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RESEARCH ARTICLE

EFFECT OF ACETONE EXTRACT OF *ZEA MAYS* L. LEAVES ON THE GROWTH OF *ASPERGILLUS FLAVUS* AND AFLATOXINS PRODUCTION.

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Abstract

Present study outlines the antifungal and anti-aflatoxigenic effect of the acetone extract of *Zea mays* L. leaves, cultivar Pioneer- 30V92. As contamination of *Aspergillus flavus* is a major challenge in agriculture and food industry, we tried to inhibit the growth of *A. flavus* along with its aflatoxins production. Acetone extract of *Zea mays* L. leaves of strain Pioneer-30V92 was investigated for its antifungal and anti-aflatoxigenic activities against the aflatoxigenic strain of *A. flavus*. Phytochemical study revealed the presence of proteins, carbohydrates, flavonoids and terpenes in the extract. The antifungal activity of this extract against the growth of *A. flavus* was investigated by agar plate diffusion method, and anti-aflatoxigenic activity was determined by using Czapek Dox Broth (CDB) medium. TLC was employed for qualitative and quantitative study of the aflatoxins. Overall experimental results indicated that acetone extract of *Zea mays* L. leaves (Pioneer-30V92) has good inhibitory effect on aflatoxins production and growth of the *A. flavus*

Introduction:-

Aspergillus flavus is a pathogenic fungus and approximately 25-40% of raw world agricultural products are susceptible to infection by this mold (Sharma and Sharma, 2012). It destroys foodstuffs and grains such as corn, peanuts, cottonseeds, tree nuts, etc. (Mishra and Dubey, 1994). *A. flavus* produces aflatoxins, which are a class of mycotoxin and considered as toxigenic, carcinogenic, mutagenic, and teratogenic in nature (Patten, 1981). Aflatoxins are named "Class 1 Human Carcinogen" by the International Agency for Research on Cancer (IARC) (Williams *et al.*, 2004). Aflatoxins inhibit seedling growth, root elongation, carotenoid synthesis, chlorophyll synthesis, seed germination and production of some enzymes in plants (Jones *et al.*, 1980) Tropical and subtropical countries are facing the problem of aflatoxins contamination at large scale due to their hot and humid climatic conditions, which are favorable for mold growth and mycotoxin production. During prolonged storage, food items are damaged by oxidative stress due to free radical generation that leads to the damage of important cellular molecules such as proteins, nucleic acid and lipids (B. Prakash *et al.*, 2015). There is direct relation between aflatoxins production, oxidative stress and free radical generation (Jayashree and Subramanyam, 2000). Aflatoxins contamination of foodstuffs and other items have imposed a challenging scenario across the world and there is an urgent need to get rid of the problem.

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Many synthetic food preservatives are being used as fungicides to prevent food items from contamination, but prolonged use of these synthetic preservatives show adverse effects on human health. For example, hexachlorobenzene (HCB) treated grains cause prophyria or poisoning in human beings (Jones *et al*. 1980). Other synthetic preservatives such as captafol, folpet and captan are used to protect the seeds and prolonged storage items, but these chemicals cause dermal sensitization, irritation in skin and several respiratory problems (Villapana and Romaguera, 1993; Royce *et al.*, 1993). Anand and Sati (2013), reported that synthetic preservatives such as formaldehyde, benzoates, nitrates, sorbates, butylatedhydroxyanisole (BHA) and butylatedhydroxytoulene (BHT), etc. cause allergy, hypersensitivity, hyperactivity, asthma, cancer and neurological damage. Plant based products/preservatives are the need of the time.

Plants contain secondary metabolites, which have antifungal, antibacterial and nematicidal activities (Sharma and Sharma, 2012). Extracts of many plants have been reported to possess antifungal activities against the infection of *Aspergillus* species (Thanaboripat *et al*., 2004; Krishnamurthy and Shashikala, 2006). Present investigation was designed to explore the antifungal and anti- aflatoxigenic activity of the acetone extract of *Zea may*s L. leaves (Pioneer-30V92) against *A. flavus* as well as aflatoxins production by the fungus.

Materials and Methods:-

Test Materials:-

Pathogen: Strains of *Aspergillus flavus* were purchased from IMTECH, Chandigarh, India and their cultures were maintained on Czapek-Dox Agar media (CDA). Prior to the experiment, strains of *A. flavus* were reinoculated on freshly prepared Czapek-Dox Agar medium and incubated for 10 days at 28^0C to ensure purity and viability.

Preparation of acetone extract: Fresh leaves of *Zea mays* L. (Pioneer-30V92) were collected and cleaned with running tap water followed by distilled water. Thereafter, leaves were dried in sunlight for two days, and then, in an oven for 24 hours. The leaves were then crushed by using mortar and pestle. 50 grams of crushed leaves were weighed on electric balance and transferred into a conical flask. 300 ml acetone was added into the flask and left for 2 days. In next step, the solution was filtered through Whatman filter paper no.1. Afterwards, filtrate was transferred into a flat bottom dish, and evaporated at room temperature till complete dryness; residue was dissolved in small amount of acetone and water (50:50) and kept at 4^oC for further analysis.

Antifungal activity:-

To evaluate the antifungal activity of the acetone extract, agar plate diffusion method was employed. Various concentrations of the extract (50µl, 100µl, 150µl, 200µl and 250µl/ml) were dissolved in 20 ml of molten Czapek Dox Agar (CDA) medium in different petri plates. Thereafter, freshly prepared fungal disc (5 mm diameter) of 10 day-old culture of *A. flavus* was spotted at the centre of each plate containing different acetone extract concentrations. One Petri plate containing only 20 ml of CDA medium and a fungal disc (5 mm diameter) was used as control. Thereafter, all petri plates were incubated for 10 days at 28^o C. After incubation period of 10 days, diameter (in cm) of the growth of the *A. flavus* was measured in each petri plate. Percentage of inhibition of the growth of the fungus was calculated by following formula:-

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

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 evaluate the anti-aflatoxigenic activity of the acetone extract of *Zea mays* L. leaves, freshly prepared Czapek Dox Broth (CDB) medium was transferred into different conical flasks (25 ml in each flask). The capacity of each flask was 100 ml. Requisite amount of the acetone extract was dissolved in 25 ml of CDB medium in each flask to get final concentration of 50µl, 100µl, 150µl, 200µl and 250µl/ml separately, and subsequently a fungal disc (5mm diameter) was inoculated into each flask containing different concentrations of the extract. A flask containing only 25 ml of CDB medium and a fungal disc (5 mm diameter) was used as a control. Thereafter, all flasks were incubated for 10 days at 28^oC. After incubation period, the fungal mycelium of each flask was filtered through Whatman no.1 filter paper and filtered mycelium was autoclaved to kill the harmful spores of *A. flavus.* Fungal mycelium was dried in an oven at 80^oC (12 hours) and weighed. The filtrate was extracted with 20 ml of

chloroform, and chloroform extract was kept on water bath to evaporate till dryness. Residue was redissolved in 1ml of chloroform. 50 µl of the chloroform extract was spotted on the thin layer chromatography (TLC) plates following Turner *et al*. (2009). Chloroform and acetone in the ratio of 9: 1 (v/v) were used as solvent system for TLC plate. After running the sample, the TLC plate was air dried and then kept under UV transilluminator (360 nm) to detect the presence of aflatoxins. The blue colour bands were observed on TLC plate under UV transilluminator, which indicated the presence of aflatoxins in the test sample. The bands were scratched from the TLC plate and dissolved in methanol (5ml), and then centrifuged at 3000 rpm for 5 minutes. Absorbance of supernatant was recorded at 360 nm, and the quantities of aflatoxins were calculated by following formula:

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

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

Phytochemical Screening:-

Test for proteins, carbohydrates, terpenes and flavonoids were carried out in the acetone extract of *Zea mays* L. leaves (Pioneer-30V92) according to the method of Harbone (1993).

Result and Discussion:-

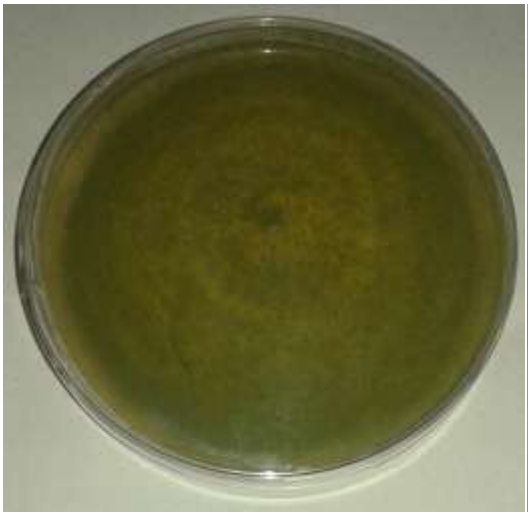

Fig. 1:-Growth of *A. flavus* in the extract untreated plate.

Fig. 2:- Growth of *A. flavus* in the extract treated plate.

Fig. 3: TLC plate of the extract untreated sample is showing the bands of aflatoxins.

Fig. 4: TLC plate of the extract treated sample is not showing the bands of aflatoxins.

Result of antifungal activity of the acetone extract of *Zea mays* L. leaves (Pioneer-30V92) has been presented in Table1. Result suggested that this extract has antifungal activity against the growth of *A. flavus* in a dose depended manner. The extract untreated sample (control) showed well growth of *A. flavus* (**Fig. 1**), but the extract treated sample showed 90.16% inhibition of the growth of this fungus at the concentration 250µl/ml (**Fig. 2**).

Concentration (µl/ml)	**Colony average growth (cm)**	**Growth inhibition (%)**
0	6.1	0
50	4.2	31.14
100	2.6	57.37
150	1.9	68.85
200	1	83.6
250	0.6	90.16

Table 1:- Antifungal activity of acetone extract *Zea mays* L. leaves (Pioneer-30V92) against the growth of *A. flavus* after 10 days incubation period at 28^{o}C.

Qualitative analysis of TLC plates under 360 nm UV light indicated the presence of two types of aflatoxins (B1 and B2). It has been reported that *Aspergillus flavus* produces two types of aflatoxins such as B1 and B2 (Ishwar and Singh, 2000). Quantitative analysis showed that the quantity of aflatoxins B1 was higher than aflatoxins B2. The extract untreated sample (control) showed the bands of aflatoxins on TLC plate (**Fig.3**), but the extract (concentration 200µl/ml) treated sample did not show the bands of aflatoxins on TLC plate (**Fig. 4**).

Concentration (µl/ml)	**Mycelial dry weight (gm)**	**Aflatoxins content (µg/g)**
0	0.411	157.79
50	0.314	68.67
100	0.236	41.76
150	0.152	24.65
200	0.108	0
250	0.098	0

Table 2:- Effect of acetone extract of *Zea mays* L. leaves (Pioneer-30V92) on *A. flavus* biomass (mycelial dry weight) and aflatoxins production.

Table 2 shows anti-aflatoxigenic effect of the acetone extract of *Zea mays* L. leaves (Pioneer-30V92). The result of anti-aflatoxigenic activity exhibited that this extract has potential to inhibit the aflatoxins production from the

toxigenic strain of *A. flavus*. The extract inhibited 100 % aflatoxins synthesis at the concentration of 200µl/ml. The mycelium growth and aflatoxins production were recorded to decrease while increasing the concentration of the extract, and it is also noticed that reduction in mycelial biomass leads to a reduction in aflatoxins synthesis. Therefore, mycelial growth must be arrested below the threshold value to inhibit the production of aflatoxins.

Qualitative phytochemical screening of the acetone extract of *Zea mays* L. leaves (pioneer-30V92) revealed the presence of proteins, carbohydrates, terpenes and flavoinds. J.E. Mellon *et al.* (2011) has reported that terpenes have inhibitory effects against the growth of *A. flavus*, and Mallozi *et al*. (1996) has reported that flavonoids have potential to inhibit the synthesis of aflatoxins. Cumulative effect of these two phytochemical compounds might be responsible for the antifungal and anti-aflatoxigenic activities of acetone extract of *Zea mays* L. leaves (Pionerr-30V92).

Conclusion:-

On the basis of the results obtained, it can be concluded that acetone extract of *Zea mays* L leaves (Pioneer-30V92) possesses pronounced inhibitory effect on the growth of *Aspergillus flavus* as well as aflatoxins production. This extract can be used as plant based preservative for safe storage of food commodities to prevent *A. flavus* contamination and aflatoxins poisoning.

References:-

1. Anand, S. and N. Sati (2013). "Artificial preservatives and their harmful effects: looking toward nature for safer alternatives." International Journal of Pharmaceutical Sciences and Research **4**(7): 2496.
2. Ishwar, S. and V. Singh (2000). "Antifungal properties of aqueous and organic solution extracts of seed plants against *Aspergillus flavus* and *A. niger*." Phytomorphology **50**(2): 151-157.
3. Jayashree, T. and C. Subramanyam (2000). "Oxidative stress as a prerequisite for aflatoxins production by *Aspergillus parasiticus*." Free Radical Biology and Medicine **29**(10): 981-985.
4. Jones, H., J. Chancey, W. Morton, W. Dashek and G. Llewellyn (1980). "Toxic responses of germinating pollen and soybeans to aflatoxins." Mycopathologia **72**(2): 67-73.
5. Harborne, J.B. 1993 Phytochemistry. Academic Press London. 89-131.
6. Krishnamurthy, Y. and J. Shashikala (2006). "Inhibition of aflatoxins B1 production of *Aspergillus flavus*, isolated from soybean seeds by certain natural plant products." Letters in applied microbiology **43**(5): 469-474.
7. Mallozi, M. A., B. Correa, M. Haraguchi and F. Brignani Neto (1996). "Effect of flavonoids on *Aspergillus flavus* growth and aflatoxins production." Rev. microbiol **27**(3): 161-165.
8. Mellon, J., C. Zelaya and M. Dowd (2011). "Inhibitory effects of gossypol-related compounds on growth of *Aspergillus flavus*." Letters in applied microbiology **52**(4): 406-412.
9. Mishra, A. and N. Dubey (1994). "Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities." Applied and environmental microbiology **60**(4): 1101-1105.
10. Patten, R.C. (1981) "Aflatoxins and disease" Am J Trop Med Hyg. 30: 422-425.
11. Patel, J., B. Gami and K. Patel (2010). "Evaluation of in vitro schizonticidal properties of acetone extract of some Indian medicinal plants." Advances in Biological Research **4**(5): 253-258.
12. Prakash, B., P. K. Mishra, A. Kedia, A. K. Dwivedy and N. Dubey (2015). "Efficacy of Some Essential Oil Components as Food Preservatives Against Food Contaminating Molds, Aflatoxins B1 Production and Free Radical Generation." Journal of Food Quality **38**(4): 231-239.
13. Royce, S., P. Wald, D. Sheppard and J. Balmes (1993). "Occupational asthma in a pesticides manufacturing worker." Chest **103**(1): 295-296.
14. Sharma, A. and K. Sharma (2012). "Protection of maize by storage fungi and aflatoxins production using botanicals."
15. Thanaboripat, D., N. Mongkontanawut, Y. Suvathi and V. Ruangrattametee (2004). "Inhibition of aflatoxins production and growth of Aspergillus flavus by citronella oil." KMITL Science Journal **4**(1): 1-8.
16. Turner, N.W., Subhramanyam, S. and Piletsky, S.A. (2009). "Analytical methods for determination of mycotoxins: A review.." Analytica chimica Acta **632: 168-180.**
17. Villapana, J. and Romagurea, C. 1993 Captan, a rare contact sentisiser in hair dressing. Contact Dermatitis. 29(2):107
18. Williams, J. H., T. D. Phillips, P. E. Jolly, J. K. Stiles, C. M. Jolly and D. Aggarwal (2004). "Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions." The American journal of clinical nutrition **80**(5): 1106-1122.