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RESEARCH ARTICLE

INHIBITORY EFFECT OF THE ACETONE EXTRACT OF MAIZE LEAVES ON THE MYCELIAL GROWTH OF *ASPERGILLUS PARASITICUS* ALONG WITH ITS AFLATOXINS PRODUCTION.

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Abstract

This study shows the anti-fungal and anti-aflatoxigenic activities of acetone extract of Maize leaves. As contamination caused by *Aspergillus parasiticus* and aflatoxin is a major challenge in agriculture and food industry, we tried to inhibit the mycelial growth of *A. parasiticus* and aflatoxins production. Here, we investigated the effect of acetone extract of hybrid stain of maize leaves (Malviya Hybrid Makka) on the growth of *A. parasiticus* and aflatoxin production. Phytochemical screening of this extract was also carried out which revealed the presence of many anti-fungal and anti-aflatoxigenic compounds such as proteins, carbohydrates, flavonoids and terpenes. The extract showed 82.89% inhibition of the growth of *A. parasiticus* mycelial and 100% inhibition of the aflatoxins production at the concentration of 250µl/ml and 200µl/ml respectively. TLC method was used for qualitative and quantitative analysis of the aflatoxins that revealed the bands of aflatoxin B1, B2, G1 and G2, and indicated that B1 is found in higher concentration than other aflatoxins. The overall study indicates that acetone extract of Maize leaves possess good potential to arrest the growth of *A. parasiticus* mycelial and its aflatoxins production.

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Introduction:-

Aspergillus parasiticus is a pathogenic fungus and many agricultural products are susceptible to infection of this mold. It affects mainly corn, peanuts, cotton seeds, tree nuts, etc (Mishra and Dubey, 1994). *A. parasiticus* produces aflatoxins that are known as carcinogenic, mutagenic, toxigenic and teratogenic in nature (Patten, 1981; Kumar *et al.*, 2017a). Aflatoxins are considered as "Class 1 Human Carcinogen" by the International Agency for Research on Cancer (IARC) (Williams *et al.*, 2004). Aflatoxins alter the root elongation, seedling growth, chlorophyll synthesis and seed germination (Jones *et al.*, 1980). Tropical and subtropical countries are facing the problem of *A. parasiticus*

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as well as aflatoxins contamination most commonly due to their hot and humid climatic conditions. These climatic conditions are favourable for fungal growth and aflatoxins production (Kumar *et al.*, 2017b). Some time food items are destroyed by oxidative stress due to free radical generation. The free radicals damage many cellular molecules such as nucleic acid, proteins and lipids (Prakash *et al.*, 2015). Aflatoxins production, oxidative stress and free radical generation are directly proportional to each other (Jayashree and Subramanyam, 2000). Contamination of *A. parasiticus* and aflatoxins in cereal crops are a matter of serious concern around the world, and there is an urgent need to solve this problem.

Many synthetic food preservatives are used to prevent the contamination of fungus and mycotoxins, but these preservatives some time show harmful effects in human life. Synthetic food preservatives such as hexachlorobenzene (HCB) cause prophyria or poisoning in human (Jones *et al.*, 1980). Other preservatives like captan, captafol and folpet cause irritation in skin, dermal sensitization and many respiratory problems (Royce *et al.*, 1993; Villaplana and Romaguera, 1993). It has been reported that synthetic preservatives like formaldehyde, nitrates, sorbates, butylatedhydroxyanisole and butylatedhydroxytoluene give the symptoms of hypersensitivity, hyperactivity, allergy, cancer and asthma (Anand and Sati, 2013).

Plants contain many types of secondary metabolites, which possess anti-fungal and anti-bacterial properties (Sharma and Sharma, 2012). Extracts of many plants showed antifungal properties against the growth of *Aspergillus* species (Thanaboripat *et al.*, 2004; Krishnamurthy and Shashikala, 2006). Present study was designed to reveal the anti-fungal and anti-aflatoxigenic properties of the acetonic extract of Maize leaves against the infection *A. parasiticus* and aflatoxins production.

Materials and Methods:-

Pathogenic organism used:-

The toxigenic strain of *A. parasiticus* was procured from Department of Food Science & Technology, Pondicherry University, Pondicherry, India. The culture was revived and maintained on fresh Czapek-Dox Agar (CDA) medium. The CDA culture plates were incubated for next 10 days at 28°C to ensure purity and viability.

Preparation of acetonic extract:-

Fresh leaves of maize (Malviya Hybrid Makka) were collected from agricultural regions of Dr. Rajendra Prasad Central Agriculture University at Pusa, in Samastipur district and kept in sterilized collecting polybag. These maize leaves were properly cleaned with running tap water followed by distilled water trice. Thereafter, leaves were dried in an oven for 72 hours. The leaves were crushed entirely by using mortar and pestle. About 60 grams of crushed leaves were weighed, and kept back into a sterilized conical flask. 300 ml of acetone was added into the flask and left for 72 hours. Afterword, the solution was filtered through Whatman filter paper no. 1, and filtrate was collected into a flat bottom dish. Evaporation of the acetonic extract was performed at standard room temperature till dryness. The dry residue was again dissolved in small amount of acetone and water in the ratio of 50:50 (v/v), and kept at 4°C for the experimental work.

Antifungal activity:-

Agar plate diffusion method was employed to analyze the antifungal activity of the acetonic extract (Kumar, 2017). Different acetonic extract concentrations (50µl, 100µl, 150µl, 200µl and 250µl/ml) were added into 20 ml of molten CzapekDox Agar (CDA) medium in different Petri plates. Freshly prepared fungal disc (5 mm diameter) of *A. parasiticus* was spotted at the centre of each petri plate containing different concentrations of the acetonic extract. One Petri plate had taken as control, containing only 20 ml of CDA medium and a fungal disc (5 mm diameter) of *A. parasiticus*. In next step, all petri plates were incubated at 28±2°C for next 10 days under controlled condition. After the incubation period, the mycelial development of the *A. parasiticus* was measured in diameter (in cm) on the medium of each petri plate. Percentage of inhibition of the growth of the *A. parasiticus* was calculated by the following formula:

$$\text{Percentage of inhibition of growth of test fungi} = \frac{DC - DI}{DC} \times 100$$

Where, DC = Average increase in mycelial growth in the control sample.

DT = Average increase in mycelial growth of the test sample.

Inhibition of aflatoxins production:-

To analyze the anti-aflatoxigenic properties of acetonetic extract from Maize leaves, Czapek Dox Broth (CDB) medium was arranged. The sterilized fresh CDB media were poured into different conical flasks (25 ml). The requisite amount of acetonetic extract of different concentrations (50µl/ml, 100µl/ml, 150µl/ml, 200µl/ml and 250µl/ml) was added into each flask containing CDB medium. The fungal discs of 5 mm diameter were inoculated into each flask containing different concentrations of the acetonetic extract. A flask containing only 25 ml of CDB medium and a fungal disc (5 mm diameter) was used as control. Subsequently, all flasks were incubated at 28±2°C for 10 days. After 10 days, the mycelium of *A. parasiticus* of each flask was filtered through Whatman filter paper no. 1. All the filtered mycelium of *A. parasiticus* was autoclaved to kill the spore pathogenicity. Then, the fungal mycelium were dehydrated entirely in an oven (at 80°C) and weighed. The dried remnants were extracted by using 20 ml chloroform, and chloroform extract was stored at room temperature to evaporate till complete dryness. Again, 1ml of chloroform was added into residue. 60 µl of the chloroform extract was loaded on the thin layer chromatography (TLC) plates following the method of Turner and his colleagues (Turner *et al.*, 2009) for confirmation of aflatoxin presence. Solvent system for TLC plate was prepared by the mixture of chloroform and acetone in the ratio of 9: 1 (v/v). After running the test sample, the TLC plate was dried and then transferred into a UV transilluminator (360 nm) to authenticate the presence of aflatoxins. The blue and green colour bands were observed on TLC plate under UV transilluminator. The blue and green colour bands indicated the presence of aflatoxins in the mold. All bands were scratched from the TLC plate, and dissolved in 5ml methanol followed by centrifugation at 3000 rpm for 5 minutes. Absorbance of supernatant was noticed at 360 nm, and the quantification of aflatoxins was done by using following formula:

$$\text{Aflatoxin concentration } (\mu\text{g/g}) = \frac{D \times M}{E \times L} \times 1000$$

Where, D = Absorbance, E = Molar extinction coefficient of aflatoxins, M = molecular weight of aflatoxins, L = Path length.

Phytochemical Screening:-

Test for different types of phytoconstituents such as proteins, carbohydrates, terpenes and flavonoids were carried out of the acetonetic extract of Maize leaves according to the method of Harbone (Harborne, 1993).

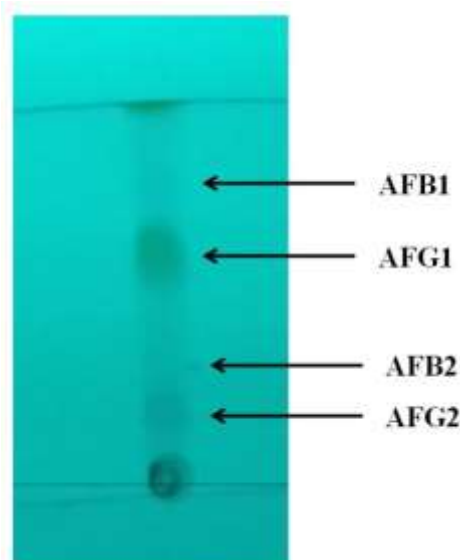
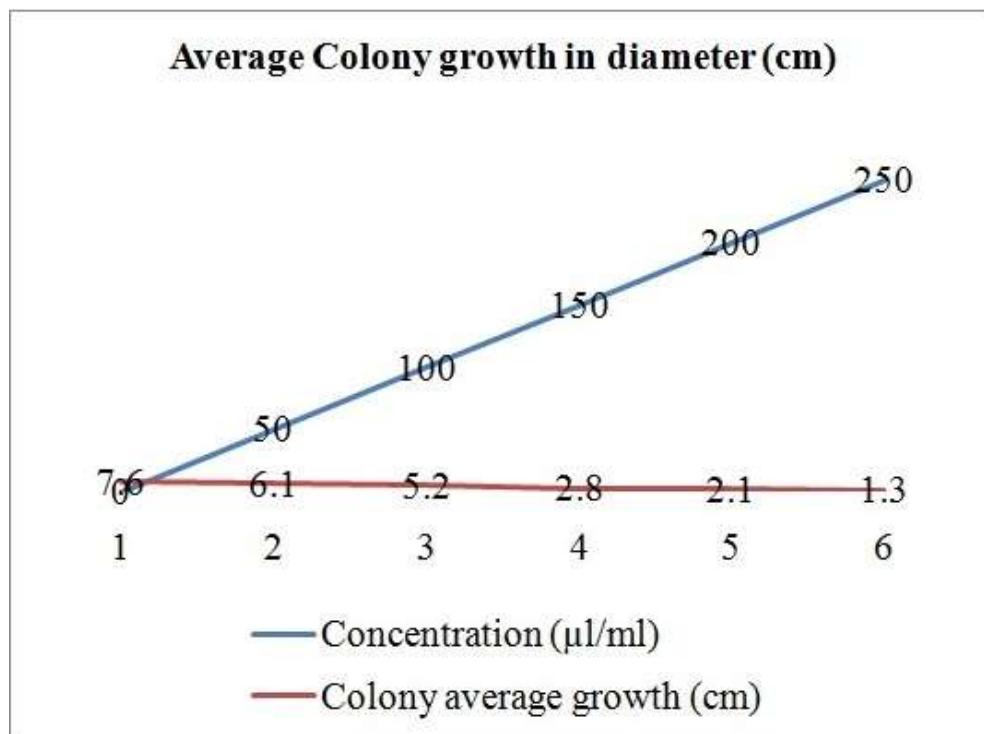


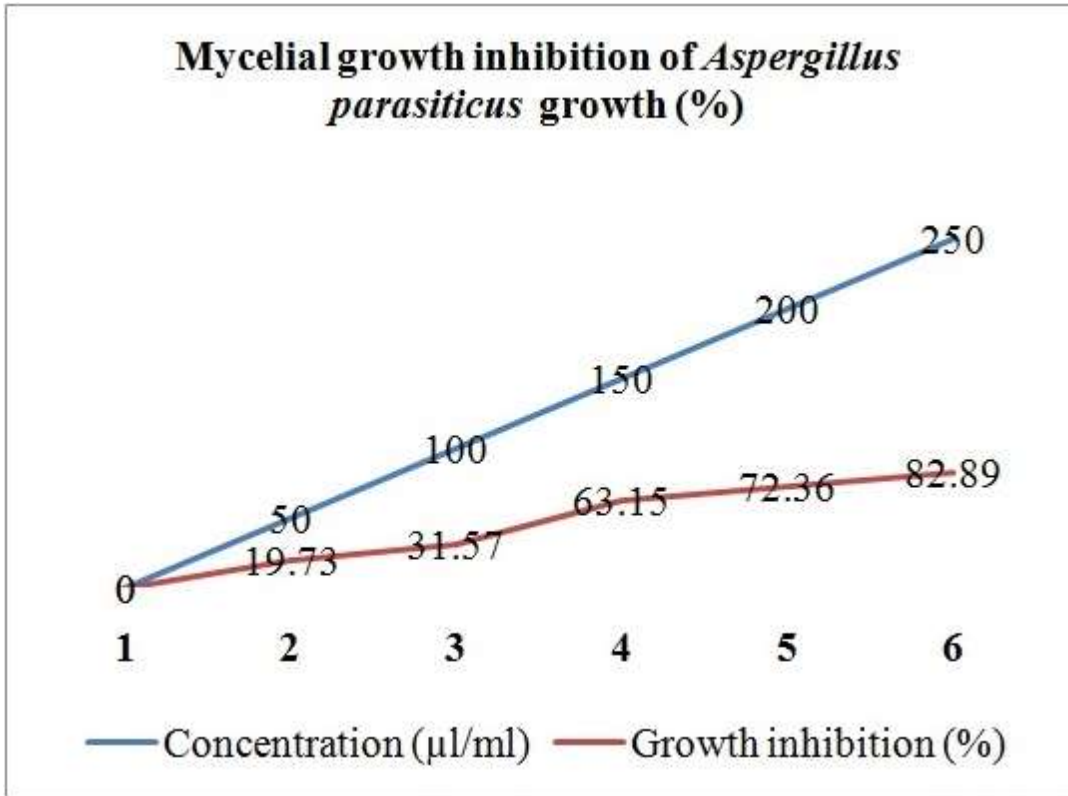
Fig1:-TLC plate of the extract untreated sample is showing the bands of aflatoxins



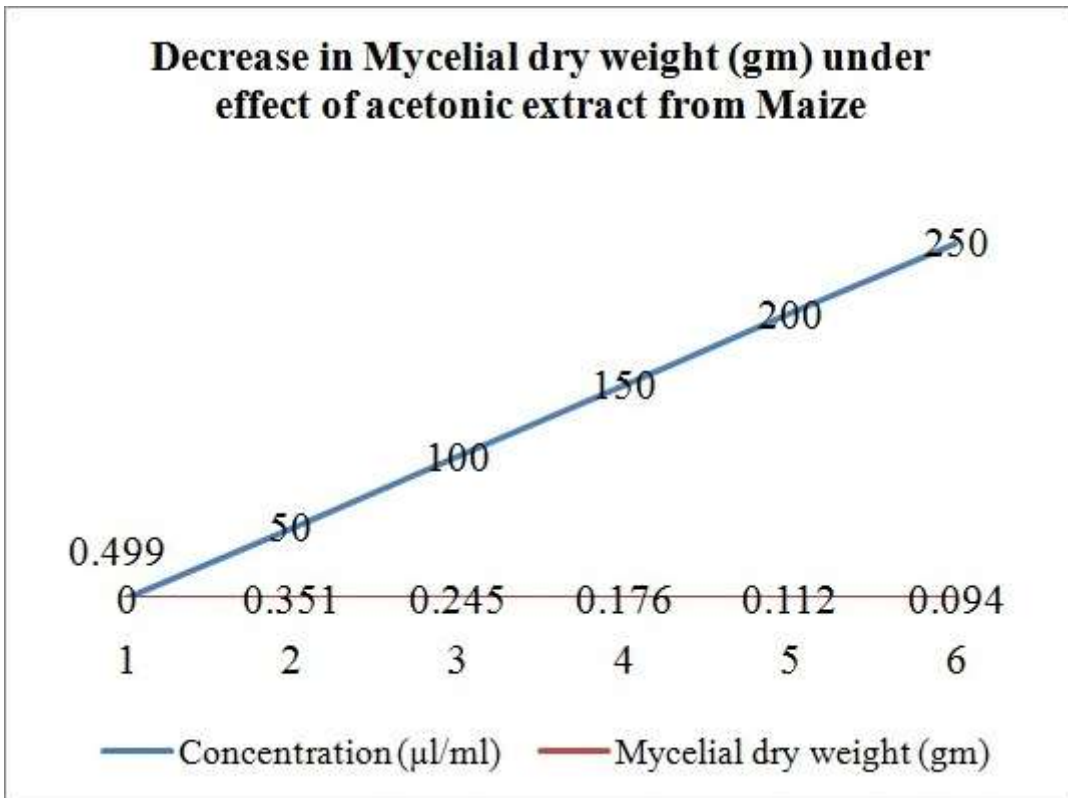
Fig2:-TLC plate of the extract treated sample is showing the sample is not showing any bands of aflatoxins.



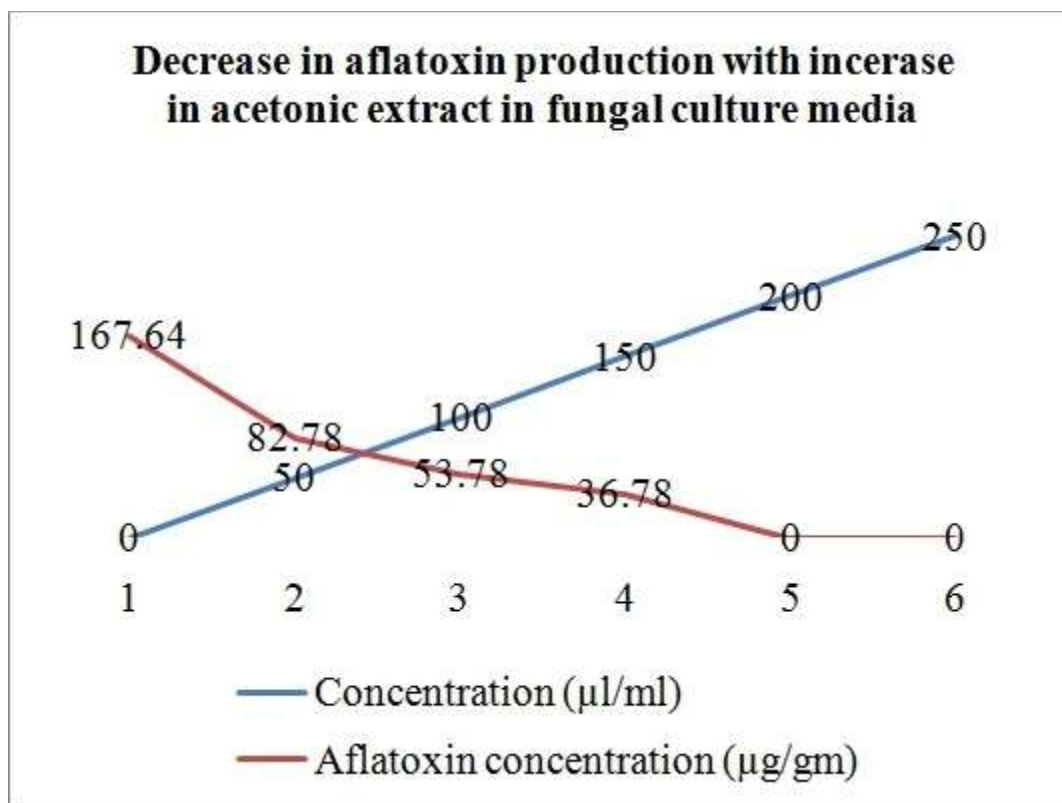
Graph1:- Average Mycelial Growth in Diameter (cm)



Graph2:- Inhibitory effect of the mycelial growth of *A. parasiticus* in percentage



Graph3:- Effect of acetic extract from Maize on mycelial dry cell mass (gm)



Graph4:- Inhibitory effect of Acetonic extract from Maize leaves on aflatoxin production.

Result and Discussion:-

Antifungal activity of the acetonic extract of Maize leaves on *A. parasiticus* has been showed in **graph1**. The result revealed that this extract has antifungal activity against the growth of *A. parasiticus* basically on dose depended manner. The inhibitory effect of the mycelial growth of *A. parasiticus* was shown in **graph2**. The extract untreated sample (control) showed well growth of *A. parasiticus* but the extract treated sample showed 82.89% inhibition of the growth of this mold at the concentration 250µl/ml.

Qualitative analysis of TLC plates under 360 nm UV light confirmed the presence of the four different types of aflatoxins such as B1, B2, G1 and G2. Quantitative studies revealed that the quantity of aflatoxins B1 was higher than other aflatoxins (B2, G1, and G2). The acetonic extract untreated sample (control) showed the bands of aflatoxins B1, B2, G1 and G2 on TLC plate (**Fig1**), but the acetonic extract treated sample did not show the bands of any aflatoxins on TLC plate at the concentration of 200µl/ml (**Fig2**).

The **graph3** is presenting the anti-aflatoxigenic effect of the acetonic extract of Maize leaves. The result of anti-aflatoxigenic activity revealed that this extract has capacity to inhibit the aflatoxins synthesis by *A. parasiticus*. The **graph4** presents that the extract showed 100 % inhibition of aflatoxins production at the concentration of 200 µl/ml. The mycelium growth and aflatoxins production were analysed to decrease on increasing the concentration of the acetonic extract. It was also analysed that decrease in mycelial biomass leads to decrease in aflatoxins production. Therefore, mycelial growth must be controlled below the limit to arrest the synthesis of aflatoxins.

Qualitative phytochemical screening of the acetonic extract of Maize leaves showed the presence of proteins, carbohydrates, terpenes and flavonoids. It has been reported that flavonoids have potential to inhibit the production of aflatoxins (Mallozi *et al.*, 1996; Patel *et al.*, 2010). Flavonoids, singly or in combination with other phytochemical compounds might be responsible for the antifungal and anti-aflatoxigenic properties of the acetonic extract of Maize leaves.

Conclusion:-

The above findings clearly indicate that acetonetic extract of the Maize leaves has contains some essential organic compounds. These compounds possess inhibitory effect on the growth of *A. parasiticus* along with its aflatoxins production. This extract may be used as plant based preservative to save foodstuff and cereal crops from the harmful effects of *A. parasiticus* and aflatoxins.

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