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RESEARCH ARTICLE

ISOLATION OF AFLATOXIN PRODUCING ASPERGILLUS SPECIES IN FOOD SAMPLES AND CONTROL OF THEIR GROWTH BY ROOT EXTRACT OF GINGER

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ABSTRACT

To isolate the Aflatoxin producing Aspergillus species in different infected food materials, ground nuts, maize and coconut endosperm are infected with different types of fungal species in which some are contaminated with aflatoxin producing aspergillus species. The aflatoxin production was observed the plates are exposed to UV light followed TLC method. Then the aflatoxin producing species are identified by their growth on coconut agar medium blue fluorescence was observed when exposed to a UV-light, Aflatoxins are highly toxic, carcinogenic compounds produced by certain fungi (Aspergillus flavus and A. parasiticus) that grow on crops, particularly in warm, humid conditions. They commonly contaminate ground nuts, maize, coconut, peanuts, corn, tree nuts, and spices, posing serious liver cancer risks to humans and animals.

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INTRODUCTION

A toxin can be defined as a substance that is synthesised by a plant species, an animal, or by micro-organisms, that is harmful to another organism. Mycotoxins are toxic metabolises produced by fungi, mostly by saprophytic moulds growing on a variety of food stuffs including that of animal feeds and also by many plant pathogens. They are potentially hazardous to man and domestic animals. It was established early in the 1960s that mycotoxins are responsible for many diseases. The name mycotoxin is a combination of the greek word for fungus mykes' and the Latin word toxicum' meaning poison. The term mycotoxin' is usually reserved for the relatively small (MW□700), toxic chemical products formed as secondary metabolites by a few fungi that readily colonise crops in the field or after harvest. These compounds pose a potential threat to human and animal health through the ingestion of food products prepared from these commodities. Contamination can occur pre- or post-harvest (e.g.deoxynivalenol (DON) and T-2 toxin produced by Fusarium pre harvest and ochratoxins (OTA) (Aspergillus and Penicillium) and Aflatoxins (AFT) (Aspergillus) post-harvest, although AFT contamination can also be a field event) [1].Generally, crops that are stored for more than a few days become a potential target for mould growth and mycotoxin formation. Mycotoxins can occur both in temperate and tropical regions of the world, depending on the species of fungi. Major food commodities affected are cereals, nuts, dried fruit, coffee, cocoa, spices, oilseeds, dried peas, beans and fruit, particularly apples. Mycotoxins may also be found in beer and wine resulting from the use of contaminated barley, other cereals and grapes in their production. They can also enter the human food chain via meat or other animal products such as eggs, milk and cheese as the result of livestock eating contaminated feed. They are often genotypically specific, but can be produced by one or more fungal species. For example OTA is produced by some species such as A.ochraceus, mainly in tropical regions and by P.verrucosum, a common storage fungus in temperate areas, and in some cases one species can form more than one mycotoxin. Most mycotoxins are chemically and structurally diverse. Since the majority of secondary metabolites are synthesised by simple biosynthetic reactions from small molecules (acetates, pyruvates, etc.), this is surprising, however, this leads to the compounds having such a diverse range of toxic effects, both acute and chronic. To describe the effects of all mycotoxins is beyond the scope of this review, and can be found in several books and reviews on the subject,

however, we have briefly covered two of the major examples to give the reader an idea of the scope of the problem.

Aflatoxins: AFTs are a group of closely related widely researched mycotoxins that are produced by fungi A. Flavus and A. parasiticus. The AFT were first heavily researched and understood after the death of more than 100,000 young turkeys on poultry farms in England, (turkey×disease) that were found to be related to the consumption of Brazilian peanut meal.AFT are difuranocoumarin derivatives that are produced by apolyketide pathway by many strains of A. flavusand A. parasiticus, however A. flavus has been reported to be a common contaminant in agricultural produce. A.bombycis, A. ochraceoroseus, A. nomius, and A. Pseudotamari are also AFT-producing species, butare encountered less frequently. From the mycological perspective, there are qualitative and quantitative differences in the toxigenic abilities displayed by different strains within each aflatoxigenic species. For example, only about half of A. Flavus strains produce AFT-producing species more than 10^6gkg−1.The four major AFT are B1, B2, G1, and G2 based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin- layer chromatography (TLC). AFTB1 is the most potent natural carcinogen known, and is usually the major AFT produced by toxigenic strains. The toxicology of AFT is both challenging and complex. The differences in susceptibility to AFT across species and between persons depend largely on the fraction of the dose that is directed in to the various possible path ways, with harmful biological exposure being the result of activation to the epoxide and the reaction of the epoxide with proteins and DNA. Aflatoxins are highly toxic, carcinogenic compounds produced by certain fungi (Aspergillus flavus and A. parasiticus) that grow on crops, particularly in warm, humid conditions. They commonly contaminate peanuts, corn, tree nuts, and spices, posing serious liver cancer risks to humans and animals

Many agricultural commodities are vulnerable to attack by fungi that produce mycotoxins. These toxic substances can persist long after the fungi have been killed and contaminate foods. Most mycotoxins are stable compounds that are not destroyed during food processing or cooking. Since they can easily enter the market place and be a hazard to public health it is important to develop effective analytical methods for the identification and quantification of mycotoxins. Among mycotoxins, four main aflatoxins B1, B2, G1, and G2 are extremely potent carcinogens and can have significant economic impacts, making them important targets for detection and quantitation. In order to detect the trace amounts of these toxins that are commonly present in agricultural products it is critical to develop highly sensitive methods. Aflatoxin-producing members of Aspergillus are common and widespread in nature. They can colonize and contaminate grain before harvest or during storage. Host crops are particularly susceptible to infection by Aspergillus following prolonged exposure to a high-humidity environment, or damage from stressful conditions such as drought, a condition that lowers the barrier to entry. The native habitat of Aspergillusis in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration, and it invades all types of organic substrates whenever conditions are favorable for its growth. Favorable conditions include high moisture content (at least 7%) and high temperature. The toxin can also be found in the milk of animals that are fed contaminated feed.

International sources of commercial peanut butter, cooking oils (i.e. oliveoil, etc.), and cosmetics have been identified as contaminated with aflatoxin. In some instances, liquid chromatography-tandem mass spectrometry (LC-MS/MS), and other analytical methods, revealed anywhere from 48-80% of selected product samples as containing detectable quantities of aflatoxin. In many of these contaminated food products, the aflatoxin exceeded FDA, or other regulatory agency, safe limits. Aflatoxins are still recognized as the most important mycotoxins. They are synthesized by only a few Aspergillus species of which A.flavusandA. parasiticusare the most problematic. The expression of aflatoxin-related diseases is influenced by factors such as species, age, nutrition, sex, and the possibility of concurrent exposure to other toxins. The main target organ in mammals is the liver, so aflatoxicos is primarily a hepatic disease. Conditions increasing the likelihood of aflatoxicos is in humans include limited availability of food, environmental conditions that favour mould growth on foodstuffs, and lack of regulatory systems for aflatoxin monitoring and control. Flavusand A. parasiticusare weedy molds that grow on a large number of substrates, in particular under high moisture conditions. Aflatoxins have been isolated from all major cereal crops, and from sources as diverse as peanut butter and marijuana. The staple commodities regularly contaminated with aflatoxins include cassava, chillies, corn, cotton seed, millet, peanuts, rice, sorghum, sunflower seeds, tree nuts, wheat, and a variety of spices intended for human or animal consumption. When processed, aflatoxins get into the general food supply where they have been found in both pet and human foods, as well as in feed stocks for agricultural animals. Aflatoxin transformation products are sometimes found in eggs, milk products and meat when animals are fed contaminated grains.
Aspergillus flavus and A. parasiticus colonize a wide variety of food commodities including maize, oilseeds, spices, groundnuts, tree nuts, milk, and dried fruit (Strosnider *et al*. 2006). Whether these fungi produce aflatoxin depends on drought stress and rainfall, suitability of crop genotype for its climate, insect damage, and agricultural practices (Wu and Khlangwiset 2010). These fungi can also produce aflatoxin in postharvest conditions: storage, transportation, and food processing. Aflatoxin contamination is a particular problem in maize, oilseeds, spices, peanuts, tree nuts (almonds, pistachios, hazelnuts, pecans, Brazil nuts, and walnuts), milk (in the form of aflatoxin B1‘s metabolite aflatoxin M1), and dried fruit (Shephard, 2008). Maize and peanuts are the main sources of human exposure to aflatoxin because they are so highly consumed worldwide and unfortunately are also the most susceptible crops to aflatoxin contamination (Wu and Khlangwiset 2010). Figure 2 (Wu 2010) depicts the pathway by which aflatoxin accumulates in food crops and contributes to various adverse human health effects. In the liver, aflatoxin may be transformed by certain P450 enzymes (CYP1A2, 3A4, 3A5, 3A7) to its DNA-reactive form aflatoxin-8,9-epoxide. This molecule may bind to liver proteins and lead to their failure, potentially resulting in acute aflatoxicosis. Alternatively, it may bind to DNA, a step that is a precursor for aflatoxin-induced hepatocellular carcinoma (liver cancer). As mentioned earlier, there may be a synergistic effect between aflatoxin and chronic infection with hepatitis B virus (HBV) that results in significantly higher liver cancer risk (Wu 2010).

Acute exposure to aflatoxins: Acute aflatoxicosis, associated with extremely high doses of aflatoxin, is characterized by hemorrhage, acute liver damage, edema, and death in humans.

Conditions increasing the likelihood of acute aflatoxicos is in humans include limited availability of food, environmental conditions that favor fungal development in crops and commodities, and lack of regulatory systems for aflatoxin monitoring and control. There have been several reported cases of acute aflatoxicos is in Africa associated with consumption of contaminated home-grown maize, including the outbreaks in Kenyain 1982, in which 12 people died, and in 2004, in which 317 people became ill and 125 people died in the central provinces (Nyikal *et al*. 2004; Azziz-Baumgartner *et al*. 2005; Probst *et al* 2007; Lewis *et al*. 2005; Stosnider *et al*. 2006; Siame and Nawa 2008).

Acute aflatoxicosis can also occur in animals. In 1960, more than 100,000 turkeys died on in the United Kingdom over the course of a few months, prompting the name ―Turkey X disease‖ (Asao *et al* 1963). Later investigation revealed that the source of the disease was toxic peanut meal. In 1981, several hundred calves that had been fed on peanut hay died in Australia (McKenize *et al* 1981), and in 2007, several hundred animal deaths occurred on a chinchilla farm in Argentina; both these occurrences were linked to aflatoxin (González Pereyra *et al*, 2008).

Chronic exposure to aflatoxins: HCC as a result of chronic aflatoxin exposure has been well documented, presenting most often in persons with chronic hepatitis Bvirus (HBV) infection (Qian *et al*. 1994, Groopman *et al*. 2008). For individuals chronically infected with HBV, aflatoxin consumption raises the risk of liver cancer up to thirty-fold, compared with either exposure alone (Groopman *et al*. 2008). Unfortunately, these two risk factors – aflatoxin and HBV – are especially prevalent in poor nations worldwide (Liu and Wu 2010). In developing countries, many people subsist largely on cereal diets. Nutritional deficiencies are very prevalent in populations consuming high levels of cereals (Bankoleetal2003), particularly children. Additionally, many children in the Developing world are also exposed to high levels of mycotoxins in their diets (Cardwell *et al* 2001). Aflatoxin and immune suppression in humans has been relatively less well-characterized, but could in fact have enormous significance from a global health perspective (Williamsetal, 2004). Several recenthumanstudieshave shown evidence of immunomodulation (Turner *et al*.2003, Jiang *et al*. 2005, Jiang *et al*. 2008), though the actual outcomes of such immune modulation have yet to be characterized in humans. Indeed, aflatoxin‘s immune toxicity may be one explanation for the stunted growth in children that appears to follow a dose-response relationship with aflatoxin exposure (Wu and Khlangwiset 2010; Gong*et al*. 2002, 2004; Turner *et al*.2003). The mechanism by which aflatoxin may result in growth impairment is not yet known; however, one possible explanation may be altered intestinal integrity through cell toxicity or immune modulation (Gong *et al*. 2008).

Similarly, decades of animal studies have demonstrated that chronic exposure to aflatoxins in animals can also cause growth inhibition and immune suppression (Khlangwiset *et al*. 2011). Nursing animals may be affected, and aflatoxin M1 maybe excreted in the milk of dairy cattle and other dairy animals. This in turn poses potential health risks to both animals and humans that consume that milk. Chronic aflatoxin exposure in animals can result in impaired reproductive efficiency, reduced feed conversion efficiency, increased mortality rates, reduced weight gain, anemia, and jaundice.

In the case of laying hens, aflatoxicosis causes an enlarged fatty liver and lowered egg production (see Lubulwa and Davis 1994 for a list of effects of aflatoxicoses on livestock: poultry, beef cattle and dairy, and pigs). The infection of plants by various fungi not only results in reduction in crop yield and quality, with significant economic losses, but also contamination of grains with poisonous fungal secondary metabolites called mycotoxins. The ingestion of such mycotoxin-contaminated grains by animals and human beings has enormous public health significance, because these toxins are capable of causing diseases inman and animals (Bhat and Vasanthi2003). Of greatest concern is the relevance of these toxins in human hepatoma and oesophageal cancer (Shephard 2008). There are hundreds of mycotoxins found in foods, but those that pose the greatest risk to human and animal health are aflatoxins (AFs), trichothecenes [e.g. deoxynivalenol (DON), T-2 toxin], fumonisins (FBs), zearalenone (ZEA), patulin (PAT) and ochratoxin A (OTA) (CAST 2003). The AFs, especially the most potent, AFB1, are primarily known hepatotoxins and hepatocarcinogens, which were the cause of death of 215 people in Kenya who consumed highly AF-contaminated maize meals in 2004 (CDC 2004). Trichothecenes are a group of about 150 related compounds that are protein inhibitors with consequent immune suppressive effects, causing severe damage to the digestive tract and death due to intestinal haemorrhage. The common esttrichothecenes are DON and T-2 toxin (Sudakin2003). Fumosins, especially fumonisin B1 (FB1) cause liver and kidney cancer, and neural tube defects in rodents, leuko encephalo malaciain horses and pulmonary oedema in pigs (Dutton 1996). Of major concern is the association of FB1 with elevated incidence of human oesphageal cancer in parts of South Africa, North Eastern Iran and China, upper gastrointestinal tract cancer in Northern Italy (Chu and Li 1994; Rheeder *et al*. 1992; Sydenham *et al*. 1990) and neural tube defects in human babies (Hendricks 1999). ZEA, an oestrogenic toxin that causes infertility in animals, is associated with outbreaks of precocious pubertal changes in children in Puerto Rico, and has been suggested to have a possible involvement in human cervical cancer (Zinedine *et al*. 2007). OTA causes kidney and liver impairment in animals (especially pigs) and man (Hussein and Brasel2001).

The established presence of these major mycotoxins and the fungi that produce them in rice in several parts of the world (Reddy *et al*. 2008) demonstrates that cereal supports growth of fungi and mycotoxin production. Apart from the suitability for fungal development and mycotoxin production, rice is highly cultivated and consumed worldwide and this makes it one of the most important principal sources of mycotoxins to human beings and animals in the world. It is the world‘s most extensively cultivated crop after wheat and a staple food of nearly 50% of the total world population (FAO 2002). In Nigeria, rice is the sixth most cultivated crop after sorghum, millet, cowpea, cassavaandyam. According to the FAO Statistics Division, Nigeria produced about 4.7 million tones of paddy rice in 2007, making it the second largest producer of rice in Africa after Egypt (FAO 2008). In spite of this amount produced, Nigeria imported about 1.6 million tones of rice (USDA2008b) and consumed about 4.9 million tones in 2008 (USDA 2008a), making it the third world‘s major rice importing country after Iran and Philippines. The deficit in the national annual demand for rice is an indication of ace real that is highly consumed in the country. Rice is grown primarily for the market in Nigeria (Erenstein and Lancon2003) and therefore has a wider distribution and hence a more likely

source of mycotoxins than most other crops. Niger State is the traditional rice growing area of Nigeria with the second highest rice yield of the six major rice producing states in the country (Erenstein and Lancon2003). It contributes about 16% of the national rice produce of the country (Ezedinma2005). It is a generally hot and humid (average annual temperature of 31.7°C and average humidity of 51.6%) middle-belt state of Nigeria. These climatic conditions are very favourable for fungal growth and mycotoxin production in food sand feeds. In spite of its strategic position as a national food basket, there is a general paucity of information on mycotoxins, particularly on rice, in the state and indeed in the whole country, except for a few reports (Makun *et al*. 2007, 2009). The present study is, therefore, a survey for the major mycotoxins (AFsB1, B2, G1 and G2,OTA, ZEA,FBs B1, DON, T-2 toxin and PAT) in rice grown in Niger State. The generated incidence data and profile of mycotoxins of the widely distributed and highly consumed staple, rice, grown in a leading food-producing state of Nigeria, from this study was aimed at establishing the quality of Nigerian grown rice. This study also aimed to elucidate the types of animal and human diseases expected from our diets and the extent of risk to mycotoxicoses.

MATERIALS AND METHODS

Collection of fungal infected food materials: Identified and collected fungal infected ground nuts, maize, and coconut materials based on their colour appearance from the farmers of near by villages. Each sample carefully stored in clean polythene bags. Isolation of aflatoxin producing Aspergillus species infected food. Prepared Potato dextrose agar medium, autoclaved the medium at 120^0C and 15 lbs pressure for 20 minute. Poured media in a sterilized Petri dishes, after solidification the fungal infected food materials are inoculated in the centre of plates. The plates have been incubated at room temperature for about 6 days.

Extraction of aflatoxin from strains grown on agar media: Aflatoxin has been isolated by grinding the mouldy agar along with grown fungal culture (20gm) in mortar and pestle with a mixture of KCl (0.5%) and methanol(100ml).Then the mixture has been filtered with the help of whatman No.1 filter paper. The filtrate thus obtained has been subjected to evaporation in a boiling water bath such that 1ml of the total volume is remained and is subjected to thin layer chromatography(TLC)for the presence or absence of fluorescence and estimated the quantity of aflatoxins by ELISA method.

Production of aflatoxins from isolated fungal species: The spores that have been grown on agar and showing fluorescence when exposed to UV light are subjected to fermentation for production aflatoxins. Prepared 100 ml of Czapeck‘s broth, autoclaved the medium at 120^0C and 15 lbs pressure for 20 minutes. Cooled the media and inoculated with isolated fungal species who is showed fluorescence when exposed to UV light and on TLC plates, incubated for about 4 days and observed for the growth .Then the sample has been taken and is subjected to TLC and estimated the quantity of aflatoxins in broth. Identification of Aflotoxinon TLC: The production of Aflatoxin has been evaluated with the help of TLC to confirm the presence or absence of Aflatoxin .Readymade silica gel coated plates have been used for TLC. The solvent mixture have been used for TLC is Benzene:Methanol:Acetone (80:15:5).The sample is spotted on the TLC sheet The sample is spotted on the TLC sheet and kept in chromatographic chamber. After the solvent reached to top edge, removed plates from chamber, air dried. Then the sheet is observed under UV-light for the fluorescence.

Estimation of Aflotoxin by ELISA: The method is based on a competitive colorimetric ELISA assay. The toxin of interest has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target toxin. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the toxin attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the toxin coated on the plate wells. The resulting color intensity after addition of substrate, has an inverse relationship with the target concentration in the sample.

Sample Preparation: Weighed out 5 g of the ground sample and placed in a suitable container. Added 25mL of 70% methanol and shaken for 20 minutes with a shaker. Centrifuged sample for 10 minutes at 4,000 rpm. Diluted1mL of the obtained supernatant with 1mL of distilled Used 50 µL of the diluted supernatant test. For growth medium extract, taken0.1ml of methanolic extract and made to1ml with distilled water from this diluted solution used 50 µl to ELISA assay.For fermented broth taken 0.1 ml fungal free filtrate and made to1ml with distilled waterfrom this diluted solution used 50 µl to ELISA assay.

ELISA Test: Added 50 µl of each Aflatoxin B1 Standards in duplicate into different wells (Added standards to plate only in the order from low concentration to high concentration). Added 50µl of each sample in duplicate into different sample wells. Added 100 µl of Antibody #1 and mix well by gently rocking the plate manually for 1 minute. Incubated the plate for 30 minutes at room temperature (20^0C –25^0C). Washed the plate 3 times with 250 µl of 1X wash Solution. After the last wash, inverted the plate and gently tapped the plate dry on paper. Added 150 µl of 1X Antibody #2 solution. Incubated the plate for 30 minutes at room temperature (20^0C – 25^0C). Washed the plate 3 times with 250 µlof 1X Wash Solution. After the last wash, inverted the plate and gently tapped the plate dry on paper towels. Added 100µl of TMB/H_2O_2 substrate. Timed there action immediately after added the substrate. Mixed the solution by gently rocking the plate manually for 1 minute while incubating. After incubating for 15 minutes at room temperature (20^0C – 25^0C)added 100 µl of Stop Buffer to stop the enzyme reaction. Transferred reaction mix into separate tubes, added 2 ml of wash buffer to each tube and read the optical density at 700nm by using spectrophotometer. Constructed standard curve, from the standard curve calculated concentrations of aflatoxin in food sample, extract of solid growth medium and fermented broth

Conformation of aflatoxin toxin producing Aspergillus species by selective media:

Coconut cream agar: Which comprised coconut cream (50%) and agar (1.5%), detected isolates of A.flavus more effectively than the synthetic media tested and was as effective as media containing desiccated coconut. Fluorescence colouring of colonies grown on coconut cream agar could be used to differentiate A.flavus from A. parasiticus and A. nomius. In

addition, conidial colour of A. flavus and A. nomius was quite distinct from that of A. parasiticus.

Aspergillus differentiation agar (AFPA): AFPA is a selective identification medium for the detection of A. flavus group strains. With this method is possible to distinguish these species from other Aspergillus based on the development of orange colour on the reverse of the plates.

Czapek Dox agar (CZ). When grown on CZ, colonies taxonomically between the two species can also be separated. Those of A.flavus being yellow-green and those of A.parasiticusa distinctly darker green, referred to as near Ivy green. Prepared AFPA, CCA and CZ media suing their respected components, autoclaved and cooled to approximately 50^{o}C and poured into petri dishes. After solidified the media inoculated each isolated fungal species in to all media and then subjected to incubation at room temperature for about one week. After completion of incubation period observed the morphology of the fungi.

Growth Control of aflatoxin producing fungi by root extract of Zingiber officinale:

Preparation of water extract from Zingiber officinale root: Fifty grams rhizome of Zingiber officinale (Ginger) was treated with 500 ml of distilled water with constant stirring for 5 hours. After stirring, the solution was filtered through 2 layers of cheese- cloth gauze and Whitman‘s (No.1) filter paper, evaporated the solvent, air dried the extract and stored in small, sterilized 5 ml screw-capped glass bottles and kept in the refrigerator (4^{o}C) until further usage. Determination of Antifungal activity of water extract of Zingiber officinale root : Taken four 250ml conical flasks and labelled as A,B,C,and D. To each flask transferred 100ml of cZpek dox broth, autoclaved the media at 120^{0}C and 15lbs pressure for 15 minutes. After completion of autoclaving process cooled the media to room temperature and added 100mgs (1mg/ml) of water extract powder to flask A, 200 mg to flask B(2mg/ml), 300mg to flask C(3mg/ml) and 400 mg to flask D(4mg/ml).Mixed thoroughly and inoculated loopful of active spores of isolated aspergillus species to each flask who is confirmed producing aflotoxins. Incubated fungal inoculated flasks at room temperature for six days.

RESULTS AND DISCUSSION

The collected food materials which are infected with fungus are exposed to UV light in which some sample are showed fluorescence are inoculated on potato dextrose agar medium. The agar is further grinded and subjected to methanol extraction. Then the extracted compounds from agar medium was further subjected to TLC, fluorescence is observed under UV light. The isolated Aspergillus species are grown on coconut cream agar (CCA), blue fluorescence was observed when exposed to a UV-light. Based on these results again conformed the isolated species belongs aflatoxins producing fungi. When grown on Czapek Dox agar (CZ), th isolated all species are showed yellow-green growth, which conforms the isolated all species belongs to Aspergillus flavus. The isolated Aspergillus species are grown on Aspergillus differentiation agar (AFPA): development of orange colour on the reverse of the plates conformed the isolated all species are belongs to Aspergillus flavus.

The isolated aspergillus species are subjected to production of aflatoxins in cZpeck Dox broth and quantified by ELISA method. Based on Quantitative ELISA, the aflatoxins producing Apsergillus species are grown in enrichment medium, the aflatoxins production rate is increased. The water extract from roots of Zingiber officinale significantly retarded the sporulation of Aspergillus Flavus in Czapek Dox broth. Flasks containing4 mg/ml concentration ofextract showed highest antifungal activity.

Fungal infected Ground nuts

Ground nuts: The collected fungal infected ground nut seeds have different colour morphology. When exposed to UV light only green and yellowish green morphology seeds are showed fluorescence the remaining seeds are not emitting fluorescence. The fluorescence emitting seeds are grown on PDA medium the colour appearance on growth media, sample-1 and sample-5 are showed green colour spores. Then the growth media extract was subjected to TLC found sample-1 and sample-5 are showed bluish fluorescence.

Fungal infected Maize

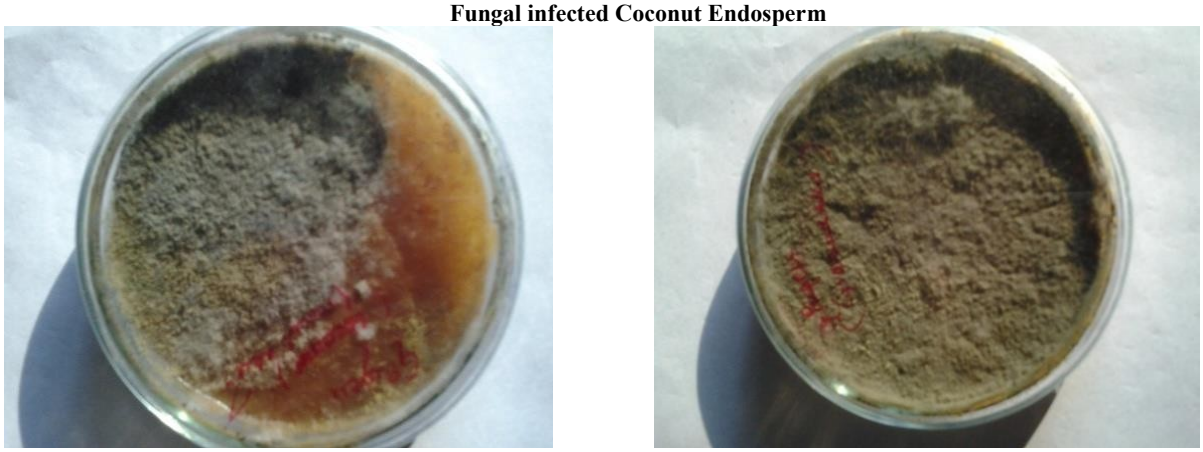
Sample	Colourof infected seeds	SeedsShowing fluorescence	Growthon PDA medium	Fluorescence of growth medium	TLCof growth media extracts
Sample-1	Green	Yes	Green	Yes	Yes
Sample-2	Black	No	--	--	--
Sample-3	Brown	No	--	--	--
Sample-4	White	No	--	--	--
Sample-5	Yellowish green	Yes	green	Yes	Yes

Sample	Colourof infected seeds	Seeds Showing fluorescence	Growthon PDA medium	Fluorescence of growth medium	TLCof growth media extracts
Sample-1	Green	Yes	Green	Yes	Yes
Sample-2	Black	No	--	--	--
Sample-3	green	Yes	Green	Yes	Yes
Sample-4	White	No	--	--	--

Sample	Colour of Endosperm	Endosperm Showing fluorescence	Growth on PDA medium	Fluorescence of growth medium	TLCof growth media extracts
Sample-1	Green	Yes	Green	Yes	Yes
Sample-2	Black	No	--	--	--
Sample-3	White	No	--	--	--

S.No	Standard/Sample	OD at 700nm	Concentration in nano grams/ml
1	Standard-1	0.02	1
2	Standard-2	0.05	2
3	Standard-3	0.09	3
4	Standard-4	0.13	4
5	Standard-5	0.17	5
6	Groundnutpowder	0.02	1
7	Maizepowder	0.02	1
8	Coconutpowder	0.01	0.5
9	Extractofsolidgrowth medium (Fromgroundnut)	0.10	3.3
10	extract of solid growth medium(Maize)	0.09	3
11	extract of solid growth medium(Coconut)	0.08	2.6
12	fermented broth (ground nut)	0.20	5.8
13	Fermentedbroth (maize)	0.15	4.3
14	Fermentedbroth (Coconut)	0.16	4.6

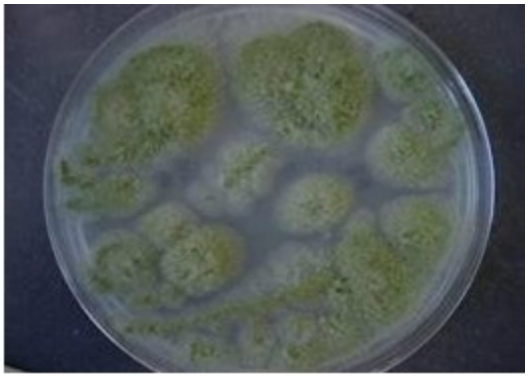
Fungal infected Coconut Endosperm



Growth on Potato Dextrose agar medium showing green colour spore formation

Emission of fluorescence light when exposed to UV light

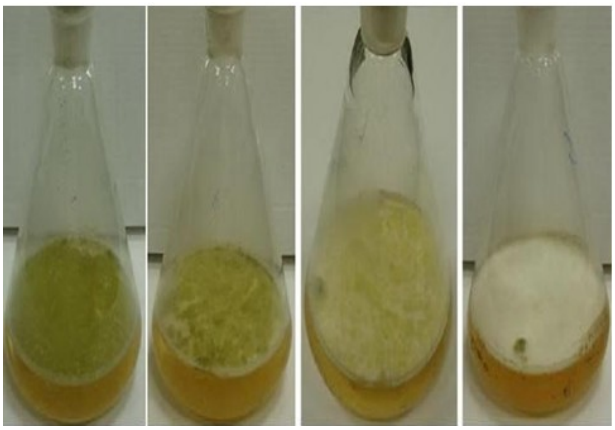
Production of Aflatoxins by using Czapek Dox broth as enrichment media



Showing blueish fluorescence byAspergillus species grown on coconut cream agar (CCA), when exposed to a UV-light

Showing orange colour on the reverse of the plate when Aspergillus flavus grown on Aspergillus differentiation agar (AFPA)

Aspergillus Flavus Showing yellow-green growth on CzapekDox agar (CZ)



Determination of Antifungal activity of water extract of Zingiber Officinale root

Maize: The collected fungal infected Maize seeds have different colour morphology. When exposed to UV light only green morphology seeds are showed fluorescence the remaining seeds are not emitting fluorescence. The fluorescence emitting seeds are grown on PDA medium the colour appearance on growth media, sample-1 and sample-3 are showed green colour spores. Then the growth media extract was subjected to TLC found sample-1 and sample-3 are showed bluish fluorescence.

Coconut: The collected fungal infected coconut endosperm have different colour morphology. When exposed to UV light only green morphology seeds are showed fluorescence the remaining seeds are not emitting fluorescence. The fluorescence emitting seeds are grown on PDA medium the colour appearance on growth media, sample-1was showed green colour spores. Then the growth media extract was subjected to TLC found sample-1showed bluish fluorescence.

CONCLUSION

The food materials, ground nuts, maize and coconut endosperm are infected with different types of fungal species in which some are contaminated with aflatoxin producing aspergillus species. The aflatoxin production was observed the plates are exposed to UV light followed TLC method. Then the aflatoxin producing species are identified by their growth on coconut agar medium blue fluorescence was observed when exposed to a UV-light, on Czapek Dox agar medium the yellow-green colour growth was observed and development of

orange colour on the reverse of the plates when the organism grown on Aspergillus differentiation agar. Based on the above results the food materials are infected with Aspergillus Flavus. From the quantitative ELISA method, estimated the quantities of aflatoxins in infected food material, solid medium and enrichment medium. Based on ELISA results the aflatoxin production rate was increased with enrichment medium. When the root extract of Zingiber officinale was used to control the growth of Aspergillus species, the extract successfully controlled sporulation of the fungi.

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