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EXTRACTION AND PURIFICATION OF FUNGAL MYCOTOXINS AND TO STUDY THEIR EFFECT ON VARIOUS PLANTS

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Abstract

Mycotoxins are secondary metabolites produced by micro-fungi that are capable of causing disease anddeath in humans, animals, and plants. Because of their pharmacological activity, somemycotoxins or mycotoxins derivatives have found use as antibiotics, growth promoters, and other kinds of drugs; still others have been implicated as chemical warfare agents This work focuses on the most important ones including A flatoxin and fumonisins, and the effects on medicinal plant widely found in India subcontinent viz Ocimum sanctum. Fungal-degraded fruits Orange, Papaya, and Raspberry were taken for extraction of mycotoxins by high-speed blending with acetone and chloroform, followed bypurification with column chromatography (Silica gel) of the crude extract. TLC performed to determine the Rf value of purified toxins were identified as Aflatoxin B1 and G1, Trichothecin, and Nivalenol. Mycotoxins were then sprayed in equal amounts (2 ml) on the leaves of the potted (Ajooba, Sizonium, Penji)plants and left for 4-5 days, changes were observed and resultedin quite impressive wilting,necrosis, and yellowing of leaves were observed showing the fatal effect of mycotoxin on these plants.

Keywords: Mycotoxin, PDA, Orange, Papaya, Raspberry, TLC, Purification

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Introduction

The term "mycology" is derived from the Greek word "mykes" meaning mushroom. Therefore mycology is the study of fungi. Fungi are abundant in the entire biosphere. They have fascinated mankind since the beginning of written history and have considerably influenced our culture. Edible and medicinal mushrooms are widely used to serve our needs, whereas we have to struggle with fungal pathogens in agriculture and forestry and as causative agents of lethal diseases in humans and animals. In biotechnology, the ability of many fungi to grow easily under controlled conditions implicates fungi as model organisms for cell biology, development, and genetics of eukaryotes (Gavin et al., 2006).Mycotoxins are now recognized as prevalently toxic compounds produced as secondary metabolites by various fungi and excreted into their substrates. These substrates frequently include plants grown and stored for human or animal consumption as well as processed food. Mycotoxins and the associated health disorders in humans and animals have been recognized as a major health and economic problem (Morgavi, 2007).

Mycotoxins are naturally secondary metabolites of filamentous fungi, specifically molds that adversely affect biological organisms (Ghazi et al., 2003).Mycotoxins such as aflatoxins, beauvericin, deoxynivalenol, moniliformin, trichothecene, and zearale none are contaminants of food commodities (Rabie et al., 2000). The most known and studied group of mycotoxins in South Africa are fumonisins which have been associated with oesophageal cancer in humans and the cause of leucoencephalomalacia (LEM) in horses, mules and donkeys (Piraica et al., 1999) It is thus necessary to eliminate or reduce the presence of mycotoxins in the food chain. An important step in controlling food production chain contaminants is identifying food-borne fungi. The conventional methods used to detect fungal contamination are based on phenotypic and physiological characteristics that use standard culture and biochemical/serological tests. (Niessen, 2007).

Materials and methods

Sample Collection and Isolation

Three samples of juicy fruits (orange, papaya and raspberry) which were collected from the local vendors of Indira Nagar, Lucknow and kept at room temperature 5-6 days for fungal growth.Fungus grown on fruits (orange/Fo, papaya/Fp, and raspberry/Fr) were used to isolate the fungus on Potato dextrose agar media by scratching.

Identification of Fungal Colonies

For identification of colonies, lactophenol cotton blue staining and various biochemical tests like carbohydrate fermentation test, carbohydrate assimilation test, casein hydrolysis test, urease test, catalase test, and gelatin hydrolysis test were performed.

Extraction of Mycotoxin from Fungal Species

The organic solvent used for mycotoxin extraction was acetone to aid the breaking of weak electrostatic bonds that bind some mycotoxin to other substrate molecules.

Purification of the Extract with the Help of Column Chromatography

The is done eluted with a series of solvents or solvent mixtures which are designed to first wash off interfering compounds and then elute the desired mycotoxins, while other interfering compounds remain strongly bound on the column

Identification of Mycotoxins by Thin Layer Chromatography

Mycotoxins were analyzed using thin-layer chromatography (TLC).For this purpose, culture filtrate wastaken. The sample were then mixed with aniline blue dye (10%) for colour reference and spotted onto the TLC strips. The toxins were identified by calculating the Rf values, as compared with the standard values. The Rf values were calculating using the following formula:

Analysis of the Effect of Mycotoxin on the Plants by Spray Methods

The effect of potent mycotoxin from different species and classes of fungi were and analyzed on selected plants such as:

1. Goosefoot (Syngoniumpodophyllum),
2. Pansy (Viola tricolor),
3. Ajooba (Bryophyllumpinatum).

The mycotoxins were isolated and purified and then sprayed on the plants leaves by different method. These spray methods provided prominent and impressive results.

Results and Discussion

Rotten fruit samples were collected for the production of mycotoxins from fungal species. The rotten fruit samples collected were orange, papaya, and raspberry.

Identification

Table1 : Biochemical test for Identification of fungal mycotoxins

S.No.	Biochemical test		Results		
			Orange fungus	**Papaya fungus**	**Raspberry fungus**
1	Carbohydrate fermentation	Glucose	Positive	Positive	Positive
		sucrose	Negative	Positive	Negative
		Mannitol	Positive	Positive	Positive
2	Carbohydrate assimilation		Positive	Positive	Positive
3	Casein hydrolysis		Positive	Negative	Positive
4	Urease		Positive	Positive	Positive
5	Catalase		Positive	Positive	Positive
6	Gelatin hydrolysis		Positive	Positive	Positive

Based on staining and biochemical test colonies were identified as *Aspergillus*spp, *Fusarium*spp. and *Trichothecium* spp.

Partial Purification of Mycotoxin by Column Chromatography

This type of chromatography based on the size of particles. Small size particles eluted later due to penetrating silica molecules, and large molecules eluted first and fastly.

Thin Layer Chromatography of Partially Purified Mycotoxin

Thin layer chromatography of partially purified mycotoxin was done by thin layer chromatography for the determination of the type of mycotoxin produced by the fungal species. The mycotoxin determination was done by Rf value.

The Rf value observed in table-2 showed the presence of Aflatoxin B1 and G1 as the Rf value for test mycotoxins were close to that for the standard G1 and B1 mycotoxins.The Rf value observed in above cases showed the presence of Trichothecin and Nivalenol as the Rfvalue for test mycotoxins were close to that for the standard Trichothecin and Nivalenol respectively.

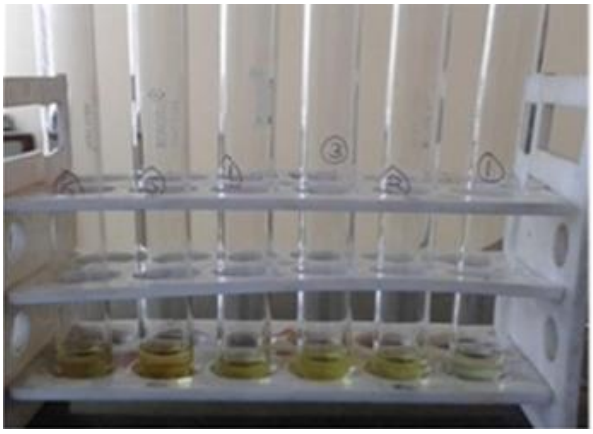

Fig-1. Purified fruit extract by using column chromatography

Table 2: Rf value of partially purified fungus sample

S. No	Fruit extract	Observed Rf value	Standard Rf value
1	Fo1	0.61	**0.62 (Aflatoxin G1)**
2	Fo2	0.62	
3	Fo3	0.70	
4	Fo4	0.76	**0.76 (Aflatoxin B1)**
5	Fo5	0.73	
1	Fp1	0.89	0.97 (Trichothecin)
2	Fp2	0.96	
3	Fp3	0.87	
4	Fp4	0.93	
5	Fp5	0.87	
1	Fr1	0.80	0.78 (Nivalenol)
2	Fr2	0.79	
3	Fr3	0.82	
4	Fr4	0.85	
5	Fr5	0.78	

Effects of Mycotoxins on Different Plants

The mycotoxin produced from the fungal species was sprayed on different parts of the selected plant like stem leaves and Root. Mycotoxin was sprayed onto the leaves of *Syngoniumpodophyllum* after 3-5 days plants became totally dead, necrosis occurred and leaf became black in color.Mycotoxin was sprayed in the root region of *Violartricolor* after 5 days of spray plant leaves became yellowish in color and wilting occurred. Mycotoxin was sprayed onto the leaves of *Bryophyllumpinatum* after 5 days leaf showed necrosis (patches). i.e., damage to the tissue.

Fig-3. Effect of mycotoxin on *Syngoniumpodophyllum*, *Violartricolor* and *Bryophyllumpinatum*

Conclusion

The present study focused on isolation of fungus and its mycotoxin extraction from fruits. The fruit samples used for the study were Orange, Papaya and Raspberry. The fungus was identified as *Fusarium*, *Aspergillus* and *Trichotheciuma* by culturing the fungus on Potato dextrose Agar and CzapexDox Agar media. The biochemical properties of the fungus

were also studied using various biochemical tests and Lactophenol cotton blue staining. Then mycotoxin was extracted from this fungus and its purification and identification was then done using Column Chromatography and Thin Layer Chromatography.

From the present investigations it can be concluded that variety of fungi harboring on fruits are potentially toxigenic and not only hazardous directly to man but also may be responsible for diseases of these fruits and in change of its flavor. Consumption of these toxins is a potential problem for humans Thus these fungi may be responsible for primary and secondary mycotoxicoses in man. Hence, more detailed investigations are desired to suggest measures to check the mould infestation of these fruits to prevent its spoilage.

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