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The Efficiency of Cuscuta (stem parasite) Plant Extracts on the Growth and Aflatoxin Production by Aspergillus flavus In SMKY Liquid Medium

Rajnigandha Rajnigandha

rajnigandha776@gmail.com

Lalit Narayan Mithila University Darbhanga

Gajendra prasad Prasad

Lalit Narayan Mithila University Darbhanga

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Abstract

The effect of different concentrations of cuscuta extract was studied on the growth and aflatoxin production by *Aspergillus flavus* in SMKY liquid medium. The effect of these compound was also verified against aflatoxin production in maize. Plant based antifungal studies, extract from the plant like cuscuta generally contain bioactive compound e.g. flavonoids, phenols that inhibit the growth and aflatoxin production of *Aspergillus flavus*. In liquid media these extract often disrupt fungal metabolism leading to reduced biomass and significantly lower the toxin synthesis. The efficiency of cuscuta extract show significant reduction in aflatoxin production and also sometime at lower than required for complete growth *A.flavus.*The study suggest that Cuscuta could act as a potential natural agent for managing *Aspergillus* in agriculture crops.

Introduction

In India maize (*Zea mays* L.) is third most important food crops after rice and wheat. Maize is considered staple food for millions poor families. Bihar is one of the 4th largest maize producer in India witnessing a maize revolution with high yielding Rabi crops. Environmental factors such as type of soil, rainfall, and temperature and ph value are known to influence the occurrence of molds and mycotoxin production in maize (Ragini & Prasad, 2022)

The contamination of food and feeds by aflatoxin B1 and other mycotoxin continues to be serious concern for human and animal health .Mycotoxin contamination in Bihar fluctuate due to climatic variation such as delayed harvesting, poor drying and storage conditions(Diksha Sultania&Gajendra Prasad, 2022)

Cuscuta sp. (dodder) also known as Akashbel or Amarbel, is a parasitic angiosperm belonging to the family Cuscutaceae. (Abu-Lafi *et .al.*, 2018; Bahadir *et.al*.2020). Cuscuta sp. have important medicinal, pharmacological and edible values while others are a threat to the natural ecosystems and agricultural crops. (Kaiser B.et.al., 2015; Macleod *et al*., 1963; Marvier 1998b; Nwakocha *et. al*., 2013). Aspergillus *flavus* which produces a class of toxic secondary metabolites called aflatoxin poses threat to most of the crops in agriculture. Preliminary work with this plant extracts showed on Aspergillus *flavus*, phytochemicals within parasitic plants like cuscuta contain bioactive compound that inhibit the growth and aflatoxin production of A.f*lavus* in liquid media. These extract can reduce mycelial growth and decrease aflatoxin B1 level acting as natural, ecofriendly antifungal agent in contaminated agricultural substrates. Extract of cuscuta contain flavonoids and other phenolic that interfere with the metabolic system of Aspergillus reducing mycelial growth by over 70 to 100% at higher concentrations. The inhibition is likely due to the presence of antioxidants and secondary metabolites in cuscuta that interfere with fungal oxidative enzyme necessary for toxin production.

In present research, investigation aimed to confirm the efficiency of cuscuta plants extract on the growth of and aflatoxin production by Aspergillus *flavus* in SMKY liquid media and to test activity against aflatoxin production.

Material and Methods

Sample collection

Maize sample variety were collected from farmer's field in Darbhanga district of Bihar during rainy season. Cuscuta plant sample were collected from different angiospermic plants for experimental purpose.

Organism and inoculum

A toxigenic strain of *Aspergillus flavus* isolates from maize kernel was used throughout the present investigation.

Inoculum was isolated by growing the organism on Potato Dextrose Agar medium for 7 days at 28 $_{+/-2}{}^{o}C$ .The spores were harvest by adding 10ml sterile distilled water and the developing colonies were examined for fungal growth. (ISTA-1999)

Aspergillus *flavus* group of fungi were tested in a SMKY liquid medium and extracted with chloroform and the extracts were used for further qualitative and quantitative analysis (Diener and Davis, 1966).The estimation and presence of aflatoxin B1in maize by Thomas et.al., 1975.

Preparation of plant extract

Fresh cuscuta (e.g. C.*reflexa*, C.*chinensis*) plant material is collected from the study area seasonally by confirming the taxonomic identity. The overall process contain following steps-

Collection of plant material and drying, size reduction, extraction, filtration, Concentration and drying and reconstitution

The quality of an extract is influenced by several factors such as plant parts used as starting material, extraction procedure and plant material .All the parameter are optimized and controlled during extraction.

Evaluation-

Growth- Mycelial mats are filtered, washed and dried to constant weight to determine dry weight reduction.

Aflatoxin production- Aflatoxins are extracted from the filtrate usually using chloroform and quantified to determine reduction in toxicity.

Control treatments-

Negative control- Medium + *A.flavus* (no extract)

Positive control – Medium + *A.flavus* + standard antifungal agent

Result and Discussion

Table 1
Isolation of Mycoflora on and in Maize seeds (*Zea mays*)

Sl.No.			Total no. of colonies	Total no. of colonies	% relative infection	% relative infection	
	Mycoflora	No. of positive sample	Surface sterilized	Non sterilized	Surface sterilized	Non sterilized	Range of kernel infection
1	*A.flavus*	22	147	201	43.16	39.04	7.40
2	*A.niger*	17	133	164	34.56	29.50	6.32
3	*Penicillium sp.*	12	21	36	4.54	6.15	6.10
4	*Rhizopus sp.*	9	29	48	6.75	7.27	5.14
5	*Fusarium sp.*	4	33	89	8.87	15.00	3.20
	Total no. of colonies	-	388	458	-	-	-

Table 2
Aflatoxin producing potentiality of *A.flavus* isolates and aflatoxin contamination in maize seed samples

No. of A.flavus isolates/maize sample screened	No. of toxigenic isolates/contaminated sample	Type of aflatoxin B1, B1B2, B1B2G1	Range of aflatoxin B1 concentration µg/kg
60 isolates	30	20 6 01	1.0-6.2
6 samples	5	02 01 -	1.2–20

A total of 5 fungal species were found to be associated under varying isolation condition as shown in table- 1.The sp.of *Aspergillus flavus* and *A.niger* were recorded in all these samples where a few genera were isolated from sterilized and non-sterilized kernels. The percentage incidence of *A. flavus* was found to be maximum followed by *A.niger* and *Fusarium* sp. The total no. of colonies recorded was 388 and 458 in surface sterilized and non-sterilized seeds respectively.

Although 60 isolates of A.*flavus* representing 6 isolates from each sample were screened for their aflatoxin producing potentialities. Out of these 30 isolates were found to be toxigenic (Table 2) .Among the toxigenic strains 20 isolates elaborated aflatoxin B1 whereas 6 isolates were capable of producing aflatoxin B1 and B2. Only one isolates could produce B1, B2, and G1. A.flavus isolates produced aflatoxin B1 in the range of 1.0-6.2µg/kg

Table – 3

Effect of plant extract on mycelium growth and production of Aflatoxin B1 in SMKY liquid media

Concentration of cuscuta extract (gm/ml)	**Mycelium growth of A.flavus (gm+/-SE)**	**% inhibition of mycelium**	**Production of Aflatoxin B1 (mg/25ml) 1.00+/-S.E.**	**% inhibition**
Control	2.61+/-SE		1+/-SE	
0.5	2.52+/-4.28	8%	1.81+/-0.005	8.3%
1.0	2.17+/-1.53	16%	1.72+/-0.09	11.5%
1.5	1.98+/-0.87	25%	1.66+/-0.042	14%
2.0	1.84+/-0.7	38%	1.59+/-0.004	18%
2.5	1+/-0.2	46%	1.33+/-0.016	30.5%

The minimum and maximum inhibition of growth of A.*flavus* were recorded at 8% and 46% in the concentration of 0.5gm/ml and 2.5gm/ml of plant extract respectively. Similarly, the minimum and maximum inhibition of aflatoxin B1 production in SMKY liquid medium were recorded 8.3% and 30.5% at 0.5gm/ml and 2.5gm/ml concentration of plant extract, respectively.Data were analysed by Dospekov, (1984) accordingly.

DISCUSSION

Cuscuta extract contain bioactive compounds that inhibit Aspergillus flavus growth and reduce aflatoxin production in liquid media. These extracts often rich in polyphenol and alkaloid disrupt fungal metabolic pathways leading to significant reduction in mycelial growth and suppressed synthesis of aflatoxin B1.The general effectiveness of phytochemicals from similar parasitic plants strongly suggest that cuscuta sp. can effectively suppress both growth and toxin.The justification of our research work already reported by Bitter plant, crucifer plants, and medicinal plants extracts in SMKY liquid medium (Ragini & Prasad ,2022; Mahesh & Prasad, 2023; Ragini *et. al*., 2025)

CONCLUSION

The aflatoxin contamination in food crops have become a serious threat to human beings and problem in severe causing health hazard. The toxin contained products are also losing economic value in the global market.

Therefore such antifungal agents are tested in liquid medium to evaluate their potential as bio preservative recommended pre and post-harvest practice should be followed by growers and food industries to minimize the aflatoxin contamination.

Declarations

Author Contribution

Both the authors have equal contribution in writing the manuscript

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