus aculeatus* 2NRC, *Penicillium nigricans* 8NRC, *Penicillium duclauxii* 9NRC, *Aspergillus niger* 12NRC and 16NRC were consider as nontoxin producing fungal isolates which showed no significant inhibition of *C. vulgaris* at different time used as compared to the control (Figure 3). These negative results might be expected should expanding different enzymes activity or decline concentration of mycotoxin which prompt decrease alternately obstacle mycotoxin activity concerning illustration specified over. The results of present study are in a harmony with Bean et al. [14] which proved that *C. vulgaris* growth are sensitive to macrocyclic trichothecenes formed by *Myrothecium* species. It was found that the crude extracts of the thirty non-toxins producing fungal isolates that refer to various species such as *A. niger, A. flavus, F. solani* and *S. elegans* which showed no significant inhibition of *C. vulgaris* at the three time intervals used when compared with the control [32].

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