IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Analysis of Sewage and Fresh Pond Water Samples from Thakur College Campus and their Treatment using Fungal Organisms

Udaybhan Yadav[1] and Kunal Thakur[2]
Co-ordinator, Microbiology ID, ZSCT's Thakur Shyamnarayan Degree College, Mumbai, India[1]
Assistant Professor, Microbiology ID, ZSCT's Thakur Shyamnarayan Degree College,Mumbai, India[2]

Abstract: *Aflatoxin biosynthesis was induced by compounds in filtrates (EF) obtained from cultures consisting of ground maize kernels colonized by Aspergillus flavus. Natural poisons in food are as much a part of our life as the oxygen we breathe. It is more important to be able to neutralize these poisons naturally from our diet. This is only possible if we are exposed to very minute and non–toxic amounts which our bodies can handle with ease. It would not only be unrealistic, but impossible to totally eradicate natural poisons from our food. Some mycotoxins directly inhibit the growth of microorganisms. The term Mycotoxin literally means poison from fungi. Mycotoxins are secondary metabolites produced by micro fungi that are capable of causing disease and death in humans and other animals. Because of their pharmacological activity, some mycotoxins or mycotoxin derivatives have found use as antibiotics, growth promotants, and other kinds of still others have been implicated as chemical warfare agents.*

Keywords: *Aspergillus flavus,* Mycotoxins, Aflatoxins, Secondary Metabolites

I. INTRODUCTION

Mycotoxins: The most important mycotoxins are the following: aflatoxins (AFB1,B2, G1, G2), ochratoxin A (OTA), tricothecenes, zearalenoneand fumonisins (B1, B2). Of the tricothecenes, attentionis paid mainly to deoxynivalenol (DON or vomitoxin), nivalenol and T2-toxin. Some laboratories also focused onHT2 toxin, 3-Ac-DON, DAS, Fusarenon-X. One laboratory also included MAS, Neosolaniol and 15-Ac-DON in the range of analyses. Other mycotoxins were also mentioned but only by a few members; these were patulin, sterigmatocystin and cytochalasin E. This review focuses on the most important ones associated with human and veterinary diseases, including aflatoxin, citrinin, ergot akaloids, fumonisins, ochratoxin A, patulin, trichothecenes, and zearalenone. They are usually named on the basis of the fungus that produces them. For instance, "Aflatoxin" uses the A for Asperigillus and Fla for the species flavus along with the word toxin. There are three major genera of fungi that produce mycotoxins: Aspergilius, Fusarium and Penicilium. Mycotoxin-producing fungi grow on a wide spectrum of feeds that include cereal grains, groundnuts, beans and peas.

Aflatoxins: Aflatoxins are toxic metabolites produced by certain fungi in/on foods and feeds. They are probably the best known and most intensively researched mycotoxins in the world.

The occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or processing periods. Aflatoxins are produced by different species of *Aspergillus niger,* flaves particularly and *parasiticus*, as well as members of the Genera *Penicillium* and *Rhizopu*s. Aflatoxins can contaminate corn, cereals, sorghum, peanuts, and other oil-seed crops. *Aspergillus*, which produces aflatoxin, is among the most common grain mold fungi. Nevertheless, aflatoxin's reputation has a potent poison may explain why it has been adopted in bioterrorism The aflatoxins were isolated and characterized after the death of more than 100,000 turkey poults (turkey X disease) was traced to the consumption of a mold-contaminated peanut meal (four major aflatoxins are called B1, B2, G1, and G2 based on their fluorescence under UV light or green) and relative chromatographic mobility during thin-layer chromatography.

Aflatoxins have been associated with various diseases , such as aflatoxicosis in livestock , domestic animals and humans throughout the world Aflatoxins have received greater attention than any other mycotoxins because of their

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans . Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. People look at the direct life threat from culprits, but the job hazards are much more than that. Aflatoxins, like other natural poisons in food, cannot be completely eradicated from food. Aflatoxin poisoning is reported from all parts of world in almost all domestic and non domestic animals and other non human primates. In addition, inhalation of aflatoxin is associated with disease and injury in both animals and human identification of genetic resistance to the production of aflatoxin in more than five major crops. Aflatoxins are recognized as serious food safety hazards by most countries of the world and more than 50 countries have established or proposed regulations for controlling them in food and feed. There is to our knowledge very little evidence of the occurrence of aflatoxins in European cereals and in some other countries37 .In the U.S. corn, cottonseed, peanuts, and other crops are routinely tested and those containing more than 20 parts per million of aflatoxins cannot be used in human food or fed to dairy cows. It is estimated that crop loss due to aflatoxin contamination costs U.S. producers more than $100 million per year on average. As it the is realized that absolute safety is never achieved , many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed.

1.1 Contents

A careful survey of the early outbreaks showed that they were all associated with feeds, namely Brazilian peanut meal. An intensive investigation of the suspect peanut meal was undertaken and it was quickly found that this peanut meal was highly toxic to poultry and ducklings with symptoms typical of Turkey X disease. Speculations made during 1960 regarding the nature of the toxin suggested that it might be of fungal origin. In fact, the toxin-producing fungus was identified as *Aspergillus flavus* (1961). This discovery has led to a growing awareness of the potential hazards of these substances as contaminants of food and feed causing illness and even death in humans and other mammals. Studies that are summarized in the following sections revealed that aflatoxins are produced primarily by some strains of A. Flavus and by A. niger .most , if not all , strains of A. parasiticus , plus related species, A. nomius Moreover , these studies also revealed that there are four major aflatoxins : **B1** , **B2** , **G1** , **G2** plus two additional metabolic products , **M1** and **M2** , that are of significance as direct contaminants of foods and feeds . The aflatoxins M1 and M2 were first isolated from milk of lactating animals fed aflatoxin preparations; hence, the M designation. Whereas the B designation of aflatoxins B1 and B2 resulted from the exhibition of blue fluorescence under UV-light, while the G designation refers to the yellow-green fluorescence of the relevant structures under UV-light. These toxins have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds. Their molecular formulas as established from elementary analyses and mass spectrometric determinations are:

- B1 : C17 H12 O6
- B2 : C17 H14 O6
- G1 : C17 H12 O7
- G2 : C17 H14 O7

Aflatoxins B2 and G2 were established as the dihydroxy derivatives of B1 and G1 , respectively . Whereas, aflatoxin M1 is 4-hydroxy aflatoxin B1 and aflatoxin M2 is 4-dihydroxy aflatoxin .Consumption of low concentrations by animals sensitive to aflatoxins can lead to death in 72 hours. In general, at nonfatal levels, the health and productivity of animals fed contaminated feed are seriousimpaired. As a result, the Food and Drug Administration (FDA) has set an action level for aflatoxins in corn at 20 parts per billion (ppb). Corn containing aflatoxin levels of 20 ppbor more cannot be sold in interstate commerce, and, in general, should not be fed to young poultry , swine, and livestock, or to lactating animals, and must not be milled**.**

Aspergillus niger is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called "black mold").Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins, aflatoxin.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Taxonomy: A. niger is included in Aspergillus subgenus Circumdati, section Nigri. The section Nigri includes 15 related black-spored species that may be confused with A. niger, including A. tubingensis, A. foetidus, A. carbonarius, and A. awamori.

Pathogenicity:

1. Plant disease: A. niger causes black mold of onions. Infection of onion seedlings by A. niger can become systemic, manifesting only when conditions are conducive. A. niger causes a common postharvest disease of onions, in which the black conidia can be observed between the scales of the bulb. The fungus also causes disease in peanuts and in grapes. The spore comes to common trees such as maple.
2. Human and animal disease: A. niger is less likely to cause human disease than some other Aspergillus species, but if large amounts of spores are inhaled, a serious lung disease, aspergillosis can occur. Aspergillosis is particularly frequent among horticultural workers who inhale peat dust, which can be rich in Aspergillus spores. Less commonly, it has been found on the walls of ancient Egyptian tombs and can be inhaled when the area is disturbed. A. niger is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss and, in severe cases, damage to the ear canal and tympanic membrane.

Aspergillus flavus is a mold fungus. It is a pathogen, associated with aspergillosis of the lungs and sometimes believed to cause corneal, otomycotic, and nasoorbital infections. It is believed to be allergenic and sometimes causes losses in silkworm hatcheries. It is particularly common on corn and peanuts, as well as water damaged carpets, and is one of several species of mold known to produce aflatoxin which is a carcinogenic substance.

Industrial uses: *A. niger* is cultured for the industrial production of many substances. Various strains of *A. niger* are used in the industrial preparation of citric acid (E330) and gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organization. Many useful enzymes are produced using industrial fermentation of *A. niger*. For example, *A. niger* glucoamylase is used in the production of high fructose corn syrup, and pectinases are used in cider and wine clarification. α-galactosidase, an enzyme that breaks down certain complex sugars, is a component of Beano® and other medications which the manufacturers claim can decrease flatulence. Another use for *A. niger* within the biotechnology industry is in the production of magnetic isotope-containing variants of

II. MATERIAL AND METHODS

1. Preparation of PDA Medium
2. Preparation of PDA plates
3. Streak Plate Method from Mixed Culture to Pure Culture

Sterilization of Maize Seeds

Take the seeds in a conical flask. Wash it in a tap water properly and then with a dilute Savlon or Dettol. Again wash 2 times in a tap water. Wash with 70% alcohol for half min. Transfer the seeds to sterile conical flask containing 0.1 % HgCL2 solution. Plug the flask and shake gently for 5-7 min. Decent the HgCL2 solution from the flask. Rinse the seeds 3-4 times with sterile DW properly. Transfer the seeds in a Petri dish. Seeds germinated in a dark, Calculate percentage of germination. Root formed kept in a light for further growth.

Sterilization of Soil

The garden soil is been taken and placed in a vessel. The vessel had been placed in a cooker so the micro-organisms or any of the contamination will not be there because of high temperature.

Extraction of Aflatoxins from Culture Media

Aflatoxin produced by fungi in culture media can be easily extracted in a chloroform and their toxicity can be assayed using bioassays.

Material

Aspergillus.niger or A.flavus grown for 10 days on Czapek's broth supplemented with casein to give 0.5g nitrogen / L at pH 4.5 as stationary culture.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Bucher funnel
Whatman No . 1 filter paper
Chloroform

For Czapek (Dox) Broth (CDB) -
Sodium Nitrate : 1gm
Potassium dihydrogen phosphate : 0.5gm
Magnesium Sulphate : 0.25gm
Ferrous Sulphate : pinch
Sucrose : 15.0 gm
Distilled water : 500ml

Method

For preparation of Czapek Broth mix all the ingredients required to prepare it, adjust Ph to 5.6 and sterilize for 15 min in an autoclave at 1.05 kg f/cm2

Filter the medium through Buchner funnel using Whatman No.1 filter paper. Extract 100ml of the culture filtrate thrice with equal volumes of chloroform. Cool the chloroform extracts and concentrate to dryness in a rotary evaporator. Dissolve the residue in minimum quantity of distilled water. If required for direct bioassay or in chloroform for chromatographic separation its toxicity on plant growth may be assayed without further purification

Bioassay of Aflatoxins

The toxicity of the aflatoxin extract obtained form culture filtrates on plant growth may be assayed using seed germination and seedling growth tests.

Materials

Aflatoxin extract
Maize seeds
Test tubes
Pipettes, 10 ml
Petri dishes
Filter dishes
Scale.

Methods

Inhibition of Seed Germination

Soak the surface sterilized seeds in 20 ml aliquots of culture filtrate on appropriate dilutions of the toxin extract for 24 hr in test tubes. Spread 100 of the treated seeds on moist filter paper placed in Petri dish. lncubate at room temperature. Maintain suitable control with seeds soaked in dist water .Count the germinated seeds after 7 days. Calculate per cent of germination.

Reduction in Seedling Vigour

Germinate the seed soaked in water in dishes. Select 12 germinated seeds with uniform shoot length measuring about 5mm and place them in dished lined with filter paper moistened with 5ml of the culture filtrate or toxin extract. Maintain control with distilled water. After 6 days, measure the total shoot growth of 10 seedlings avoiding both shortest and longest ones and measure elongation during 5 days incubation period.

When the aflatoxin extract is applied on the maize seed and the growth of specific fungus has been seen. The 40% seed's were germinated out of which only 15% showed development of seedlings . The growth of the maize plant was shunt and slow as compaired to the standard seedlings.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

III. OBSERVATION AND RESULT

Calculation

Percentage of germination of :
Percentage of germination = no. of germinated seeds/ Total no of seeds × 100
Percentage of germination of the standerd seed of maize is = 70/100 × 100 = 0.70
Percentage of germination of the infected seed of maize by Aspergillus . niger = 25/100 × 100 = 0.22
Percentage of germination of the infected seed of maize by Aspergillus .flavus = 45/100 × 100 = 0.45

Result :

Percentage of germination of the standerd seed of maize is = 0.70
Percentage of germination of the infected seed of maize by Aspergillus . niger = 0.22
Percentage of germination of the infected seed of maize by Aspergillus .flavus = 0.45

Thin Layer Chromatography

The separation and identification of organic compounds is a routine work in many service laboratories. This layer chromatography (tlc) is an easy technique to adopt for the said purpose. It is highly useful in research laboratories to separate, identify and characterize unknown compounds. A variety of small molecules like amino acids, sugar, organic acids, organic acids, lipids etc are separated by tlc technique. The greater advantage of tlc is the spread at which separation is achieved. When volatile solvents are used the time required to effect separation is only about 30 min and with nonvolatile solvents it is seldom longer than 90 min.

Principle

The general priniciple involved in tlc is similar to that of colume chromatography i.e, adsorption chromatography. In the adsorption process, the solute competes with the solvent for the surface sites of the adsorbent. Depending on the distribution coefficients, the compounds are distributed on the surface of the adorbent. Of course, in tlc the partition effect in the separation is also not ruled out. The adsorbent normally used contains a binding agent such as calcium sulphate which facilitates the holding of the adsorbent to the glass plate.

Materials

- Thin Layer Chromatography (tlc) Kit
- Ultra – Violet (UV) Chamber
- Mechanical Shaker
- Toluene
- Ethyl Acetate
- Formic Acid
- Chloroform
- Silica Gel G (tlc Grare)

Procedure

Extraction of Toxins

1. Weigh exactly 50g of ground sample material and transfer it into a 250ml conical flask.
2. Moist the material uniformally by adding 10 -15ml of distilled water and add about 200ml chloroform, stopper the mouth with a cotton plug in aluminium foil.
3. Shake the flask for one hour mechanically. (It is important that the oil – containing materials are defatted prior to extraction.)
4. Filter the slurry through a Buchner funnel under mild suction. Equal amount of a filtering aid such as celite may be mixed before filtering in order to ease filtration. Wash the flask and the slurry thoroughly with additional chloroform (25 ml) and collect the filtrate.
5. Transfer the filtrate quantitatively to a separatory funnel and shake with water one-half volume of

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

chloroform.After the phases separate,drain the bottom (chloroform) phase into a flask containing about 10g sodium sulphate (anhydrous) to absorb any water.

6. Concentrate the clear, chloroform extract over a warm water bath. Make up the concentrate to a known volume with chloroform and store in amber – coloured vials under refrigeration until analysis

Preparation of the Plates

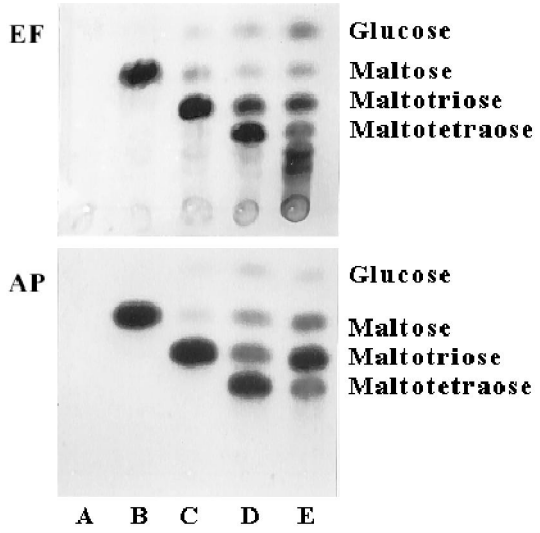
1. Place 30g silica gel G in a stoppered flask, share vigorously with 60-65ml distilled water for about one minute, transfer to the applicator and spread uniformally on glass plates (20 × 20 cm). The exact quantity of chloroform to get a good slurry . The thickness of layer should usually be 0.25 mm.
2. Allow the plates to dry for 1 hr in dust free conditions.
3. Divide the gel into a number of lanes by drawing lines on the gel with a sharp needle.
4. Spot different volumes of the sample extract in various lanes carefully with a capillary on a imaginary line 2.5cm away from one end of the plate.
5. Develop the plate in a solvent system of toluene: ethyl acetate: formic acid (6: 3: 1) in a chromatographic tank for about 50min. By then, the solvent front might have moved up to 20mm below the top end of the plate.
6. Dry the plate at room temperature to remove the solvent. Visualize the fluorescing spot of toxin under UV light; otherwise, eye sight will be affected.
7. Identify each fluorescing spot of the sample extract .Determine the Rf value of each spot.

Calculation:

Rf = Distance (cm) moved by the solute from the origin
Distance (cm) moved by the solvent from the origin
For a fluoresce green (G) G1 $ G2
Rf of Aspergillus niger = 14.6/20 = 0.73
Rf of Aspergillus flavus = 15.3 / 20 = 0.77

Result :

Rf of Aspergillus niger = 0.73
Rf of Aspergillus flavus = 0.77



Photoplate.1: Thin-layer chromatographic (TLC) analysis of malto-oligosaccharides digested by maize EF and 2% amylopectin (AP) culture filtrates. Culture filtrates were incubated for 8 h at 40°C with **A,** no substrate; **B,** 0.6% maltose; **C,** 0.6% maltotriose; **D,** 0.6% maltotetraose; or **E,** 0.6% maltoheptaose. After chromatographic development, products were detected by spraying the TLC plates with 30% sulfuric acid solution and charring at 100°C.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Extraction and Detection of Nuleic Acids

The nucleic acid consist of Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).

Extraction and Detection of DNA from Infected Seeds

Requirement:

Racicals of germinating seeds

15 % TCA (Trichloro acetic acid)
10 % NaCl (Sodium chloride)
Chilled etanol
EDTA (Etylene diamine tetra acetic acid) powder
Grinder (motor and pestle)
Centrifuge
Test tubes.

Extraction of DNA

Weigh 1gm radicals of germinated seeds and cut into small pieces. To this add a pinch of EDTA powder and grind it in a grinder or motor pestle. Then add 5ml of TCA (removes fats and carbohydrates). Take the contents in a centrifuge tube and centrifuge the solution at 5000rpm. Discard the supernatant and to the ppt and 10ml of 10%NaCl (to dissolve DNA solution). Boil the content in a water bath for 30 mins. Cool the solution and again centrifuge at 5000rpm for 5mins.Take the supernatant and chill it. To this add equal quantity of chilled ethanol. DNA appears at the junction of two liquids. Shake the tube and keep it in ice-box for 30mins. After 30mins centrifuge the content to separate the DNA ppt. Discard the supernatant and collect the ppt.
Put a drop of Acetocarmine stain. The DNA ring becomes purplish pink in colour.

Chemical Preparation:

15%TCA :1.5gm of TCA in 30ml of distilled water.
10%NaCl : 10gms of NaCl in 100ml of distilled water.

Detection of nucleic acid

The nucleic acids absorb strongly in the ultra violet region of the spectrum (due to conjugate double bond system). They show characteristic maxima at 260 nm and minimum at 230nm but such a low range is not possible so minimum OD at

Result of OD:

Standard material: 0.56
Aspergillus. flavus : 0.66
Aspergillus .niger : 0.45

Extraction and Detection of RNA from Plant Material

Requirement:

Maize seeds, saline EDTA (Ethylene diamine tetra acetic acid), Phenol 80%, chilled ethyl alcohol, centrifuge, pestle and mortar.

Procedure:

Weigh 1gm of maize and cut it into small pieces. Crush the maize seeds grained in 5ml of saline EDTA or distilled water using pestle and mortar. To this add 10ml 0f 80% Phenol. Shake the mixture and centrifuge it. Take the supernatant and 2 volumes of chilled ethanol. Keep it in refrigerator overnight. Centrifuge the next day and collect the RNA ppt.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Detection of RNA

Dissolve the RNA crystals from the previous experiment in 1ml of water. Also dissolve 0.5gmsof RNA crystals. Use these two materials for descending chromatography on Whatman No .1 PAPER. After running the chromatogram dry it in dry air for 4 hours. And locate the bases under ultra violet light. Both will be same.

Preparation:

80% Phenol. Take 80ml of Phenol and 20ml of distilled water.

Calculation :

Rf = Distance (cm) moved by the solute from the origin
Distance (cm) moved by the solvent from the origin
Rf of Pure maize = 8.1/8.9 = 0.91
Rf of Aspergillus niger = 7.2/8.4 = 0.84
Rf of Aspergillus flavus = 7.3/8.2 = 0.89

Result :

Rf of Pure maize = 0.91
Rf of Aspergillus niger = 0.84
Rf of Aspergillus flavus = 0.89

REFERENCES

[1]. Agricultural science and Technology, Report No.116 pp91.
[2]. Eaton,D.L. and Groopman,J.D.1994.The Anon.1989.Mycotoxins , Economic and Health Risks.Council for Toxicology of Aflatoxins. Academic Press, New York.pp383-426.
[3]. Finley,J.W.,Robinson,S.F. and Armstrong ,D.J.1992.Food Safety Assessment. American Chemical Society, Washington, D.C. pp261-275.
[4]. Goldbatt, L.A.1969.Aflatoxin.Academic Press, New York. Pp1-40.
[5]. Heathcote,J.G. and Hibbert,J.R. 1978. Aflatoxins : Chemical and biological aspect. Elsevier, New44. Thrasher JD (2000) Are chlorinated pesticides a causation in maternal mtDNA mutations? Arch Envir Hlth 55:292.
[6]. Niranjan BG *et al* (1982) Preferential attack of mitochondrial DNA by aflatoxin B1 during hepatocarcinogenesis. Science 215:73.
[7]. Niranjan BG *et al* (1986) Protection of mitochondrial genetic system against aflatoxin B1 binding in animals resistant to aflatoxicosis. Cancer Res 56:3637.
[8]. Meki ET *et al* (2001) Aflatoxin B1 induces apoptosis in rat liver; protective effect of melatonin. Neuroendocrinol 22:417.
[9]. Baron CC *et al* (2000) Lipopolysaccharide augments aflatoxin B(1)-induced liver injury through neutrophil-dependent and -independent mechanisms. Toxicol Sci 58:208.
[10]. Liener,I.E.1969.Toxin constituents of plant foodstuffs. Academic Press , New york.pp392-394.
[11]. Wyllie,T.D. and Morchause,L.G. 1978.Mycotoxin Fungi, Mycotoxins, Mycotoxicoses-An Encyclopedic Handbook.Vols.1,2, and 3.Marcel Dekker,Inc.New york.
[12]. Abdollahi, A., and Buchanan, R. L. 1981. Regulation of aflatoxin biosynthesis: Characterization of glucose as an apparent inducer of aflatoxin production. J. Food Sci. 46:143-146.
[13]. Abdollahi, A., and Buchanan, R. L. 1981. Regulation of aflatoxin biosynthesis: Induction of aflatoxin production by various carbohydrates. J. Food Sci. 46:633-635.
[14]. Blanco-Labra, A., and Iturbe-Chinas, F. A. 1981. Purification and characterization of an a-amylase inhibitor from maize (*Zea maize*). J. Food Biochem. 5:1-17.
[15]. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

[16]. Brown, R. L., Cleveland, T. E., Payne, G. A., Woloshuk, C. P., Campbell, K. W., and White, D. G. 1994. Determination of resistance to aflatoxin contamination in maize kernels and of fungal colonization using an *A. flavus* transformant expressing *Escherichia coli* b-glucuronidase. Phytopathology 85:983-989.

[17]. Brown, R. L., Cotty, P. J., Cleveland, T. E., and Widstom, N. W. 1993. Living maize embryo influences accumulation of aflatoxin in maize kernels.J. Food Prot. 56:967-971.