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A Study on Aflatoxin Contamination and Induced Biochemical Changes in *Syzygium Cumini*

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Abstract

Mycoflora incidence and mycotoxin contamination in fruits/seeds of *Syzygium cumini* (L.) skeels collected from different localities of Uttarakhand (India) were investigated. A total of seven fungi namely *Aspergillus flavus, A. niger, A. repens, Fusarium avenaceum, F. semitetum, Rhizopus stolonifer* and *Trichothecium roseum* were recorded on fresh and stored samples of *Syzygiun cumini. Aspergillus flavus* and *A. niger* was predominant. Nine percent of *Aspergillus flavus* isolates were toxigenic and produced aflatoxin B_1 in the up to 6.2 µg/ml in the liquid media. Results of natural occurrence of mycotoxins revealed that twenty-five percent of the stored samples was naturally contaminated with aflatoxins and the range of aflatoxin produced was 0.46-0.93 µg/g. Out of 17 positive samples,14 samples exhibited presence of aflatoxinB_1, 2 samples aflatoxin B_1,B_2 and one sample exhibited presence of aflatoxin B_1,B_2 and G_1. A significant reduction in the level of alkaloid, starch and protein content of *S. cumini* seeds was recorded during fungal infection.

Keywords: *Syzygium Cumini,* Mycoflora, *Aspergillus Flavus,* Aflatoxins, Biochemical Changes

Introduction

Syzygium cumini (L.) skeels commonly known as Black plum or Jamun is one of the most important forestry species widely used as herbal medicine. It is an evergreen tropical tree of family Myrtaceae associated with many health and medicinal benefits. Various parts of *Syzygium cumini* are used for medicinal purpose such as bark, fruit, seed and leaves. While the bark and seeds are known for their hypoglycemic, anti-inflammatory, anti-diarrhoea and diuretic properties, leaves are used to treat diabetes, constipation etc. However, one of the best medicinal benefits of *S. cumini* seeds is its anti-diabetic properties. The seeds are reported to contain alkaloid, jambosine, and glycoside jambolin (antimellin), which halts the diastatic conversion of starch into sugar. Besides multiple medicinal properties, black plum is an important summer fruit having many vital nutrients like carbohydrates, protein, dietary fibers, fats etc.(Swami 2012).

Review of Literature

Mycotoxin contamination of various foodstuffs i.e. agricultural produce, forest produce and tree borne oil seeds has already been established (Hesseltine 1974; Bilgrami and Sinha 1984; Singh *et al*. 2001; Shukla and Singh 2006; Singh 2012). Medicinal plant materials have also been found prone to fungal infection and mycotoxin contamination and reports are available on presence of mycotoxin producing fungi and natural occurrence of mycotoxins in medicinal plants and traditional herbal medicines (Rani and Singh 1990; Roy and Chourasia 1990; Singh et al.1999; Singh 2003). These plant materials get contaminated during harvesting, transportation, processing, storage or while still in the field. It has been established that fungal infection and mycotoxin contamination cause deterioration in the chemical constituents of the plant material under storage which may result in reduced efficacy and unsafe herbal preparations (Dutta and Roy,1987). Gupta *et al.*(2016) also reported fungal contamination in powdered samples of leaves, stem bark and seeds/fruits of *S. cumini*.

The chemical composition and antioxidant activity of *S.cumini* have been studied in detail (Warrier et al.1996; Benherlal and Arumughan 2007; Jadhav et al*.* 2009; Affity et al.2011), but there is scant information available on mycotoxin contamination and induced biochemical changes in *S. cumini.*

Aim of The Study

To investigate aflatoxin contamination and induced biochemical changes in *S. cumini*.

Material and Methods

Samples collected from different forest areas and storage centres/ pharmaceutical industries of Uttarakhand (India) were screened for incidence of mycoflora, analyzed for the natural occurrence of aflatoxins and biochemical changes due to fungal contamination and mycotoxin production.

Mycoflora analysis

Mycoflora associated with the seed samples were examined using the blotter test and the agar plate method as recommended by International Seed Testing Association (ISTA, 1999). The developing fungal colonies were isolated, identified and maintained on potato dextrose agar (PDA) and Czapek's-dox agar media. Percentage incidence of fungi was determined on the basis of occurrence of a particular species in hundred seeds/samples.

Natural occurrence of Aflatoxins and toxigenic producing potential of Aspergillus flavus

Aflatoxin producing potentials of *Aspergillus flavus* isolates were tested in SMKY liquid medium (Diener and Davis, 1966). The constituents of the medium were, Yeast extract–7g; Sucrose– 200g; Magnesium Sulphate ($MgSO_4$ $7H_2O$)-0.5g; Potassium Nitrate (KNO_3)-3g; Distilled water-1litre. Culture filtrate was extracted with chloroform and the chloroform extract was used for qualitative and quantitative detection of aflatoxins.

Samples were extracted chemically for the presence of aflatoxins by standard procedure (AOAC, 1984). Samples were powdered in a grinder and 50 g flour was blended with 250 ml methanol: water (60:40,v/v) for 2 minutes at high speed. The extract was filtered through Whatman No. 1 filter paper. 125 ml of this filtrate was than extracted with 30 ml saturated sodium chloride (NaCl) solution and 50 ml n- hexane in a 250 ml separating funnel for 2 minutes. The lower methanol layer was transferred to another separating funnel. Finally, the lower methanol layer was extracted with 40 ml chloroform. The chloroform extract was used for qualitative detection and quantitative estimation of aflatoxins. Qualitative and quantitative estimation of the mycotoxins were carried out using Thin Layer Chromatography (TLC). The spots of aflatoxins were identified by comparing with the spots of standards obtained from Sigma, USA.

To study the changes in alkaloid, protein and starch content of the *S. cumini* seeds, surface-sterilized seed samples were inoculated with spore suspensions (250-300 spores/ml) of *A. flavus* and incubated for 11 days at 96% r.h. Estimation of alkaloid, protein and starch content in the healthy and infested samples was done according to the standard recommended procedures (Harborne, 1998).

Results of mycoflora incidence, aflatoxin estimation and biochemical parameters was statistically analyzed using SPSS (1977) package. Standard error of means, t-value and critical difference were computed to draw appropriate inferences.

Results

A total of seven fungi namely *Aspergillus flavus, A. niger, A. repens, Fusarium avenaceum, F. semitetum, Rhizopus stolonifer* and *Trichothecium roseum* were recorded on fresh and samples of *Syzygiun cumini.* While fresh fruits exhibited presence of only two fungi *A. niger* and *Fusarium avenaceum*, six fungi *A. flavus, A. niger, A. repens, F. semitectum, R. stolonifer* and *T. roseum* were recorded on stored samples. Occurrence of *A. niger* was the most common on all samples followed by *A. flavus.* While *A. candidus* was recorded only in monsoon season, *T. roseum* was recorded in summer and monsoon season only. Source and seasonal interaction was significant for *A. flavus, F. semitectum, R. stolonifer* and *T. roseum* and non-significant for *A. niger* and *A. candidus* (Table-1).

Aflatoxins were elaborated by *A. flavus* isolated from samples of *Syzygiun cumini,* 3 isolates produced aflatoxin B_1 out of 32 screened and the amount of aflatoxin B_1 produced by the toxigenic isolates of *A. flavus* was in the range of 0.3-6.2 µg/ml. Results of natural occurrence of mycotoxins revealed that aflatoxins were not detected in fresh samples of *Syzygiun cumini* while 25% of the stored samples were naturally contaminated with aflatoxins. Out of 17 positive samples, 14 samples exhibited presence of Aflatoxin B_1, Aflatoxin B_1,B_2 in 2 samples and one sample exhibited presence of Aflatoxin B_1,B_2 and G_1 and the range of aflatoxin production was in the range of 0.46-0.93 µg/g(Table-2).

Changes in the level of starch, protein, and total alkaloid during infestation of toxigenic strain of *Aspergillus flavus* have been carried out. *A. flavus* caused considerable changes in the starch, protein, and total alkaloid content during their infestation (Table-3). The amount of starch content in healthy seeds/ fruits was 11.15g/100g, however, the amount of starch in infested substrates was 9.61g/100g. Protein level was also depleted due to the infestation with *A. flavus*. The amount of protein content in healthy fruits/seeds was 1.03 g/100g, whereas in the infested sample, the amount of protein was 0.94 g/100g. There was an indication of inhibition in the total alkaloid content due to infestation with toxigenic strain of *A. flavus*. The alkaloid content decreased from 0.047% in control samples to 0.044 g/100g in the *A. flavus* infested samples. Statistical analysis of the results showed decline in the level of starch, protein, and total alkaloid significant at 5% level of significance.

Discussion

The results of the present investigation indicate that *S.cumini* seeds are subject to fungal contamination including that of *Aspergillus flavus*, a known mycotoxin producing fungi. Seasonal variation directly had an impact of percentage incidence of fungal flora. These medicinal plant produce get contaminated by various fungi during the process of harvesting, storage and further distribution causing spoilage and reducing the efficacy of the produce. Occurrence and incidence of mycoflora on a certain commodity is directly governed by the nature of the substrates, methods of storage and prevailing

environmental conditions. Earlier reports also indicate a varied pattern of fungal incidence and mycotoxin contamination in important medicinal species including *Aegle marmelos, Azadirachta indica, Emblica officinalis, Terminalis belerica* and *T.chebula* (Aziz et al. 1988; Singh et al.1999; Chourasia 1995). Gupta *et al.*(2016) also reported fungal contamination in powdered samples of leaves, stem bark and seeds/fruits of *S. cumini*.

Screening of *A. flavus* isolates showed presence of Aflatoxin B_1 in higher concentration, besides, natural occurrence of aflatoxins is also quite high. The natural contamination of mycotoxins in these samples correspond to the incidence of toxigenic fungi associated with them and their respective potentiality to produce aflatoxins in the synthetic media. *Aspergillus flavus* group of fungi cause deterioration in the nutritive quality and medicinal property of the plant produce by producing aflatoxins and by changing some of the chemical constituents. Marked depletion in the alkaloid, starch and protein was recorded due to infestation with *A. flavus*. Aflatoxins and aflatoxin producing fungi have been reported to inhibit the synthesis of protein during plant metabolism (Truelove et al.1970; Dashek and Llewellyn 1974). Aflatoxin producing fungi have been known to degrade the protein level of some common dry fruits and fleshy fruits (Bilgrami et al.1983; Abdel-Hafez and Sabah 1993). Singh (2008) reported deterioration in nutritive quality of walnut kernels due to fungal infection and mycotoxin contamination. There is an indication of decline in the level of total alkaloid in samples infested with toxigenic strain of *A. flavus*. Inhibition in the level of total alkaloid might be due to their utilization or degradation into simpler forms. Dutta (1988) worked on deterioration in total alkaloid content of *Strychnos vomica* by some fungi and reported that *A. flavus* and *P. citrinum* significantly inhibited the level of alkaloid content.

Conclusion

Presence of mycotoxin producing fungi and natural occurrence of aflatoxins in the fruits/seed samples of *S. cumini* beyond tolerance level is a matter of great concern as it forms basic constituent of a large number of ayurvedic preparations. High incidence of naturally occurring aflatoxins in medicinal plant produce is a great threat to the quality and safety of the medicines. As a substantial part of the demand of herbal based pharmaceutical industries is met through wild collections from the forests, there is an urgent need to develop a mechanism to screen the samples for presence of toxigenic fungi and analyze for their level of mycotoxins before being consumed or marketed for use in the pharmaceutical industries.

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Table-1 Mycoflora Incidence in Stored Samples of *S. Cumini*

Mycoflora	**Mean Value of Mycoflora Incidence in Different Season**			
	Season	Mean	Effect	$CD_{(0.05)}$
A. flavus	Summer	43.75	Season	1.17
	Monsoon	47.5	Source	1.36
	Winter	17.15	Season x Source	2.35
A. candidus	Summer	0.00	Season	0.76
	Monsoon	18.6	Source	NS
	Winter	0.00	Season x Source	NS
A. niger	Summer	46.95	Season	1.29
	Monsoon	47.95	Source	1.50
	Winter	15.25	Season x Source	NS
F. semitectum	Summer	11.25	Season	1.07
	Monsoon	11.15	Source	1.24
	Winter	3.15	Season x Source	2.15
R. stolonifer	Summer	14.55	Season	1.41
	Monsoon	12.1	Source	1.63
	Winter	6.90	Season x Source	2.82
T. roseum	Summer	2.35	Season	0.71
	Monsoon	8.15	Source	0.82
	Winter	0.00	Season x Source	1.41

Table-2 Natural occurrence of aflatoxins in *S. cumini*

Total No. of samples		Percentage contamination	Aflatoxins detected	No. of samples	Conc. of Aflatoxin B_1 (µg/g)
Screened	Contaminated				
68	17	25	Aflatoxin B_1	14	0.46- 0.93
			Aflatoxin B_1,B_2	2	
			Aflatoxin B_1,B_2,G_1	1	

Table-3: Changes in Starch, Protein and Alkaloid Content Due to Fungal Infection

Biochemical parameters		g/100g (Mean±S.E.)	t-value	Significance $CD_{(0.05)}$
Starch	Control	11.15±0.0091	7.85	0.004
	Infested	9.612±0.1903		
Protein	Control	1.032±0.0811	8.73	0.001
	Infested	0.938±0.0759		
Alkaloid	Control	0.0472±0.000	21.36	0.022
	Infested	0.0435±0.000		