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EXTRACTION OF MYCOTOXINS FROM FUNGI CONTAMINATING FOOD SAMPLES, ASSESSMENT OF THEIR ANTIBACTERIAL ACTIVITY AND EFFECT OF SPICES OIL ON MYCOTOXIN PRODUCING FUNGI

Priyanka Ashish Jariwala[1*] and [2]Hetal K. Panchal Ph.D

[1,2]Dolat Usha Institute of Applied Sciences and Dhiru Sarla Institute of Management and Commerce, Tithal Road, Valsad-396001.

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***Corresponding Author**
Priyanka Ashish Jariwala
Dolat Usha Institute of Applied Sciences and Dhiru Sarla Institute of Management & Commerce, Tithal Road, Valsad-396001.

ABSTRACT

The contamination of food materials by fungi and their mycotoxins are of major importance to humans and animals health. Therefore, our study was focused to investigate the contamination of various food materials with mycotoxin producing fungi. 21 isolates were obtained from 10 different food samples. From morphological and microscopic observations, 15 fungi were suspected to be *Aspergillus spp*., 3 *Fusarium spp*., 1 *Cunninghamella* and 2 *Penicillium spp*. They were further tested for their ability to produce mycotoxins on Coconut agar. Fluorescence was observed under UV light after 7-10 days of incubation. Out of 21 isolates, 10 isolates showed fluorescence on Coconut agar medium. Later, the mycotoxin was extracted by using Yeast Extract Sucrose broth. The extracted mycotoxin was further tested for its antibacterial activity against 3 different test organisms out of which isolate F1 gave maximum inhibition zone against *Escherichia coli*, F14 against *Bacillus cereus*. Also, the inhibition of mycotoxin producing fungi was studied by using different spices oils. Clove oil showed highest antifungal activity against isolate F14 and no antifungal activity was given by sesame oil.

KEYWORDS: Mycotoxins, Fluorescence, Coconut agar, Antibacterial activity, Antifungal activity.

INTRODUCTION

Fungi are eukaryotic micro-organisms that absorbs it nutrients directly from cell wall which is made up of chitin. Fungi are capable of producing some secondary metabolites like antibiotics, pigments and toxins. The toxins produced by fungi are known as "MYCOTOXINS". Mycotoxins are low molecular weight of fungal secondary metabolites, produced mainly by species of *Aspergillus*, *Penicillium* and *Fusarium*.[3] There are many different type of mycotoxins produced by various fungi like Aflatoxin B1, B2, G1, G2, OchratoxinA, B, C, Citrinin, Patulin, Fumonisin. Mycotoxins are capable to cause serious health issues in humans as well as in animals and sometimes even they can cause death. The detection of mycotoxins can be done by antibody based assays and chromatographic techniques. Mycotoxins are not broken down in digestion so they persist in the food chain. Some of the mycotoxins are not destroyed by the temperature treatment.

Now-a-days, due to the development of multi resistant drug organisms, world-wide deaths are taking place. Many pathogenic bacteria are becoming resistant to drugs and hence an alternative strategy is very much needed. Mycotoxins- a secondary metabolite of fungi has inhibitory activity on some selected pathogenic bacteria. The best period of incubation to inhibit the growth of toxin forming fungi is about 48 hours and the best temperature is from 25 to 30˚C. Also the growth of mycotoxin forming fungi are inhibited by the essential oils. Many essential oils such as clove oil have antioxidant activity and anti-fungal properties.[7] If there is any possible way by which mycotoxin forming fungi enters the human body, then by consuming spices in food, the growth of mycotoxin producing fungi would be inhibited. Therefore this study apart from isolation of mycotoxin producing fungi; was also focused on assessment of antimicrobial activity of mycotoxin on test pathogens as well as effect of spices oil on growth of mycotoxin producing fungi.

MATERIALS AND METHODS

Sample Collection

Different food samples like vegetables, cereals, stored food like papdi, masur dal, capsicum, pickles, coriander were collected from local vegetable market of Valsad district. The samples were collected in clean plastic bags and were processed to isolate fungi.

Also several grains including rice, maize, sorghum, wheat and feed samples were taken in bowel, moisture content was maintained in it by sprinkling water and kept open until there was visible fungal growth found on surface. Later they were processed to isolate fungi.

Isolation of Fungi

The samples were inoculated onto sterile Sabouraud`s agar plate (Peptone - 10gm, Glucose - 40gm, NaCl - 5gm, Agar - 24gm, D/W - 1000ml, pH - 5.2). The plates were incubated at room temperature for 7 days. After incubation, the plates were observed for fungal colonies and different fungi were purified. Fungal mounting was performed for morphological identification

Mycotoxin Identification

The isolated fungi was inoculated on sterile Coconut agar plate (20% desiccated coconut, 1.5% agar pH – 6.1 to 6.7).[3] The plates were incubated at room temperature for 7 days. After incubation period, the plates were exposed under UV light to check fluorescence. Fluorescence observed was due to the production of mycotoxins.

Extraction of Mycotoxin

Spore suspension of each mycotoxin positive fungus was prepared in sterile Tween 80 solution. 1 ml spore suspension was inoculated in 50 ml Yeast Extract Sucrose broth (YES: 2% yeast extract and 20% sucrose).[7] The broth was incubated at room temperature for 7-8 days. After incubation, equal amount of chloroform was added to the broth. The mixture was then kept on the rotatory shaker for 24 hours. The mixture was separated in separator funnel. The upper layer contains spores and mycelia and the lower layer contains chloroform and mycotoxin. The chloroform was then evaporated in water bath at 50˚C.

Characterization of Mycotoxins by Fourier transform infrared spectroscopy (FTIR)

To understand the overall chemical nature of the extracted mycotoxins, Fourier Transform Infrared Spectroscopy (FTIR) was used. It was done at Center of Excellence, Vapi. Infrared absorption spectra were recorded on a Thermo Niocolet, AVATAR 330 FTIR system with a spectral resolution and wave number accuracy of 4 and 0.01cm -1, respectively.

Antibacterial activity of mycotoxin against test organisms

24 hours old culture of test organisms like *Escherichia coli*, *Bacillus cereus* and *Candida albicans* was streaked on sterile nutrient agar plate. 3 wells were made in each plate at equidistant from each other to perform this experiment in triplicate. 50 µl of extracted mycotoxins were poured into each well. The plates were incubated at 37ºC for 24 hours. After incubation, the plates were observed for clear zones surrounding the wells. The zones were measured with the zono-meter scale.

Antifungal activity of spices oils against mycotoxin producing fungi

Wells were made at the center of the plate. The isolated fungi was streaked on sterile Sabouraud`s agar plate. 100 µl of spices oil was poured into each wells. The plates were incubated at room temperature for 6-7 days. After the incubation period, the plates were observed for zone of inhibition surrounding the wells. The zone diameter was measured using zono-meter.

RESULTS

Result on Isolation of fungi

21 presumptive fungal isolates has been isolated and purified from 10 different food samples analysed as shown in figure 1. On the basis of morphological characterisation 15 fungi were suspected to be *Aspergillus spp*., 3 *Fusarium spp*., 2 *Penicillium spp*. and 1 *Cunninghamela spp* as shown in figure 2.

Figure 1: shows Sabouraud`s agar plate with fungal growth.

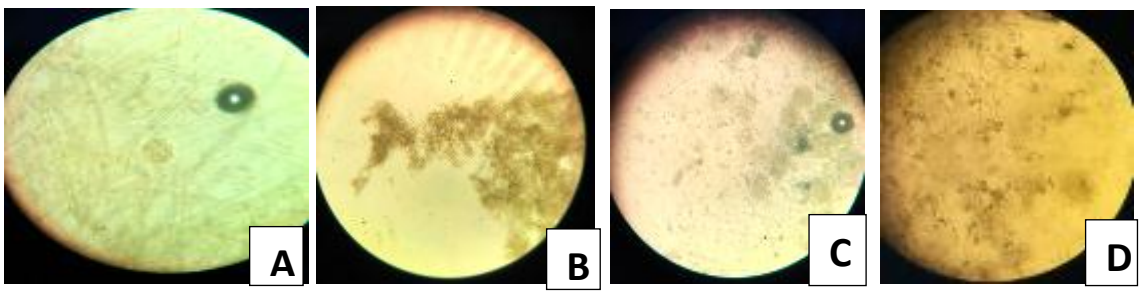

Figure 2: shows morphological identification of isolated fungi (A) *Cunninghamela spp.* (B) *Fusarium spp*. (C) *Penicillium spp*. (D) *Aspergillus spp.*

Screening of mycotoxin production

After 7 days of incubation at room temperature on coconut agar medium, the plates were observed for fluorescence under UV light. The fluorescence indicates that the isolates have the mycotoxin producing capability. Out of 21 isolates, 10 isolates had the capacity to produce mycotoxins and thus selected for further studies. (figure 3).

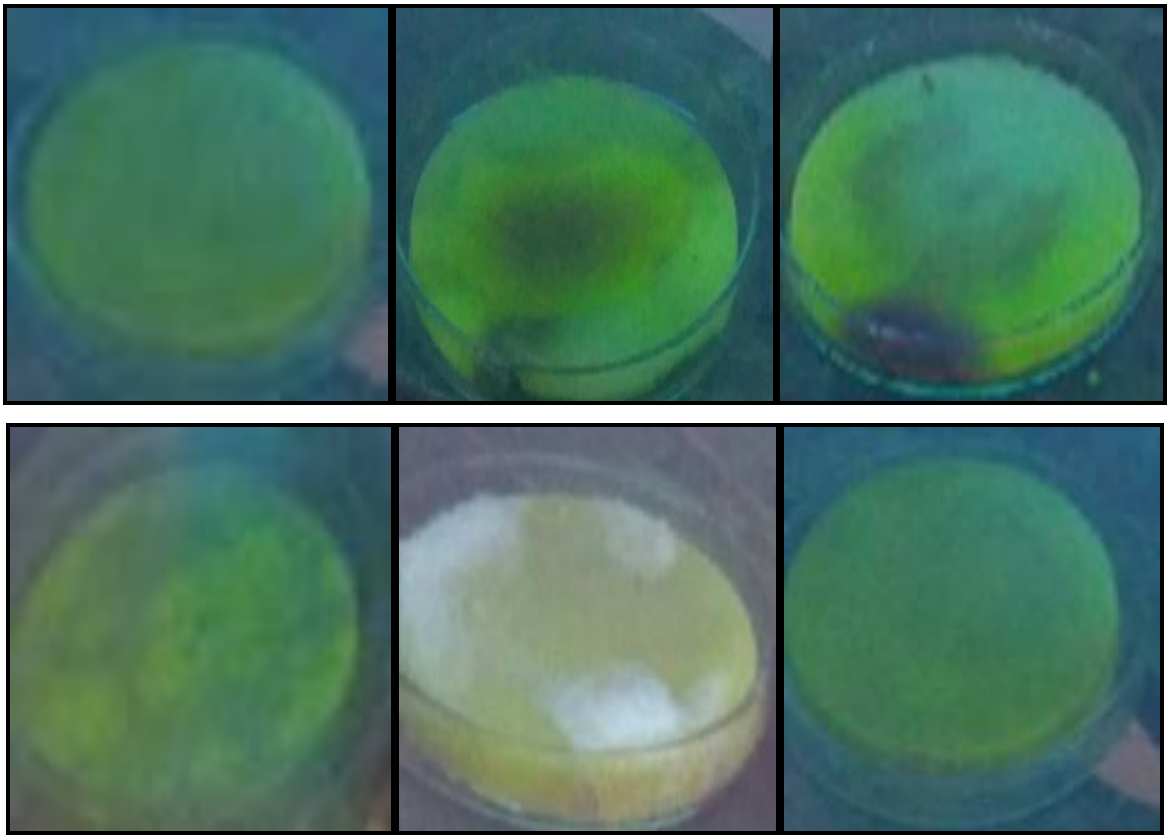

Figure 3: shows mycotoxin production showing fluorescence on coconut agar.

Antibacterial activity of mycotoxins

Out of 10 extracts of mycotoxins, isolate F1 showed maximum antibacterial effect against *Escherichia coli*, isolate F14 showed maximum inhibition against *Bacillus cereus* and no zone of inhibition was observed against yeast *Candida albicans* (Figure 4). The zone diameter against *Escherichia coli*, *Bacillus cereus* and *Candida albicans* were 30mm ± 0.47, 14mm ± 0.47 and no zone respectively (Table 1, Table 2).

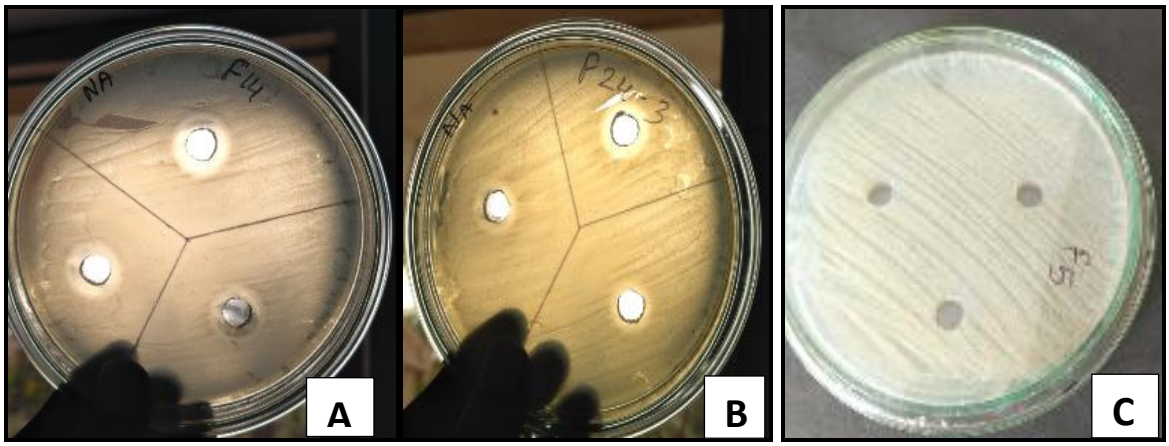

Figure 4: showing antibacterial activity of mycotoxins against (A) *Escherichia coli* (B) *Bacillus cereus* (C) *Candida albicans.*

Table 1: Antibacterial activity of mycotoxin against *Escherichia coli.*

Sample no.	Average zone diameter in mm ± SD
F1	30 ± 0.47
F5	30 ± 1.24
F9	27 ± 0
F11	16 ± 0.47
F14	12 ± 0.47
F15	10 ± 0.47
F21	9 ± 0.47
F23	-
F24-2	10 ± 0.47
F24-3	11 ± 0.47

Table 2: Antibacterial activity of mycotoxin against *Bacillus cereus*.

Sample no.	Average zone diameter in mm ± SD
F1	13 ± 0.81
F5	-
F9	13 ± 0.47
F11	14 ± 0.47
F14	14 ± 0.47
F15	14 ± 0.47
F21	-
F23	-
F24-2	11 ± 0.47
F24-3	11 ± 0.81

Sensitivity of fungal isolates to spices oils

In this study it was found that the fungal isolates were highly sensitive against clove oil rather than sesame oil and curd. Isolate F14 was highly sensitive to clove oil and the zone of inhibition was observed to be 36 mm. While sesame oil showed no antifungal activity against the mycotoxin producing fungal isolates.

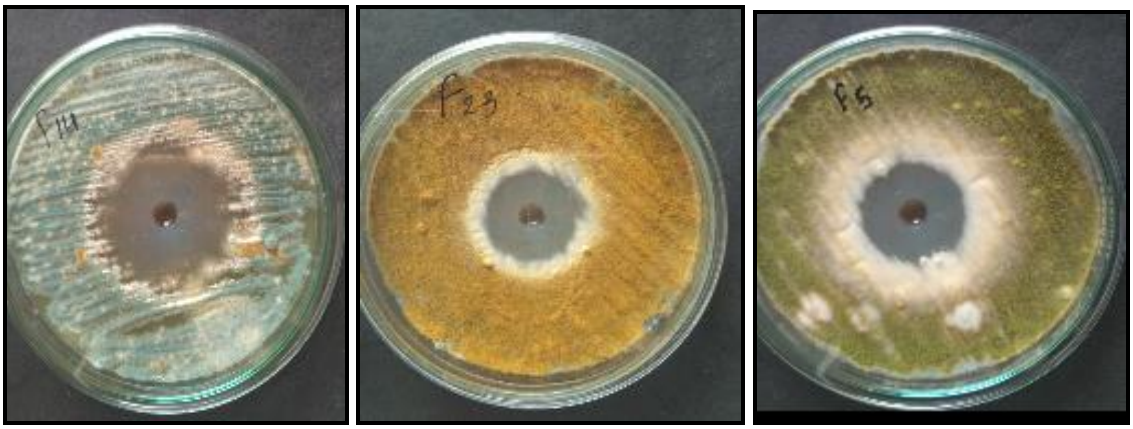

Figure 5: Antifungal activity of clove oil.

Table 3: Antifungal activity of clove oil against mycotoxin producing fungi.

Sample no.	Clove oil (Diameter of zone in mm)
F1	21
F5	27
F9	32
F11	30
F14	36
F15	27
F21	21
F23	26
F24-2	27
F24-3	25

FTIR Analysis

Fourier transform infrared spectroscopy is a technique employed for the identification of the functional groups present in the sample. FTIR spectra of 10 different mycotoxins produced by 10 fungal isolates was performed. Absorption bands corresponding to functional groups were observed. Characterization absorption peak were found between range of 1634 cm^{-1} to 1640 cm^{1}. This confirms the presence of C=C stretching characterized to alkene group. Also other characterization absorption peak were found between range of 2370 cm^{-1} to 2372 cm^{-1}. This confirms the presence of O=C=O stretching characterised to ketone group. The information from the respective wave number confirmed that the sample were of mycotoxins.

DISCUSSION

The contamination of food, feed and other products by fungi varies according to the geographical area, moisture, temperature and hygienic conditions.[7] Here, we report the isolation of many different types of fungi, out of which *Aspergillus spp*. was most common.

In this study, from 10 different samples, 21 different fungal isolates were obtained. Agustina del Palacio *et.al*., in the year 2016 isolated fungi from wheat sample. In his study, the main

fungi isolated were from genera *Fusarium* (43%), *Aspergillus* (36%), *Alternaria* (33%), *Sporothrix* (24%), *Eppicoccum* (12%) and *Penicillium* (6%).[3]

In this study for the identification of mycotoxin producing fungi, Coconut agar was used. 10 isolates gave yellow green fluorescence on coconut agar under UV light. Fakruddin *et.al* in the year 2015 performed the similar study and obtained lime green color fluorescence on PDA medium.[4] Sumanee Kuntawee and Angsana Akarapisan in the year 2015 performed the similar study which resulted in the production of blue and yellow green fluorescence on coconut agar.[5]

Fungi and their mycotoxins are known to exhibit antibacterial activity. The emergence of drug-resistant organisms has increased, so the use of mycotoxins can be done as it has antibacterial activity against some organisms. Therefore, this studies shows the effect of mycotoxin on different test organisms like *Escherichia coli, Bacillus cereus* and *Candida albicans.* The extracted mycotoxin was effective against *Escherichia coli* and *Bacillus cereus* while it was not active against *Candida albicans*. Satpal singh bisht *et.al* in the year 2011 performed experiment on similar study. They used Aflatoxin, Gliotoxin and Penicillin against different test organisms like *Escherichia coli, Pseudomonas spp., Staphylococcus aureus, Bacillus subtilis and Micrococcus luteus*. The variation in the size of inhibitory zone in well diffusion tests can be attributed to the differences in diffusion of oil in contaminated medium with fungi under laboratory conditions and may be due to the resistance of some isolates.[7]

As mycotoxins are toxic and carcinogenic to humans as well as animals, if mycotoxins producing fungi enters the body of humans or animals, then the growth of the fungi can be inhibited by the consumption of spices oil as it has antifungal property. Our study focuses on the use of different spices oil like clove oil and sesame oil. Out of all the two used, clove oil was only effective against the mycotoxin producing fungi. Clove oil showed maximum inhibition against isolate F14. Sesame oil showed no zone of inhibition against any of the isolate.

Mohamed *et.al* in the year 2017 isolated *Aspergillus flavus* from fish and fish feed and used clove oil in different concentration for the inhibition of growth of this fungi. He got maximum growth of inhibition in 4% concentration of clove oil.[7] Adam Perczak *et.al* in the year 2018 used lactic acid bacteria to inhibit the growth of mycotoxin producing fungi.[8]

CONCLUSION

Our study shows the detection of fungi that has the capability to produce mycotoxins in different food samples, the extraction of the mycotoxin, its antibacterial activity and the effect of spices oil and curd microflora on mycotoxins producing fungi. The emergence of drug-resistant organisms has increased, so the use of mycotoxins can be done as it has antibacterial activity against some organisms. As mycotoxins are toxic and carcinogenic to humans as well as animals, if mycotoxins producing fungi enters the body of humans or animals, then the growth of the fungi can be inhibited by the consumption of spices oil and curd as it has antifungal property.

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