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Research Article

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 to be candidate mycotoxins 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 the more attention was given to those have 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 genotoxic [1].

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 activity [2-6] cytokine release by leukoyctes[7] and T-lymphocyte-mediated cytotoxicity [8]. It is genotoxic and also causes apoptosis in macrophages[9]. 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 synthesis or (c) inhibition of the mitochondrial electron transport system.

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 intoxication. 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 organisms[11]. Trichorzins HA and MA isolated from two strains of the widespread soil fungus *Trichoderma harzianum* which have been shown to exhibit antibiotic activity against phytopathogenic fungi [12].

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 have been 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%, $(NH_4)_2SO_4$ 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%, Ca (H_2PO_4) 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 were 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 addition. Malformin was purified by silica gel column chromatography using water: 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 have been subjected on to TLC plates 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, Patuin; toluene: ethyl acetate: formic acid 6:3:1, Terrecyclic acid; Benzne: 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 bacteria[13-17]. Antimicrobial activity of certain plant extracts were also reported against *Bacillus subtilis Staphylococcus aureus Proteus species, E.coli* that were found sensitive to the mycotoxins[18,19].The total 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

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Escherchia coli	24.33	27.00
Pseudomonas sp.	32.66	33.66
Staphylococcus aureus	29.66	31.66
Bacillus subtilis	24 .00	26.66
Micrococcus luteus	33.00	33.66

Gliotoxin

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Escherchia coli	30.00	31.33
Pseudomonas fluorescence	25.33	26.33
Proteus sp.	27.00	29.00
Staphylococcus aureus	25.00	27.00
Bacillus subtilis	19.00	23.00

Penicillin

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Escherchia coli	27.66	53.00
Proteus sp.	25.00	28.33
Staphylococcus aureus	26.66	30.00
Bacillus subtilis	21.00	22.00
Micrococcus luteus	28.00	32.00

Antimicrobial activity of Patulin was observed highest against *Micrococcus luteus* (Fig.. 4), *Proteus s*p. 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).

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

Patulin

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Escherchia coli	24.33	27.66
Pseudomonas fluorescence	23.33	25.00
Proteus sp.	24.33	25.66
Staphylococcus aureus	24.33	27.33
Micrococcus luteus	25.00	28.00

Terrecyclic acid

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Proteus sp.	36.33	45.66
Staphylococcus aureus	28.33	41.33
Bacillus subtilis	18.66	32.00
Micrococcus sp.	28.33	48.00
Bacillus cereus	22.66	28.66

Deoxynivalenol

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Escherchia coli	25.00	29.00
Pseudomonas fluorescence	21.00	24.66
Staphylococcus aureus	25.33	28.00
Bacillus subtilis	20.33	30.00
Micrococcus sp.	32.66	47.66

Malformin

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Escherchia coli	19.00	29.66
Pseudomonas fluorescence	19.33	21.66
Proteus sp.	32.00	33.66
Staphylococcus aureus	22.66	32.00
Bacillus subtilis	17.00	22.00

The Fumonisin and Trichorzin were found to be candidate mycotoxins against *Bacillus megaterium*, *Micrococcus* sp., *Pseudomonas aeuroginosa* 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 need to be screened on various cell lines and animal models.

Table 3: Antimicrobial activity of Fumonisin, Trichorzin

Fumonisin

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Escherchia coli	23.00	29.00
Pseudomonas fluorescence	23.66	24.33
Bacillus subtilis	18.33	19.66
Bacillus cereus	21.00	27.33
Bacillus megaterium	31.33	34.00

Trichorzin

Test microorganism	Zone of inhibition (mm)	
	Crude Extract	Purified extract
Escherchia coli	25.66	29.33
Staphylococcus aureus	23.33	38.33
Bacillus subtilis	18.00	25.00
Micrococcus sp.	33.00	37.00
Pseudomonas aeruginosa	32.33	50.33

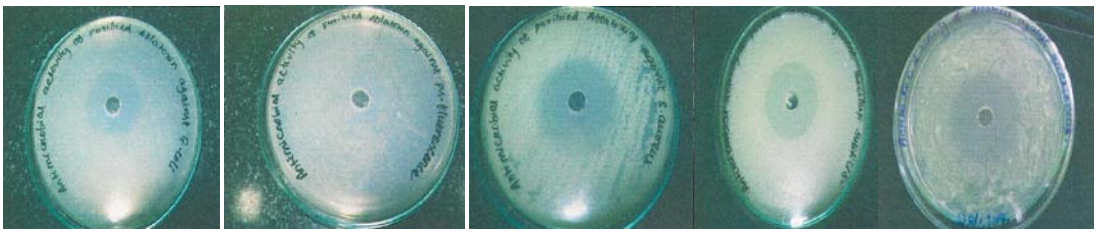

Fig. 1: Inhibition zones of aflatoxin against test organisms

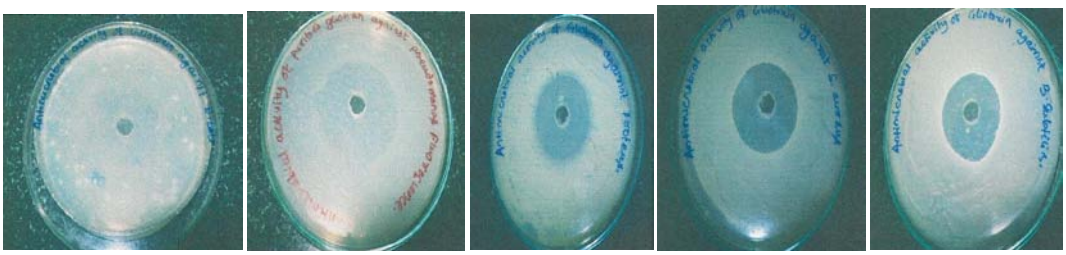

Fig. 2: Inhibition zones of gliotoxin against test organisms

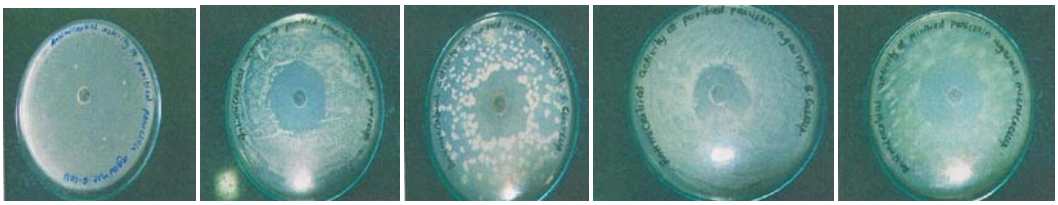

Fig. 3: Inhibition zones of penicillin against test organisms

Fig. 4: Inhibition zones of patulin against test organisms

Fig. 5: Inhibition zones of terrecyclic acid against test organisms

Fig. 6: Inhibition zones of deoxynivalenol against test organisms

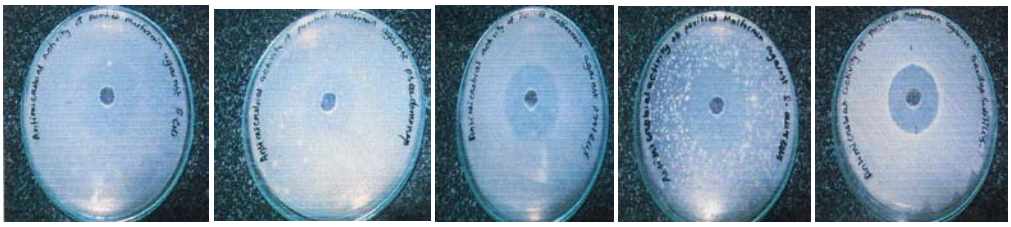

Fig. 7: Inhibition zones of malformin against test organisms

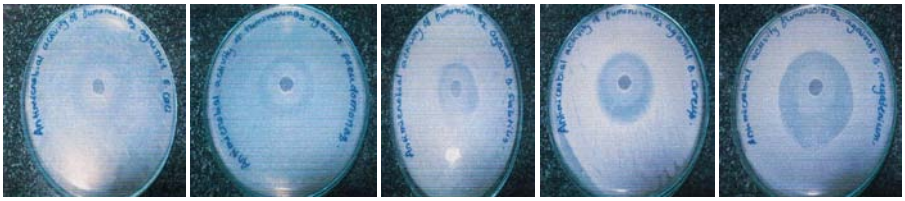

Fig. 8: Inhibition zones of fumonisin b2 against test organisms

Fig. 9: Inhibition zones of trichorzin against test organisms

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