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The Inhibitory Action of *Parthenium Hystrophorous* Plant Extract Against Aflatoxin and Aflatoxin Producing Fungus *Aspergillus Flavus*

Neha Guttikonda, Chetan Godale* and Shrutin Yemul
Walchand College of Arts of Science, (Affiliated to Solapur University), Solapur-413006, Maharashtra, India.
***Chetan Godale, Assistant Professor, Department of Genetics, Walchand College of Arts and Science, Solapur-413006, Maharashtra, India.**

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***Corresponding Author Email:** chetanhg87@gmail.com

Abstract
Aflatoxins are toxic, mutagenic, carcinogenic and teratogenic secondary metabolites posing health hazards to humans, animals and also adversely affect agricultural product. These are mycotoxins that are produced by *Aspergillus flavus* and *Aspergillus parasiticus* species of fungi that classically affect cereals which are components of both foods as well as feed products. Aflatoxins B1, B2, G1 and G2 are commonly found in food. It has a cluster of approximately 20 related fungal metabolites. *Parthenium hysterophorus L.* is an aromatic yearly and obnoxious invasive herb under Asteraceae family which shows anti-inflammatory, antimicrobial, anti-cancerous, pesticidal, thrombolytic activities. The antifungal activity was checked against *Aspergillus flavus* by agar well diffusion method. The antifungal effect shows positive results i.e , zone of inhibition against selective fungal species. The results showed leaves of *Parthenium hysterophorus* are effective against selective fungal species. Hence it is effective to decrease the amount of aflatoxins. The Aflatoxin was detected using TLC.

Keywords
Aflatoxin, *Parthenium hysterophorus* and *Aspergillus flavus.*

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INTRODUCTION
Aflatoxins are a group of naturally occurring mycotoxins that are produced by *Aspergillus flavus and Aspergillus parasiticus*, species of fungi that typically affect staple food, which are ingredients, used in both food and feed products [1]. Mycotoxins consist of a group of relatively 20 related fungal metabolites, although only aflatoxins B1, B2, G1 and G2 are

normally found in foods. Aflatoxins consist of a group of approximately 20 related fungal metabolites, although only aflatoxins B1, B2 and G1 are normally found in foods [5]. They are produced by at least three species of Aspergillus, *Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius*, and can occur in a wide range of important raw food commodities, including cereals, nuts, and dried fruit [4]. Mycotoxins are crystalline substances, freely soluble in moderately polar solvents such as chloroform, methanol and dimethyl sulfoxide, and dissolve in water to the extent of 10-20 mg/litre. They fluorescence under UV radiation. Aflatoxins are both acutely and chronically toxic. Aflatoxin B1 is most potent hepato-carcinogens known, and hence the long-term chronic exposure to extremely low levels of aflatoxins in the diet is an important consideration for human health [5]. In the collected, advanced areas of the world, acute poisoning in animals is rare and in man is extremely unlikely. The 'Turkey-X disease', which caused the deaths of 100,000 turkeys and other poultry in the United Kingdom in 1960, was caused by extremely high concentrations of aflatoxins in imported groundnut meal [5]. The liver is the main target organ, although the section of the hepatic effect alters with species. Effects on the lungs, myocardium and kidneys have also been detected and aflatoxin can aggregate in the brain. Aflatoxins have been involved in sub-acute and chronic effects in humans. These effects consist of primary liver cancer, chronic hepatitis, jaundice, and cirrhosis through repeated ingestion of low levels of aflatoxin. Aflatoxin B1 is an effective mutagen causing chromosomal aberrations in a diversity of plant, animal and human cells [4].

*Parthenium hysterophorus*L. is an aromatic yearly and disagreeable invasive herb under Asteraceae family, now commonly known as feverfew, *Tanacetum parthenium, Chrysanthemum parthenium* [2]. The regular height of this straight plant is up to 1 m but under favorable conditions the height may reach up to 2 m having deeply penetrating taproot with many barely separate feeding roots and an angular, longitudinally grooved and profusely branched hairy stem [2]. Besides the harmful effects, *P. hysterophorus* shows anti-inflammatory, antimicrobial, anti-cancerous, pesticidal, thrombolytic activities. The decoction of *P. hysterophorus* has been used in ancient medicine to treat fever, diarrhoea, neurologic disorders, urinary tract infections, dysentery, malaria [3].

MATERIAL AND METHODOLOGY

Collection of plant material

The leaves of the *P. hysterophorous* collected from the of Walchand College, Solapur, for the study of anti-fungal activity.

Microorganism and maintenance of culture

Fungi *A. flavus* was procured from the college microbiology lab of walchand centre for biotechnology, solapur, Maharashtra., India which has a special feature of aflatoxin production. The strain was subcultured on Potato Dextrose Agar (PDA) medium at 37°C for 7 days under facultative aerobic conditions [4].

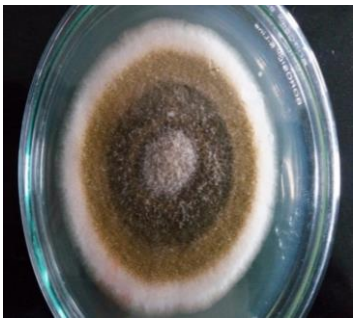


Figure 1- Maintained subculture

Detection of *A. flavus* toxin

Inoculum preparation

The test organism *A. flavus* was subcultured for 4 days. The spores from the slant was suspended in 3 mL of distilled water. This was used as the inoculum for the fermentative production of aflatoxin [4].

Aflatoxin production

50 mL of production medium (Yeast extract sucrose medium) whose pH adjusted to 6.0-6.4 was taken in 250 mL Erlenmeyer flask and sterilized by autoclaving at 15 psi, 120°C for 15-20 minutes and then allowed to cooled. The flasks were inoculated with 1mL of the above spore suspension and thoroughly mixed. These flasks with the inoculum were incubated for 8 days at room temperature as stationary culture [4].

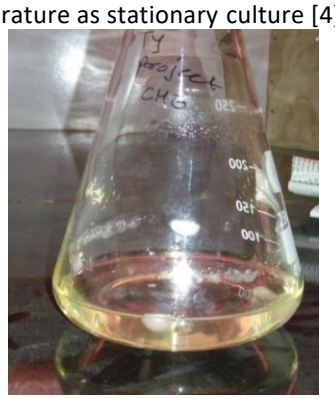


Figure 2- Aflatoxin production by submerge fermentation

Toxin extraction

45 g of ground sample material was collected from the medium which was moistened uniformly by adding 10-15 mL of distilled water followed by 200 mL of chloroform and kept for shaking in a shaking incubator for 1h. The material was filtered through a Buckner

funnel under mild suction and the filtrate was collected. The filtrate was transferred quantitatively to a separatory funnel and it was shaked with water, one half volume of chloroform. After the phase separates, the chloroform phase was drained into a flask containing about 10 g sodium sulfate (anhydrous) to absorb any water. By using Quick fit distillation set up, the aflatoxin was extracted from the chloroform and stored in amber-colored vials under refrigeration for analysis [4].

Detection of toxin

For the detection of aflatoxin, the extract was subjected to TLC. Thin layer chromatographic technique of the clean. extract was done on seep silica gel plates (Merck, Silica Gel 60, 25 mm, 20x20). Detection of the different aflatoxins was carried out according to standard procedures. A mixture of methanol: acetic acid: ethanol (50:40:10) was used as a mobile phase. Aflatoxins were visualized under UV light at 365 nm in a chromatovisor. Aflatoxin had a retention time and fluorescent spot similar to the standard aflatoxins being tested. Trifluroacetic Acid (TFA) was directly super-imposed on to the aflatoxin extract spot before development. After reaction, the plate was developed and examined under UV light for the presence of the blue fluorescent spot of B2, which can be recognized with the comparison to the B1 standard [4].

Antifungal Activity Assay

APA media preparation

The 20g of grated coconut was taken and 1% aspargine (1 gm) was added.The volume was make up by 100ml sterile distilled water, PH was adjusted to 4.5.The media was autoclaved and distributed in 4 test tubes under aseptic conditions [4].

Antifungal activity using APA broth

The inoculum was prepared by suspending spores of 7days culture of A.flavus in 0.1% Tween 80 solution.Then the spore suspension was poured in broth as inoculum (1ml) using micropipette in coconut broth. The tubes were labelled as control and in increasing concentration, the leaf extract was added in increasing concentration as per label and incubated for 7days in dark at 25^{o}C [4].

Antifungal activity using well diffusion method

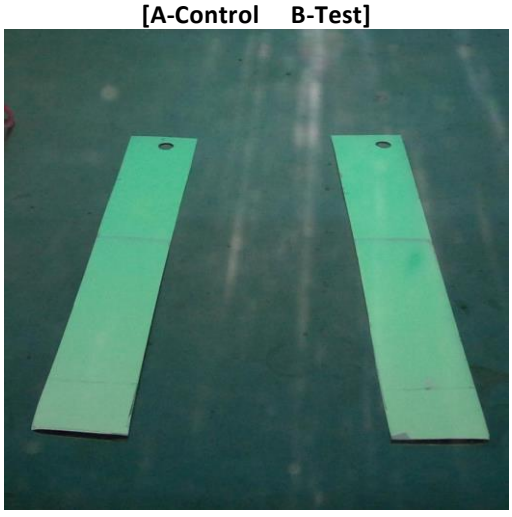
After the preparation of leaf extract using solvents like methanol and ethanol. The PDA plates were prepared. 0.1µl inoculum of A. flavus was spreaded on PDA plates. Then wells were prepared and 100µl leaf extract was put into the wells and plates were incubated for 24 hours.

Results and Discussion

Detection of toxin

The detection of the toxins was carried out by performing TLC. We observed blueish green florescent bands under UV. similar results for aflatoxin detection under UV light by using TLC were observed by Basil D'Mello *et al* 2014. Greenish colored bands observed by Basil D'Mello *et al.*

[A-Control B-Test]



A **B**

Figure 3 -Aflatoxin bands observed under UV

Antifungal activity using well diffusion method

As we added leaf extract made using solvents as Methanol and Ethanol, after incubation we had observed that the leaf extract shows very small amount of zone of inhibition.

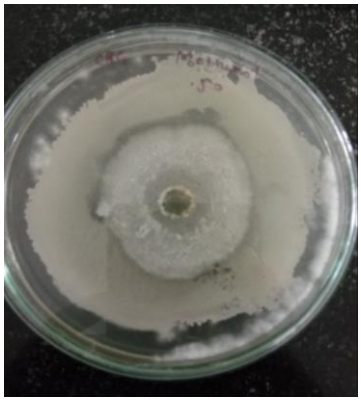


Figure 4: zone of inhibition shown by *P.hysterophorous* against *A. flavus* (methonal)

APA broth

According to the graph we observed that as the leaf extract concentration increases, the rate of mycelial growth decreases. From which we concluded that amount of Aflatoxin also gets decreased.

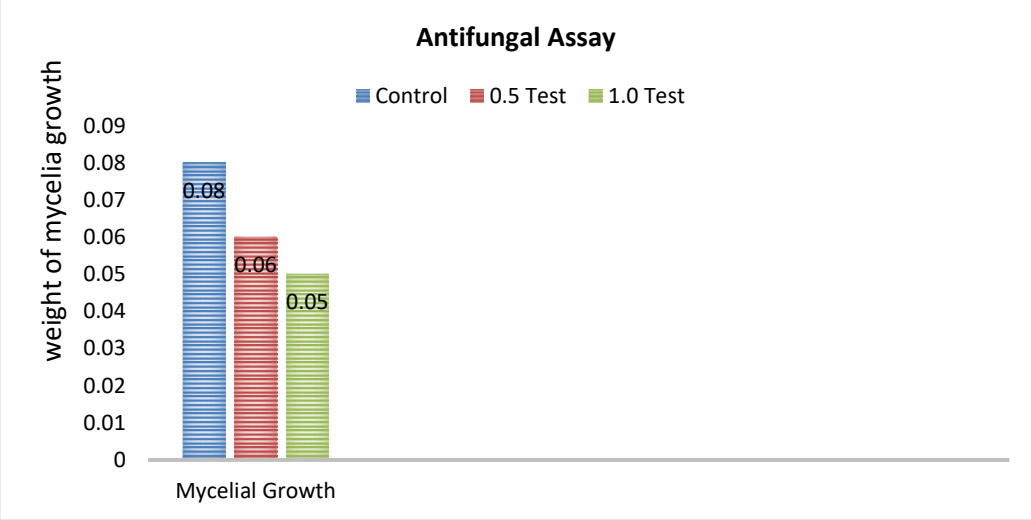


Figure 5: Graphical representation of inhibition of Aflatoxin.

CONCLUSION

From the results of antifungal activity assay, we can conclude that methanolic leaf extract gives better results i.e. zone of inhibition. With increase in amount (concentration) of leaf extract, the rate of mycelial growth decreases which indicates the decrease in production of Aflatoxin. So, we can use the *parthenium hysterophorous* leaf extract to prevent the growth of aflatoxin producing *A. Flavus.*

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