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Quality analysis and prevention of aflatoxin contamination in *Capsicum annum* by selective spices extract

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ABSTRACT

In the present study the anti aflatoxin activity of common spices were analysed. Chilli is the major spice contributing 40-42% by volume and 20-22% by value of total spices exported from India. In curry, chilli is used as a paste, powder, broken split or whole form. Aflatoxin belongs to a group of fungal toxins usually known as mycotoxins, and is prevalent in agricultural products and foodstuff. Aflatoxin is connected with both acute and chronic toxicity in animals and including acute liver injury, liver cirrhosis and cancers. Aflatoxins, produced by A. flavus, A. parasiticus, A. nomius, A. tamari, A. bombycis and A. pseudotamarii are both acutely and chronically toxic to both humans and animals. The Aflatoxin producing fungi Aspergillus flavus was isolated from the chillies and chilli powders. The isolates were identified and conformed by biochemical and selective agar plating methods. The effects of extracts of spices like pepper, garlic, onion and ginger on aflatoxin arrest were analyzed by Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) Methods. The pepper showed the maximum anti-aflatoxigenic activity of Aspergillus flavus.

INTRODUCTION

Red pepper (*Capsicum annum L.*) is harvested in huge level worldwide as the oldest, most essential and it was extensively used for flavor and as colorant [1,2]. It is used further in shampoos, gravies, cheese, sauces, salads condiments, baked goods, direct compression tablets, cereals, lipsticks *etc.*[3]. The red pepper's red color is because of the existence of carotenoids, chiefly capsanthin, zeaxanthin, capsorubin, and cryptoxanthin [4] and the yellow color due to cucurbitaxanthin A, cis-zeaxanthin, violalaxanthin, criptoxanthin, carotene and cis-carotene[5].

The orange red and yellow-pigmented fruit in Capsicum sp. is the effect of increased level of carotenoids in the pericarp [6]. These pigments have valuable dietary importance as precursors for vitamin A and as antioxidants [7,8,9]. The red pigments in particular are the chief sources of non-toxic red dyes [10] included to many processed foods and cosmetics to increase their outward show [11]. The xanthophylls extracted from Capsicum fruit have become a vital cost-effective source of red pigments.

Chilli besides imparting pungency and red colour to the dishes, is also a good source of vitamins (175 mg/100 g), vitamin A (870 IU/100 g) and vitamin B (0.59 mg/10 g). Apart from these, small quantity of protein, fats, carbohydrates and traces of minerals are also present. The active principle of pungency which was earlier believed

to be a crystalline volatile alkaloid called capsaicin is now found to be a mixture of 20 allied components. The capsaicin is used in pain cosmetics, balms and medicines connected to heart diseases.

A. flavus infects many foods, particularly groundnut and produce a group of compounds called aflatoxins. These are exceedingly toxic and carcinogenic to avian and mammalian species [12]. *A. flavus* is a specific infecting agent during and after harvest. The infection occurs on store fruits and the contamination with aflatoxin deteriorates the quality and make the product unfit for consumption, thereby hitting the export trade in the international market. Aflatoxin contamination has posed serious problems in commerce and international trade of chillies because of stringent quality standards imposed on aflatoxin contamination by many importing countries [13].

Aflatoxin can be detoxified by physical, chemical or biological methods [14]. However, limitations such as nutrient loss, low organoleptic qualities, new pollution, expensive equipment and small-scale treatments caused by physical and chemical methods have encouraged recent emphasis on biological treatments [15]. Some srtains of microbes like lactic acid bacteria, *A.parasiticus, Trichoderma viride, A.niger, Mucor ambiguus* and other fungi have been abserved for different AFB_1 degradation abilities [16,17,18]. If one strain of the microbes can inhibit the growth of *Aspergillus* species, it will reduce aflatoxin production [19].

Consequently, minimizing aflatoxin production using suitable techniques is probably the most excellent policy to work against the effects of aflatoxins [20, 21]. But there is a limited work on the detoxification of Aflatoxin. So the present study mainly focus the anti-aflatoxin activity of selected spices like pepper, onion, ginger and garlic extract.

EXPERIMENTAL SECTION

Isolation of Aflatoxin producing fungi

A total of 20 Chilli powder and Chilli samples of different varieties were randomly collected during February - May 2013. To minimize the loss of water content, the samples were collected in a sterile polyethylene bags and sealed, transferred without delay to the laboratory, kept at 4ºC until analysis. One gram of each Chilli powder and Chilli sample was mixed in 9 ml of sterilized distilled water aseptically and shaken robustly. Suitable tenfold serial dilution was made. 0.1 ml of the dilution was transferred to sterilized Petri plates aseptically, which contains growth media for the isolation of aflatoxin producing *A. flavus*. Freshly prepared potato dextrose agar (PDA) (200g peeled potatoes, 20g agar and 15g agar in 1000ml of distilled water) and czapek dox agar medium (2g $NaNO_2$, 1g K_2HPO_4, 0.5g $MgCl_2$, 0.5g KCl, 0.01g $FeSO_4$, 30g sucrose and 15g agar in 1000ml distilled water) were used. Each sample was incubated for 7 days at 25 ± 2°C in triplicates. Then they were examined visually as well as under a compound light microscope daily for preliminary identification of fungal genera. On suitable agar plates, the identified genera were sub-cultured for species detection. Identification of fungal species was done on the basis of cultural and morphological characteristics. Macroscopic features like colony colour, texture and margins, as well as microscopic such as size of conidia and conidiophores and their arrangements were examined for species differentiation.

Extraction and identification of aflatoxin from

A. flavus isolated from chilli powder and chilli samples were grown on sterilized PDA at 25 ± 2°C for 5 days. Triplicates were maintained for every isolate. Aflatoxin was isolated by blending the moldy agar (20g) in blender for 5 min with methanol (100ml) containing 0.5% potassium chloride. The mixture was cleaned through Whatman No.1 filter paper. The clarified filtrate was concentrated and recognized on thin layer silica gel chromatography (TLC) for presence of aflatoxin. Two replicates were examined by point outing crude extract of aflatoxins. The used TLC plates were layered with silica gel 60 F254 on aluminum sheet, 20 x 20 cm and kept in chloroform/methanol (97:3) mixture [22]. These were then examined at 365 nm under UV light and the R_f values were noted on the same plate.

Anti aflatoxigenic activities of selected spices extract

Preparation of spices extract

Fifty gram of fresh onion, ginger, garlic and pepper were mixed and it was minced using a domestic blender for 1 min at average speed with 300 ml distilled water. The mixture was macerated at 4ºC for 24 hrs. Then extracts were filtered and uncontaminated using a cellulose acetate membrane filter (47mm) of 0.45µm pore size. For the antifungal analysis, the extracts were used directly after dilution. The distilled water used as control.

Preparation of inoculum

A. flavus was refined on Potato Dextrose Broth (PDB) (Hi media, India) for 22 hours at 30ºC. Test fungi in PDB were enumerated by serial dilution method. $10^4 - 10^5$ cfu/m was the final cell concentration of culture.

Antifungal activity test

Requisite amount of different spices like onion, pepper, garlic were mixed with 20ml PDA medium to get final concentration of 0.1, 1, 5mg/ml. Antifungal screening was done by poison food technique. Two types of control were used for experiment, 20ml of PDA served as a negative control whereas 20ml of PDA containing 100 mg of bavistin so as to get a concentration 100 mg/ml were used as positive control. The 7day old culture of each test fungus was used as inoculum and plates were incubated for 7 days at 30 ± 1ºC. Diameter of fungal growth on each petri dish was measured daily. Three replicates of the experiment were maintained. Mycelial growth inhibition was calculated by following:

$$\text{Percentage of mycelial growth inhibition} = \frac{C-T}{C} \times 100$$

Where, C - average increase in mycelial growth in control; T - average in mycelial growth in treatment.

Determination of Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC)

5 ml of sterilized extracts at various concentrations were taken into a sterile tube. To the extracts 1 ml of *A. flavus* culture was added and mixed. Then this mixture was added into the 5 ml of sterile PDB. Then the tubes were incubated for 15 days at 30ºC. The remarks were completed for visible growth of fungi. The maximum dilution (lowest concentration) showing no noticeable growth was considered as MIC during 15 days. Cells from the tubes showing no growth were sub cultured on PDA plates and kept for 5 days at 30ºC to find out the inhibition was reversible or not. MFC was determined as the maximum dilution (lowest concentration) at which no growth noticed on the plates. In control tubes 1 ml extract and *A. flavus* culture were added into the 5 ml of solvent (ethyl alcohol or acetone or water) separately. All the tests were done in triplicates.

Inhibition of *A. flavus* growth by selected spices on YES medium

For the inhibitory growth of *A. flavus*, 50ml of yeast extract sucrose (YES) medium was placed in a 250ml flasks and then autoclaved for 15 min at 120°C. Inoculation was done by adding 1ml of a suspension of spores of a toxigenic *A. flavus* strains without (control) and with the Pepper, Ginger, Onion and Garlic extracts were added at various concentrations (0.1%, 1%, 5%). The extract was added in the flasks were incubated in the dark for 7days at 25°C. After the incubation period, the growth of *A. flavus* in all flasks was visually examined. Flasks having mycelia were cleaned through Whatman filter no.1 and washed with distilled water. The mycelia were placed on pre weighed petri plates and were permitted to dry for 6 hrs at 50 °C and then at 40°C over night. The net dry weight of mycelia was then strong-minded. The percent mycelial inhibition was evaluated using the following:

$$\text{Percent mycelial inhibition} = \frac{C-T}{C} \times 100$$

Where, C is the dry weight of the control (g), and (T) is the dry weight of the treatment with the tested extract.

Mycelia weight assay

The most effective extracts including Pepper, Onion, Garlic and Ginger were used to determine MIC, MFC and their anti-aflatoxigenic efficiency using yeast extract sucrose broth (YES) medium supplemented with 0.8% sodium chloride [23]. Various concentrations of each spices extract (5, 10, 15, 20 and 25mg/ml) were taken separately and dissolved required amount in 10ml of YES broth.Sterilized through millipore filter and mixed with 40ml of sterile YES broth in 150ml Erlenmeyer flasks. Three replicates were performed for each concentration and the control set was maintained similar to the treatment without plant extract. The flasks were inoculated with discs of the toxigenic *A. flavus (*6mm diameter) and incubated at 25 ± 2°C for 7days. After incubation, substance of each flask was cleaned (Whatman No. 1) and biomass of cleaned mycelium was dried out for 4days at 70°C till their weights leftovers constant. For treatments and control mycelial dry weights was determined then MIC and MFC were calculated for each sample extract.

Analysis of aflatoxin by Vicam Aflatest assay

The YES broth culture (25 ml) was taken and placed in blender jar. Five gram of sodium chloride was added with 100ml methanol:water (80:20) into the jar and covered blender jar and mixed at elevated speed for one minute. 50ml of this blended sample was poured into a fluted filter paper and collected the filtrate in a dirt free container. In a clean cup, 10ml of filtrate was pipetted out and diluted the extract with 40ml of distilled water. The contents were mixed well and filtered the extract through glass microfibre filter (Vicam 31956). Collected pure filtrate in a clean cup and arranged Aflatest column for affinity chromatography. 10ml filtered pure extract was pipetted through the column and pushed the extract through the column slowly. Repeatedly wash the column with 25% ethanol for 4 times. Eluted the aflatoxins from the Aflatest column with HPLC Grade Methanol and elute was collected in a glass cuvette. 1 ml of diluted Aflatest developer (Make up fresh solution daily. 5ml of developer was mixed with 45ml distilled water) was added directly to elute in the cuvette. Mixed well and placed the cuvette in a calibrated Flurometer and recorded the digital readout after 60 seconds. Readout will be in parts per billion total aflatoxins for the sample extracted.

RESULTS AND DISCUSSION

In the present study, a toxigenic strain of *A. flavus* isolated from chilli was chosen because of its toxigenic productivity and its strong affinity to colonize Chilli and various food commodities render them unfit for human consumption. The isolated *A. flavus* was stored in PDA plates at 4°C for further identification and analysis.

Macroscopic and microscopic identification

The identification of different *Aspergillus* species isolated from Chilli powder and Chilli samples were tested for morphological and cultural characteristics. The results showed that there was variation in the colony colour, margins, texture and colony reverse colour. After the incubation period of the PDA agar plates, the morphological characteristics of isolates were further conformed by macroscopic observation.The isolated *Aspergilus* sp. were examined for their microscopic characters and are presented in Table 1.

Chillies were commonly infected with *A. flavus* in storeroom conditions. From the dried out fruits, chilli powder and red chillies were prepared and they could be clearly infected with aflatoxin B_1 [24]. The mold occurrence on chillies kept in cold storage for few years and the degree of aflatoxin B_1 in those samples are observed in the present study. Since toxigenic *A. flavus* is related with chillies kept in cold storage, attempts should be done to stop the mold attack and elaboration of mycotoxins by the molds. The regular supervision of these mold occurrence and aflatoxin B_1 level are very essential to increase the worth of the red pepper kept in the cold condition.

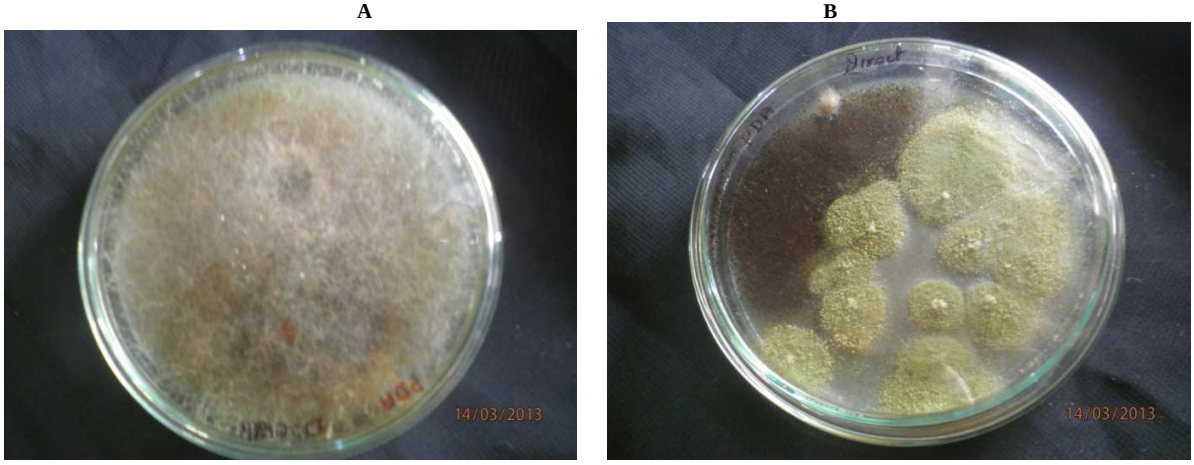
Table 1. Morphological and Microscopic characteristics of *A.flavus*

Characteristics	Observation
Surface colour	Yellow and greyish green
Margins	Entire
Reverse side	Colourless to yellow
Elevations	Umbonate
Growth	Moderate to rapid
Hyphae	Branched septate
Conidiospore	
a)Length	600 to 800µm
b)Diameter	15 to 20µm
c)Vesicle	Globose to subglobose
Conidia	20 to 45µm
a)Heads	Yellow/grayish green
b)Diameter	2 to 6µm
c)Ornamentation	Almost smooth
Phialides	Two series (Biseriate) covering nearly entire vesicle
	7 to 10µm
a)Primary	7 to 10µm
b)Secondary	

Plates showed yellow and greyish green coloured surface colonies, entire margins and pale yellowish coloured reverse side was observed (Figure 1-A,B). The rapid and moderate growth was observed. The hyphae of *A. flavus* were branched and septate. The conidia were yellow in colour.

Figure 1: Morphological characterization of *A. flavus* on Potato dextrose agar

A **B**

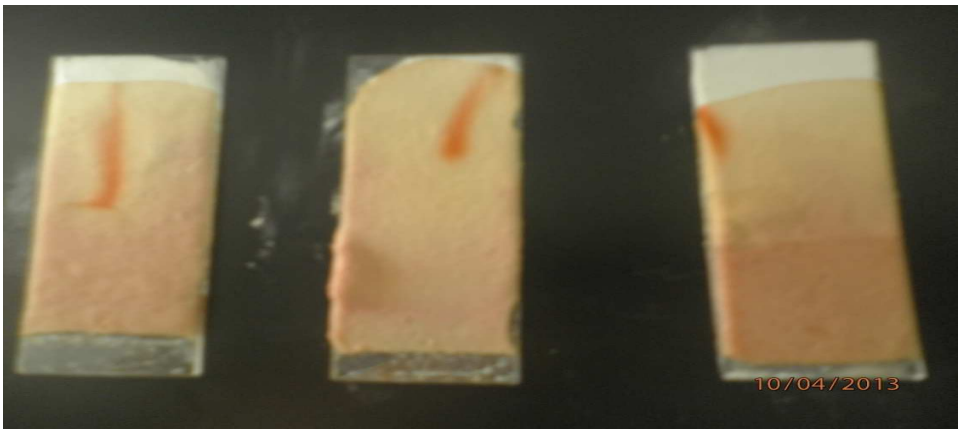


A. The plate showed branched septate hyphae of *A. flavus*
B. The plate showed grayish green surface colour and grayish conidia of *A. flavus*

Screening of Aflatoxin production by *A. flavus* in TLC Plate method

Out of 20 strains of *A. spices*, 14 strains were identified as *A. flavus.* In preliminary screening of aflatoxin production analysis by TLC method more than 12 isolates were positive in aflatoxin production. It produced the band at R_f ranging between 0.47 and 0.49 (Figure 2). It confirmed the creation of aflatoxin by the strains of *A. flavus.* Finally the quantity (PPB) of aflatoxin production was confirmed by Vicam aflatoxin analyzer.

Figure 2: Aflatoxin production by *A. flavus* isolates on TLC Plate



Determination of MIC and MFC of spices by PDA plate methods

The MIC and MFC (mg/ml) concentrations of selected spices Pepper, Garlic, Onion and Ginger extracts were tested and the mycelial inhibitory zones (mean ± SD) of *A. flavus* were analyzed. It is seen that Pepper and Garlic has antifungal effects on *A. flavus*. The inhibitory activities were observed in the order of Pepper > Garlic > Onion > Ginger (Table 2). The mycelial growth inhibition was observed at the 5mg concentration is effective in Pepper and Garlic showed in 5mm and 7mm of mycelial growth (Figure 3, Figure 4).

Table 2: Showed the Minimal Inhibitory Concentrations and inhibitory zones diameter of selected spices extract against *A. flavus*

Type of spices extract	Concentration (mg/ml)	Mycelial Inhibition Zones (mm)
Pepper	Control	$65_{\pm}3.61$
	0.1mg/ml	$15_{\pm}1.26$
	1 mg/ml	$11_{\pm}0.95$
	5 mg/ml	$5_{\pm}0.43$
Onion	Control	$65_{\pm}3.61$
	0.1mg/ml	$25_{\pm}2.95$
	1 mg/ml	$19_{\pm}2.48$
	5 mg/ml	$12_{\pm}1.52$
Garlic	Control	$65_{\pm}3.61$
	0.1mg/ml	$17_{\pm}2.18$
	1 mg/ml	$12_{\pm}1.28$
	5 mg/ml	$7_{\pm}0.58$
Ginger	Control	$65_{\pm}3.61$
	0.1mg/ml	$20_{\pm}2.65$
	1 mg/ml	$17_{\pm}2.26$
	5 mg/ml	$10_{\pm}1.24$

$\pm$ *Values showed are standard deviation n=3*

Figure 3: The graph showed the percentage of Minimal Inhibitory Concentration (5mg/ml) of selected spices extract against *A. flavus*

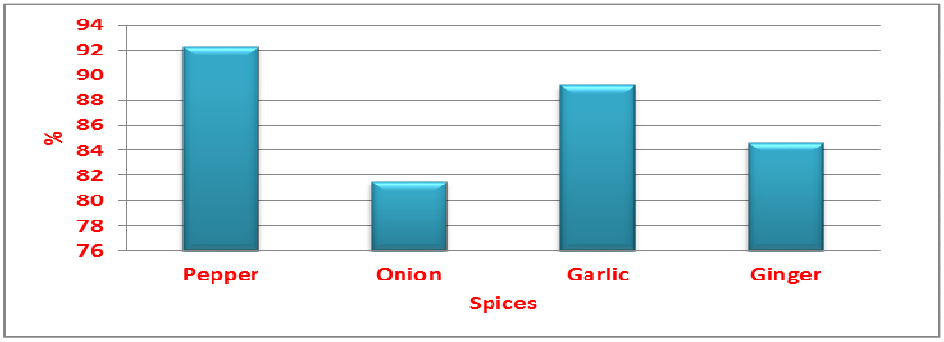
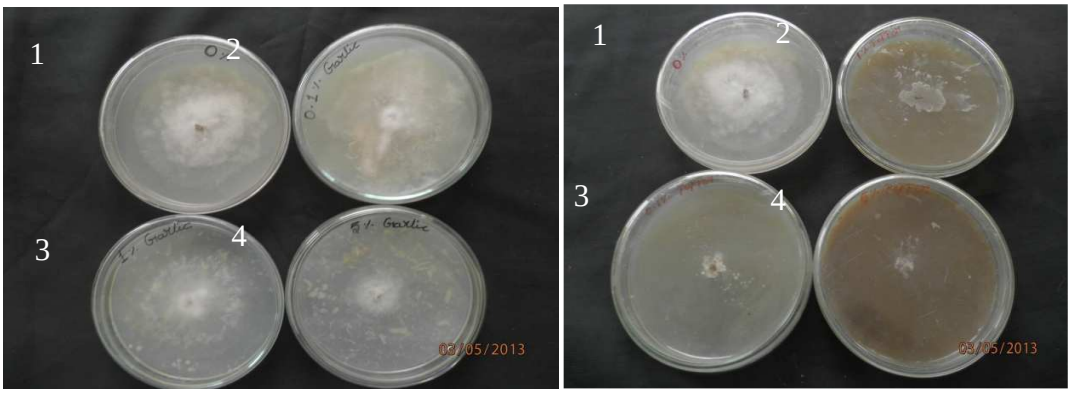
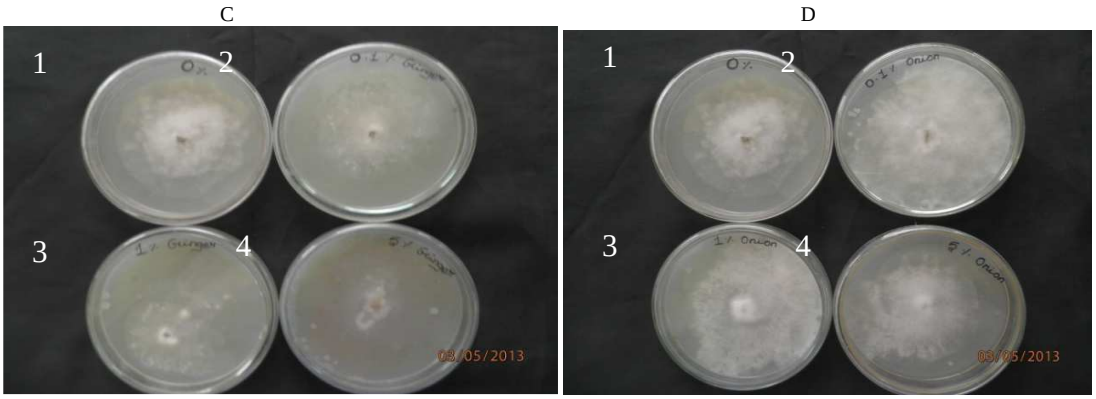


Figure 4: Showed the Minimal Inhibitory Concentrations of selected spices extract against *A. flavus*

A B





A - Garlic, B - Pepper, C - Ginger, D - Onion
1 = 0mg/ml-(Control), 2 = 0.1mg/ml, 3 = 1mg/ml, 4 = 5mg/ml

Antifungal possessions of onion and garlic against a number of fungi have been reported. *A. flavus* is less repressed by small concentrations of fundamental oils of yellow and green onions but red onion and garlic necessary oils give an idea about strong inhibitory effects [25]. Several studies declared that the phenolic compounds in spices and herbs might take part in a vital role in their antimicrobial effects. An extremely positive connection between antibacterial activity and total phenolic content in sufficient number of spice and herb extracts [26]. They also confirmed that numerous plant extracts having elevated levels of phenolics also acquire tough antibacterial activity. Aflatoxin-producing molds are broadly dispersed in nature and are recurrently the reason for infectivity in human food possessions. Aflatoxins are less important metabolites produced by toxigenic strains of *A. flavus* and *Aspergillus parasiticus* .

Mycelial growth inhibition assay in YES broth culture

The mycelial growth inhibition assay was employed by poisoned food technique to evaluate fungistatic and fungicidal properties. The effective of Pepper, Onion, Garlic and Ginger extract was analyzed. The inhibitory activity of Pepper, Onion, Garlic and Ginger extracts showed various capabilities to suppress *A. flavus* grown on broth medium after 7days incubation period. Although, the inhibitory effect of the Pepper, Onion, Garlic and Ginger extracts increased in proportion to their concentrations and reached to a maximum in the final concentration of 25 mg/ml, the extract of Pepper showed absolute growth inhibition at concentration 10 mg/ml followed by Garlic which inhibited mycelial growth at 25 mg/ml respectively (Table 3).

Table 3: Antifungal screening of spices extract at (5, 10 and 25 mg/ml) concentrations against the mycelia weight (g) of *A. flavus* isolated from Chilli

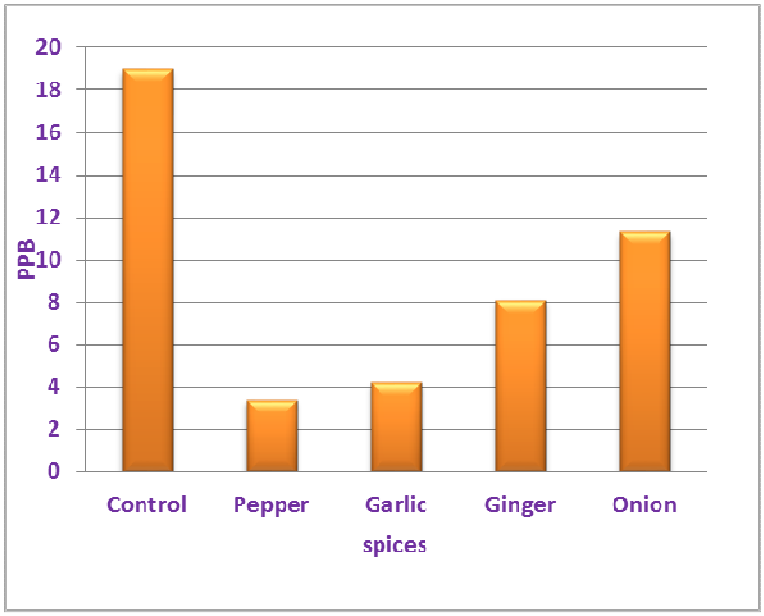
Spices Extract	Mycelial Weight			
	Control	5mg	10mg	25mg
Pepper	3.20 ± 0.18	1.12 ± 0.02	0.04 ± 0.00	0.00 ± 0.00
Onion	3.20 ± 0.18	2.27 ± 0.11	1.96 ± 0.09	0.60 ± 0.06
Garlic	3.20 ± 0.18	1.60 ± 0.06	0.30 ± 0.03	0.03 ± 0.00
Ginger	3.20 ± 0.18	2.15 ± 0.10	1.86 ± 0.08	0.44 ± 0.04

The result of the YES broth culture inhibitory experiment was used to conform the Antiaflatoxigenic activities of spices Pepper and Garlic. Pepper was powerfully energetic at MIC of 5 mg/ml and at MFC of 10 mg/ml. The extracted YES broth culture containing *A. flavus* was analysed with Vicam Aflatoxin analyser. The observed results were tabulated (Table 5, Figure 8). The Garlic showed the fungi static activity against the toxigenic *A. flavus* with MIC of 10 mg/ml and MFC of 25 mg/ml.

Table 5: Aflatoxin analysis of YES broth containing spices treated 10mg/ml concentration

S. No	Sample	Result in PPB
1.	Control	19
2.	Pepper	3.4
3.	Garlic	4.2
4.	Ginger	8.1
5.	Onion	11.3

Figure 8: Aflatoxin analysis-Spices treated with 10mg/ml concentration



None of the tested spices extracts were able to suppress fungal growth at 5 mg/ml concentration as shown in the (Figure 7). In addition, extract of Pepper was found efficient to arrest the aflatoxin produced by *A. flavus* and totally inhibited at 10 mg/ml (Table 3). The mycelial weight of *A.flavus* was observed before and after drying. The results were showed in Table 3,4 and Figure 5,6.

Figure 5: The influence of spices extract on mycelial weight at the concentration at 5mg/ml showed in Percentage

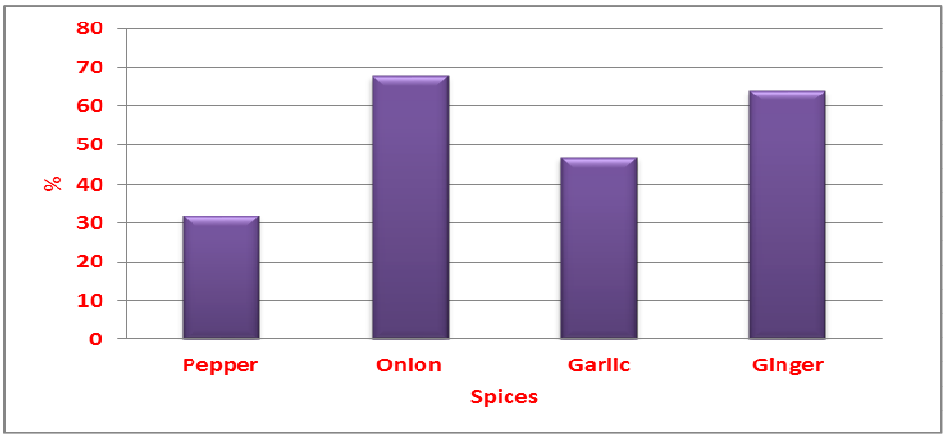


Figure 6: The influence of spices extract on mycelial dry weight at the concentration at 5mg/ml showed in percentage

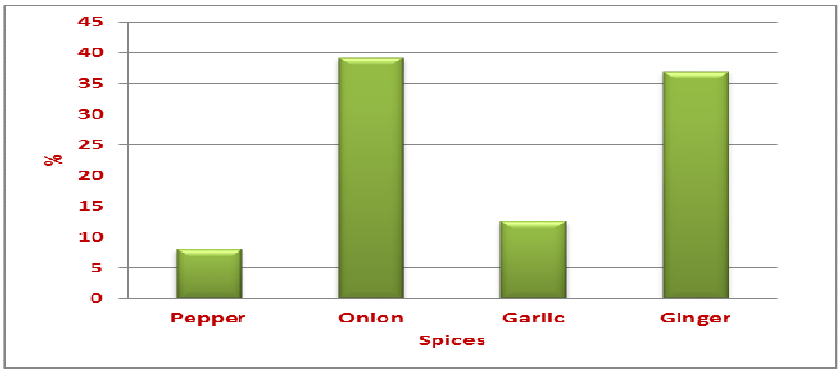
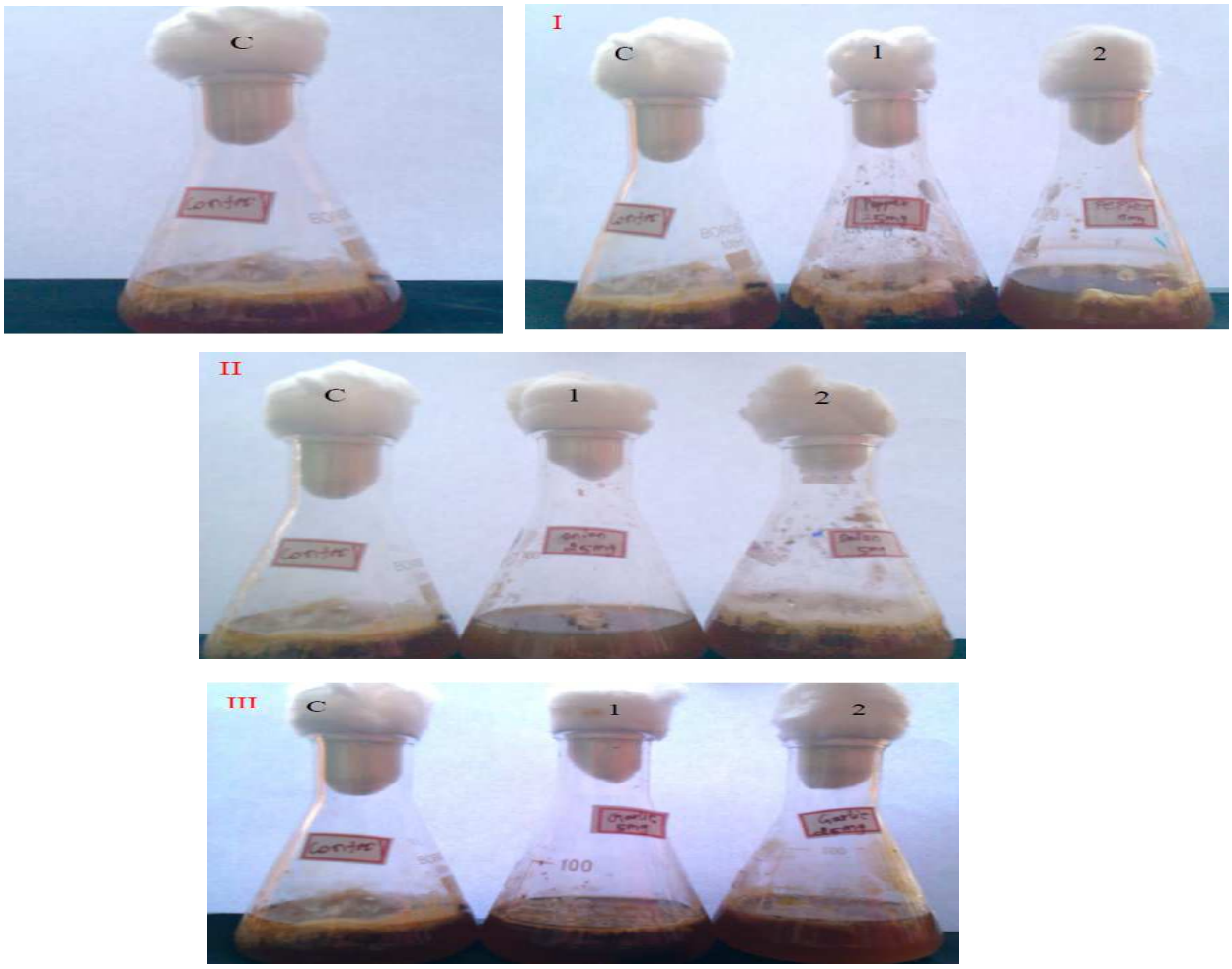


Figure 7: Control flask (Flask C) showing the growth of *A. flavus* in YES medium



I - Pepper, II - Onion, III - Garlic, IV – Ginger **1- 5mg/ml, 2-25mg/ml**

Table 4: Antifungal screening of spices extract at (5, 10 and 25 mg/ml) concentrations against the mycelial dry weight (g) of *A. flavus* isolated from Chilli

Spices extract	Mycelial Dry Weight			
	Control	5 mg	10mg	25mg
Pepper	2.63 ± 0.10	0.28 ± 0.02	0.01 ± 0.01	0.00 ± 0.00
Onion	2.63 ± 0.10	1.10 ± 0.06	0.30 ± 0.03	0.20 ± 0.02
Garlic	2.63 ± 0.10	0.40 ± 0.05	0.06 ± 0.02	0.01 ± 0.00
Ginger	2.63 ± 0.10	1.04 ± 0.07	0.55 ± 0.05	0.30 ± 0.03

These fungi develop speedily on a series of natural substrates, and the usage of contaminated food can cause severe health hazards to both animals and humans. *Apergillus* species are recognized to create aflatoxins in a range of food and feedstuffs. The chemicals are at present applied to decrease the growth of risky fungi such as *A. flavus* and *A.parasiticus* in stored foods. In recent days there has been extensive curiosity in the protection of grains through the usage of essential oils that are known to delay growth and mycotoxin production. Several researchers used necessary oils like basil, cinnamon, peppermint and thyme to save from harm maize kernels against *A. flavus* infection, without disturbing germination and corn growth [27].

Mould growth and mycotoxin incidences in food grains are relatively high in tropical and subtropical regions [28, 29]. AFB1 is well-known as one of the most toxic and carcinogenic compound among the mycotoxins, which have been determined in maize, maize-based food and feeds in various parts of the world. The infectivity of maize by toxigenic fungi and mycotoxins can happen in the field, during yield and storage, and at any instant until use [30]. The use of chemicals has been very effective in decreasing the incidences of yield losses in the field and during storage. Mould growth and mycotoxin contamination are commonly controlled using synthetic fungicides. However, random use of such chemicals has led to a number of ecological and medical harms due to toxicity and resistance may also exhibited by pathogenic microbes [31]. The increasing knowledge of constant residues, carcinogenic and toxic effects of synthetic fungicides has resulted in the need to search for new antifungal compounds applicable to food and feeds as preservatives [32]. There are no results reported for anti-aflatoxigenic activity of pepper.

Lawson and Hughes reported 200 mg/mL MIC for *Candida albicans* [33] from onion powder but, the inhibition of dried out onion flakes by ethyl alcohol and acetone were 100 and 150 mg/mL MIC for *C. albicans*. Phenolic and sulphur compounds are present in ethyl alcohol extracts of Allium species [34] and have MIC in low level. In studies of Naganawa *et al.* an ajoene compound in garlic – a derivative of alliicin was examined after ethyl alcohol extraction [35]. Ajoene was observed to be extremely inhibitory against *A. niger*, *C. albicans* and *Paracoccidioides brasiliensis*. Korukluoglu and Irkin observed MFC of 450 mg/mL (fresh garlic), 275 mg/mL (fresh onion), and > 900 mg/mL (fresh leek) after ethyl alcohol extraction and MFC of 875, >900, and >900 mg/mL after acetone extraction for *A.niger [36]*. The extracts of dried Allium slow down fungi growth much more than the fresh forms since they contain considerable amounts of sulphur compounds connected to their dry weight [37]. It was recommended that analogous study about Allium plant, as a common stabilizer, could be a alternate to synthetic antimicrobial substances. Hence, crude extracts in prospect experiments should be done and evaluated with respect to their antifungial compounds. Dried garlic, leek and onion extracts could securely be applied as substitute for fungicides to partly or totally slow down the development of fungi yielding mycotoxins[27].

CONCLUSION

Regular supervision of aflatoxin B_1 and mold incidence are extremely necessary to enhance the worth of the red pepper reserved in cold stores. A diversity of contaminants are found in foods. Of these mycotoxins, the secondary metabolites of different species of fungi have gained lot implication both in the health and economic sector. In conclusion it can be said that among all the spices, red chillies is the most frequently contaminated spice. Much attention has been focused on aflatoxins formed by *A. flavus* and *A. parasiticus* in stored grains. AFB_1 the most toxic and plentifully found among the series of aflatoxins, continues to be a chief problem for maize, groundnut, and chillies. General research should to be performed to identify the exact factors responsible for aflatoxin contamination in spices and take essential precautionary measures to lessen the occurrence of contamination. So the present work established the anti-aflatoxigenic activity of pepper and garlic by MIC studies. In future, the work may broaden by the product based studies. The inhibition may analysed in spices mixed with chillies or other products.

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