

COMPARATIVE STUDY OF VARIOUS MYCOTOXINS AGAINST FEW BACTERIAL TEST ORGANISMS

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ABSTRACT

The present investigation is an attempt to isolate various fungal sources producing mycotoxins from air and soil. Mycotoxins produced by various fungal strains are aflatoxin, gliotoxin, Penicillin, Patulin, Terrecyclic acid, Deoxynivalenol, Malformin, Fumonisin and Trichorzin. The antibacterial potential of the mycotoxins was tested against various test organisms and it was found that the aflatoxin is effective against *Micrococcus luteus* (MTCC No. 1538) and *Pseudomonas* sp. (MTCC No. 7021), in case of Gliotoxin it was observed that it has a very good antibacterial property against *E. coli* (MTCC No. 40), penicillin was found equally good for *Micrococcus luteus* (MTCC No. 1538) *E. coli* (MTCC No. 40). Antimicrobial activity of Patulin was observed highest against *Micrococcus luteus* (MTCC No. 1538), *Proteus* sp. (MTCC No. 426) was found to be more sensitive to Terrecyclic acid, *Micrococcus* sp. (MTCC No. 7527) was found most sensitive against Deoxynivalenol generating zone of inhibition 32.66 mm in case of crude and 47.66 mm in case of purified extract and Malformin was found highly effective against *Proteus* sp. (MTCC No. 426). The Fumonisin and Trichorzin were found as a candidate mycotoxin against *Bacillus megaterium* (MTCC No. 2412), *Micrococcus* sp. (MTCC No. 7527), *Pseudomonas aeruginosa* (MTCC No. 424) respectively.

Keywords: *Mycotoxins; Zone of inhibition; Antibacterial activity.*

INTRODUCTION

Mycotoxins are biologically active secondary metabolites produced by some filamentous fungi or molds under suitable temperature and humidity conditions causing serious risks for human and animal health. More than 300 mycotoxins are known but more attention was given to those having proven to be carcinogenic and toxic. These include a metabolite of *Aspergillus flavus* and *Aspergillus parasiticus*, **Aflatoxin B₁**, the most potent hepatocarcinogenic substance known, which has been recently proved to be genotoxic¹.

Aspergillus fumigatus is known to produce various immunosuppressive mycotoxins including gliotoxin. **Gliotoxin** is an alkaloid with a low molecular size known to possess a number of immunosuppressive activities, such as inhibition of superoxide release, migration, microbicidal activity²⁻⁶, cytokine release by leukocytes⁷ and T-lymphocyte-mediated cytotoxicity⁸. It is genotoxic and also causes apoptosis in macrophages⁹. **Penicillin** is a beta- lactam antibiotic derived from *Penicillium* fungi. **Patulin** a relatively small molecule produced by a wide range of species of *Penicillium* and *Aspergillus*. It is commonly found in rotting apples and is genotoxic.

Terrecyclic acid is a new antibiotic isolated from a fungus *Aspergillus terreus* having a wide antimicrobial spectrum and anti tumor activity. **Trichothecenes** are present in crops, food, and animal feed contaminated with *Fusarium* species. Their mechanism of toxicity can be due to (a) inhibition of protein synthesis (specifically in eukaryotes)¹⁰ (b) inhibition of DNA synthesis¹¹ or (c) inhibition of the mitochondrial electron transport system¹². The most important trichothecenes are **deoxynivalenol**, T-2 toxin, and zearalenone. **Fumonisin B₂** the carcinogenic mycotoxin was detected in the industrially important *Aspergillus niger*. In animals fumonisin provoke cerebral edema and liquefaction necrosis, and even centrilobular necrosis (hypoxic hepatitis) and hepatic fibrosis, Liver diseases including tumors of the liver have also been associated with fumonisin intoxication¹³. **Malformin** is a metabolic product of *Aspergillus niger* and shows antibacterial activity towards *Bacillus subtilis*, *Xanthomonas stewartii* and against a variety of gram-positive and gram-negative organisms¹⁴. Trichorzins HA and MA isolated from two strains of the widespread soil fungus *Trichoderma harzianum* have been shown to exhibit antibiotic activity against phytopathogenic fungi¹⁵.

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Material and Methods

Isolation of Microorganisms

Aspergillus flavus, *Penicillium chrysogenum*, *Penicillium expansum*, *Aspergillus terreus*, *Fusarium* sp., *Aspergillus niger* and *Trichoderma* sp. have been isolated from soil sample by serial dilution method on potato dextrose agar (PDA) and from air sample. The fungal strains were identified by lactophenol cotton blue staining and sub cultured at regular intervals to maintain the pure culture.

Production of Mycotoxins by Submerged Fermentation

Nine different basal production media were taken as follows: Aflatoxin (Starch 5%, Corn steep 4%, pH 4.0 incubation at room temperature); Gliotoxin (Yeast extract 2%, Sucrose 4%, pH 5.6 incubation for 7 days at room temperature); Penicillin (Corn steep liquor dry basic 1.5%, lactose 2.5%, Calcium carbonate 0.2%, Sodium sulfate 0.05%, pH 5.8-6.0); Patulin (Glucose 2%, Yeast extract 0.5%, NaCl 1%, NaH_2PO_4 0.23%, $(\text{NH}_4)_2\text{SO}_4$ 0.5% pH 5-5.6); Terrecyclic acid (Glucose 30g/l, Soyabean meal 2.5 g/l, Yeast extract 0.5 g/l, KH_2PO_4 1 g/l, MgSO_4 1 g/l, NaCl 0.5 g/l, CaCl_2 0.5 g/l, FeCl_3 2 mg/l, ZnSO_4 2 mg/l pH 5.5 incubation at 30°C for 4 to 5 days); Deoxynivalenol (Sucrose 3%, Peptone 0.1%, Yeast extract 0.1%, pH 5.0-5.6 incubation for 7 days at room temperature); Malformin (Corn steep liquor 5g/l, Na_2HPO_4 0.12g/l, glucose 10g/l pH 5.5-5.8); Fumonisin (Yeast extract 2%, Sucrose 15%, MgSO_4 0.05%, ZnSO_4 0.001%, CuSO_4 0.0005%) and Trichorzin (Glucose 0.5%, KH_2PO_4 0.08%, KNO_3 0.07%, $\text{Ca}(\text{H}_2\text{PO}_4)_2$ 0.02%, MgSO_4 0.05%, MnSO_4 0.001%, pH 5.0-5.6). After incubation culture broth was filtered and the filtrate was used as crude mycotoxin.

Purification of crude Mycotoxins

Purification of mycotoxins was done by solvent extraction method and column chromatography technique. The crude aflatoxin was purified by silica gel column chromatography by using chloroform with 0.7% ethanol eluent. Gliotoxins was purified by silica gel column chromatography by using toluene and ethyl acetate (8:2) eluent. Penicillin purification was done by solvent extraction by mixing equal volume of penicillin and chloroform in separating funnel. Patulin was purified in solvent extraction method by mixing equal volume of patulin with equal volume of ethyl acetate and chloroform. Terrecyclic acid was purified in a separating funnel after mixing with butanol and ethyl acetate in 60:40 ratio. Deoxynivalenol was initially mixed with water: methanol in 60:40 ratio followed by methanol evaporation in boiling water bath and half volume of saturated NaCl solution addition. Malformin was purified by silica gel column chromatography using water and ethyl acetate in 1:1 ratio. Fumonisin was purified using acetonitrile and water in 87:13 ratio by silica gel column chromatography. Trichorzin was

purified by using ethyl acetate and hexane in 1:1 ratio as eluent in silica gel column chromatography.

Mycotoxins were subjected TLC and separated by using various solvents (Aflatoxin; Benzene: ethanol:water 4:27:20, Gliotoxin; ethanol:water 9:1, Penicillin; toluene:ethyl acetate:acetic acid 40:40:20, Patulin; toluene:ethyl acetate:formic acid 6:3:1, Terrecyclic acid; Benzene:Methanol 80:20, Deoxynivalenol; Chloroform: Methanol: water 90:10:2, Malformin; Benzene:ethyl acetate:formic acid 100:40:10, Fumonisin; acetonitrile:Methanol:water 1:1:2 and Trichorzin; Chloroform:Methanol 7:3).

RESULTS AND DISCUSSION

The present investigation reveals the action of various mycotoxins against bacterial test organisms. Many in-vitro studies have been made to screen the antibacterial activity and the industrial applications of various mycotoxins and their derivatives against bacteria¹⁶⁻²⁰. All the nine mycotoxins i.e. aflatoxin, gliotoxin, Penicillin, Patulin, Terrecyclic acid, Deoxynivalenol, Malformin, Fumonisin and Trichorzin were used for their antibacterial activity and the highest zone of inhibition was observed in case of aflatoxin against *Micrococcus luteus* and *Pseudomonas* sp. (Fig.1), in case of Gliotoxin it was observed that it has a very good antibacterial property against *E. coli* (Fig.2), penicillin was found equally good for *Micrococcus luteus* and *E. coli* (Fig. 3). The findings of aflatoxin, gliotoxin and penicillin are tabulated comparing the zone of inhibition in Table 1.

Table 1 : Antimicrobial activity of Aflatoxin, Gliotoxin, Penicillin

Aflatoxin

Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Escherichia coli</i>	24.33	27.00
<i>Pseudomonas</i> sp.	32.66	33.66
<i>Staphylococcus aureus</i>	29.66	31.66
<i>Bacillus subtilis</i>	24.00	26.66
<i>Micrococcus luteus</i>	33.00	33.66

Gliotoxin

Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Escherichia coli</i>	30.00	31.33
<i>Pseudomonas fluorescens</i>	25.33	26.33
<i>Proteus</i> sp.	27.00	29.00
<i>Staphylococcus aureus</i>	25.00	27.00
<i>Bacillus subtilis</i>	19.00	23.00

Penicillin

Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Escherichia coli</i>	27.66	53.00
<i>Proteus</i> sp.	25.00	28.33
<i>Staphylococcus aureus</i>	26.66	30.00
<i>Bacillus subtilis</i>	21.00	22.00
<i>Micrococcus luteus</i>	28.00	32.00

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Antimicrobial activity of Patulin was observed highest against *Micrococcus luteus* (Fig. 4), *Proteus* sp. was found to be more sensitive to Terrecyclic acid (Fig. 5), *Micrococcus* sp. was found most sensitive against Deoxynivalenol generating zone of inhibition 32.66 mm in case of crude and 47.66 mm in case of purified extract (Fig. 6) and Malformin was found highly effective against *Proteus* sp. (Fig. 7). The findings of patulin, Terrecyclic acid, deoxynivalenol, Malformin are tabulated comparing the zone of inhibition in crude and purified form of the extract (Table 2).



Fig. 1 : Inhibition Zones of Aflatoxin against Test Organisms



Fig. 2 : Inhibition Zones of Gliotoxin against Test Organisms



Fig. 3 : Inhibition Zones of Penicillin against Test Organisms



Fig. 4 : Inhibition Zones of Patulin against Test Organisms

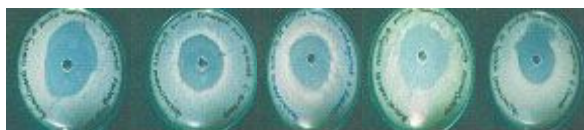


Fig. 5 : Inhibition Zones of Terrecyclic Acid against Test Organisms



Fig. 6 : Inhibition Zones of Don against Test Organisms

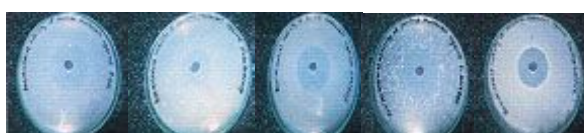


Fig. 7 : Inhibition Zones of Malformin against Test Organisms



Fig. 8 : Inhibition Zones of Fumonisin B2 against Test Organisms



Fig. 9 : Inhibition Zones of Trichorzin against Test Organisms

Table 2 : Antimicrobial activity of Patulin, Terrecyclic acid, Deoxynivalenol, Malformin

Patulin		
Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Escherichia coli</i>	24.33	27.66
<i>Pseudomonas fluorescens</i>	23.33	25.00
<i>Proteus</i> sp.	24.33	25.66
<i>Staphylococcus aureus</i>	24.33	27.33
<i>Micrococcus luteus</i>	25.00	28.00

Terrecyclic acid		
Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Proteus</i> sp.	36.33	46.66
<i>Staphylococcus aureus</i>	28.33	41.33
<i>Bacillus subtilis</i>	18.66	32.00
<i>Micrococcus</i> sp.	28.33	48.00
<i>Bacillus cereus</i>	22.66	28.66

Deoxynivalenol		
Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Escherichia coli</i>	25.00	29.00
<i>Pseudomonas fluorescens</i>	21.00	24.66
<i>Staphylococcus aureus</i>	25.33	28.00
<i>Bacillus subtilis</i>	20.33	30.00
<i>Micrococcus</i> sp.	32.66	47.66

Malformin		
Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Escherichia coli</i>	19.00	29.66
<i>Pseudomonas fluorescens</i>	19.33	21.66
<i>Proteus</i> sp.	32.00	33.66
<i>Staphylococcus aureus</i>	22.66	32.00
<i>Bacillus subtilis</i>	17.00	22.00

Fumonisin and Trichorzin were found to be a candidate mycotoxin against *Bacillus megaterium*, *Micrococcus* sp., *Pseudomonas aeruginosa* respectively (Fig. 8 & Fig. 9) (Table 3). It is concluded from the present study that there are many mycotoxins which can be used against various bacterial strains of live stock and human interest effectively and simultaneously the toxicity of these mycotoxins needs to be screened on various cell lines and animal models.

Table 3 : Antimicrobial activity of Fumonisin and Trichorzin

Fumonisin		
Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Escherichia coli</i>	23.00	29.00
<i>Pseudomonas fluorescens</i>	23.66	24.33
<i>Bacillus subtilis</i>	18.33	19.66
<i>Bacillus cereus</i>	21.00	27.33
<i>Bacillus megaterium</i>	31.33	34.00

Trichorzin		
Microorganisms used	Zone of inhibition in mm	
	Crude Extract	Purified extract
<i>Escherichia coli</i>	25.66	29.33
<i>Staphylococcus aureus</i>	23.33	36.33
<i>Bacillus subtilis</i>	18.00	25.00
<i>Micrococcus</i> sp.	33.00	37.00
<i>Pseudomonas aeruginosa</i>	32.33	50.33

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