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Fruit rot of *Datura innoxia* and microbial deterioration of tropane alkaloids

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ABSTRACT : The fruit rot was found to be the most prevalent disease in the experimental fields of *Datura innoxia* at CIMAP, Lucknow during the month of January to April, 1995. The isolations from necrotic tissues and seeds of infected fruit samples invariably produced six fungal cultures. Based on cultural and morphological characters, they were identified as *Alternaria alternata, Alternaria solani, Bipolaris zeae, B. hawaiiensis, Curvularia lunata* and *Fusarium pallidoroseum.* The pathogenicity of all fungi was tested on detached fruits under lab conditions. *A. alternata, B. zeae, B. hawaiiensis* and *F. pallidoroseum* were highly pathogenic, whereas *A. solani* and *C. lunata* were weak pathogens. Two major tropane alkaloids viz., hyoscyamine and scopolamine in *D. innoxia* fruits were considerably reduced under infestation with each of the six fungal pathogens. Maximum deterioration in hyoscyamine and scopolamine content was recorded by *B. zeae* and *F. pallidoroseum,* respectively. However, *C. lunata* and *A. alternata,* showed minimum deterioration in hyoscyamine and scopolamine, respectively.

Key words : *Datura innoxia,* fruit rot, microbial deterioration, hyoscyamine, scopolamine

Datura innoxia Mill. is very useful medicinal plant because its leaves, fruits and seeds contain many important tropane alkaloids, including hyoscyamine, scopolamine and atropine which are widely used in several pharmaceutical preparations (Anomymous, 1952; Trease and Evans, 1976). Therefore, different species of *Datura* have been grown on large scale at several places in India to fetch the growing demands of tropane alkaloids by the pharmaceutical industries. The experimental fields of *D. innoxia* at the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow were found to be severely affected with a fruit rot disease in between January and April, 1995. It also appeared that alkaloid content in the infected fruit diminished due to heavy fungal infestation under moist condition. The association of mycoflora with the fruits and seeds is a natural phenomenon and under favourable environmental conditions they rapidly multiply using them as a source of nutrient (Bucherer, 1965). There are several reports on the biodeterioration of nutritive contents of cereals, pulses and oil seeds (Bilgrami *et al.,* 1979; Singh *et al.,* 1990), fruits and vegetables (Sinha and Singh, 1984; Singh and Prashar, 1984). However, the reports on microbial deterioration of active constituents of medicinal plants are meagre (Dutta and Roy, 1987, 1992). In the present communication, we report on fungal pathogens associated with fruit rot of *D. innoxia* and biodeterioration of alkaloid contents in the infected fruit.

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MATERIALS AND METHODS

Disease incidence and symptoms

Datura innoxia plantations in the experimental fields of CIMAP at Lucknow were observed to determine incidence of fruit rot between January and April, 1995. Infected fruits showing mild to severe symptoms of dry rot were randomly collected from different sites and brought to the laboratory in polyethylene bags to study the symptoms and isolation of fungi associated with pericarp and seeds of the infected fruits.

Isolation and identification of fungi

Isolations were made from the infected seeds and advance region of necrotic tissue of infected fruits. They were surface disinfected with 1% NaOCI for 5min, washed twice in sterile distilled water (SDW), blotted dry, placed onto Potato-Dextrose-Agar (PDA) and incubated at 25±2°C for 7 days. The hyphal tips of fungal colonies growing around the infected seeds and tissues were transferred to fresh PDA slants and single spore cultures were maintained on PDA under mineral oil at 25°C. The cultural as well as morphological characters were studied to identify different fungal isolates from the infected fruits and seeds of *D. innoxia*. The identifications were later confirmed by CAB, International Mycological Institute, Surrey, UK.

Pathogenicity tests

The inoculum was prepared from 7- to 10-day-old fungal colonies on PDA. The spore suspension prepared in distilled water was filtered through sterile cotton wool and concentration of spore was adjusted to 3×10^6 spores/ml by using a haemocytometer. Pathogenicity of all the isolates was tested on healthy green detached fruits of *D. innoxia*. Fruits were surface disinfested with 1% NaOCl for 1 min, washed thoroughly with SDW, and placed in sterile Petri dishes. Thereafter, they were injured gently with a sterile scalpel at 3 sites and 0.5 ml spore suspension was dispensed to the wounds. Fruits treated with SDW served as control. Both inoculated and control fruits were kept in humidity chamber for 10 days. Fruit rot symptoms on five fruits was determined after 7 and 10 days of incubation at 25°C and a mean of necrotic lesion sizes was recorded.

Microbial deterioration of tropane alkaloids

D. innoxia fruits were inoculated individually with six fungal pathogens, namely *Alternaria alternata, A. solani, Bipolaris zeae, B. hawaiiensis, Curvularia lunata* and *Fusarium pallidoroseum* and kept in humidity chamber. Infected fruits were taken out after 10 days, dried at 45°C and powdered. Total alkaloid content was extracted and estimated by following the method of Chiu and Okamoto (1987). Quantitative analysis of major tropane alkaloids, hyoscyamine and scopolamine, was carried out by using gas chromatography (GC) method of Chiu and Okamoto (1987). The analytical gas chromatography was performed on Perkin Elmer GLC model 3920 B fitted with a 2m x 3mm stainless steel column packed with 3% OV-17 on 80/100 chromosorb WHP and N_2 flow rate was 40 ml/min. Analysis was done at 235°C isothermal operation with injector/detector temperature 280°C. The data were processed by HP-3390 integrator. Standard deviation calculations are based on five injections of standard hyoscyamine and scopolamine solutions.

RESULTS AND DISCUSSION

Disease incidence and symptoms

The fruit rot was found to be the most prevalent disease in the experimental fields of *D. innoxia* at CIMAP, Lucknow in between January and April, 1995. During the first week of January, disease incidence was 25-40%, where infected fruits developed dark brown spots varying 2-3 x 0.5-1mm in size and became yellowish. By the end of March, these spots enlarged and 70-80% infected fruits produced dry rot symptoms (Fig. 1A). Severely infected fruits shrinked, turned black and dehisced prematurely (Fig. 1B). Under moist conditions, fungal growth was observed on the seeds before dehiscence. Severely infected seeds turned black and lost viability (Fig. 1C).

Isolation and identification of pathogen

Isolations from the infected fruits on PDA consistently yielded species of *Alternaria, Bipolaris,*

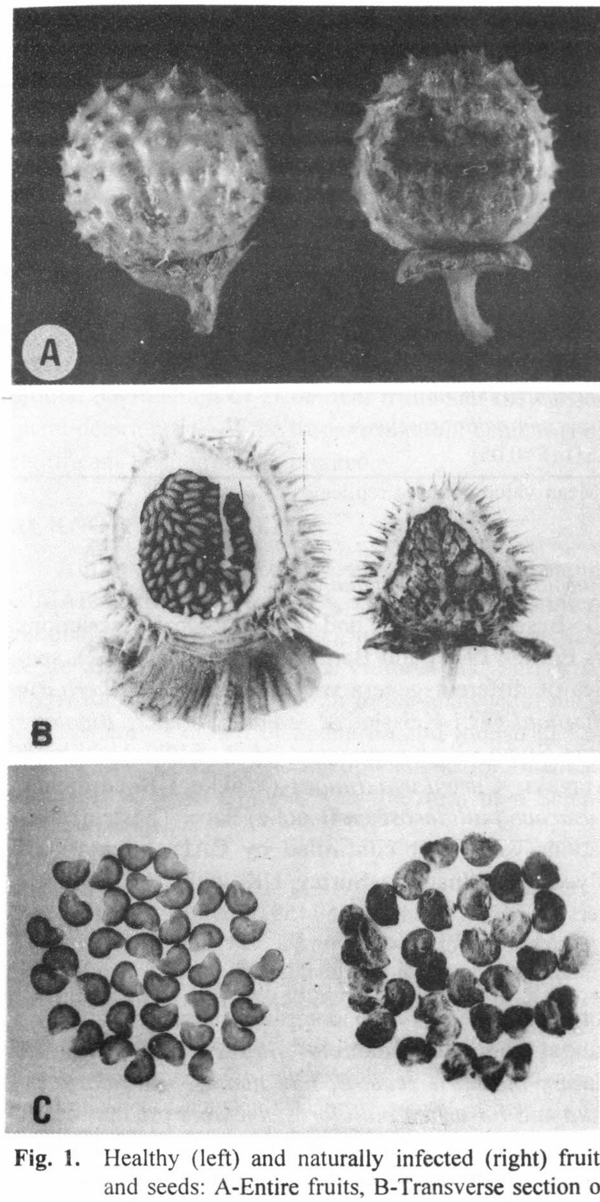

Fig. 1. Healthy (left) and naturally infected (right) fruits and seeds: A-Entire fruits, B-Transverse section of the fruits and C-Seeds collected from the field

Table 1. Disease severity on *D. innoxia* fruits caused by different fungal pathogens under artificial inoculation

Fungal pathogens	Isolate No.	Mean lesion diameter (mm)* after	
		7 days	10 days
Alternaria alternata	DI-7	8.5	12.8
A. solani	DI-8	3.6	4.8
Bipolaris zeae	DI-3	9.6	13.0
B. hawaiiensis	DI-4	8.2	12.6
Curvularia lunata	DI-6	2.8	3.4
Fusarium pallidoroseum	DI-5	7.1	10.0
Control		0.0	0.0
LSD (P=0.05)		2.6	3.2

*Mean of lesions sizes produced on the fruits of *D. innoxia* by different pathogens after inoculation with 0.5 ml of $3x10^6$ spores/ml

Table 2. Microbial deterioration of hyoscyamine and scopolamine contents of *D. innoxia* fruits under artificial infestation

Fungal pathogens	Alkaloid contents			
	Hyoscyamine concentration*		Scopolamine concentration*	
	(mg/g dry wt.)	% loss	(mg/g dry wt.)	% loss
Healthy	12.5	-	3.1	-
Alternaria alternata	4.7	62.3	2.1	34.6
A. solani	9.2	26.4	1.4	56.2
Bipolaris hawaiiensis	1.7	86.0	1.0	61.6
B. zeae	1.5	87.7	1.0	67.9
Curvularia lunata	9.7	22.1	1.7	45.4
Fusarium pallidoroseum	2.2	82.3	0.1	97.1
LSD (P=0.05)	0.3	1.3	0.1	2.8

*Mean value of three replicates

Curvularia and *Fusarium*.

Based on cultural and morphological descriptions by Booth (1971) and Barnett and Hunter (1972), species of different genera were identified as *Alternaria alternata* (Fr.) Keissler, *A. solani* Sorauer, *Bipolaris zeae* Sivan, *B. hawaiiensis* (M.B. Ellis) Uchida & Aragaki, *Curvularia lunata* (Wakker) Boëdijn and *Fusarium pallidoroseum* (Cooke) Sacc. These identifications were later confirmed by CAB, International Mycological Institute, Surrey, UK. with CABIMI numbers 357463, 357464, 357459, 357460, 357462 and 357461, respectively.

It is evident from the experimental results that fruit rot of *D. innoxia* is a complex disease because six fungal pathogens, namely *Alternaria alternata, A. solani, Bipolaris zeae, B. hawaiiensis, Curvularia lunata* and *Fusarium pallidoroseum* were predominantly involved in the infection of healthy fruits and subsequently in the development of disease syndrome. The average frequencies of these fungi were 40%, 32%, 40%, 28%, 21% and 18%, respectively. The incidence of the disease on unriped green fruits suggests that fruits with high moisture content favoured microbial infection.

Pathogenicity tests

Of the six fungi employed in pathogenicity tests, *A. alternata, B. zeae, B. hawaiiensis* and *F. pallidoroseum* were highly pathogenic producing large, dark brown necrotic lesions (>10 mm in size) at the site of inoculation after 6 days. These necrotic lesions enlarged, coalesced and produced dry rot symptoms 10 days after inoculation. The isolates of *A. solani* and *C. lunata* were weak pathogens because they produced necrotic lesions of limited size (2-7 mm) causing partial rotting of the fruits after 10 days of inoculation. All control fruits were free from disease symptoms.

Microbial deterioration of tropane alkaloids

The results on GC analysis of alkaloid contents shown in Table-2 indicated that the hyoscyamine and

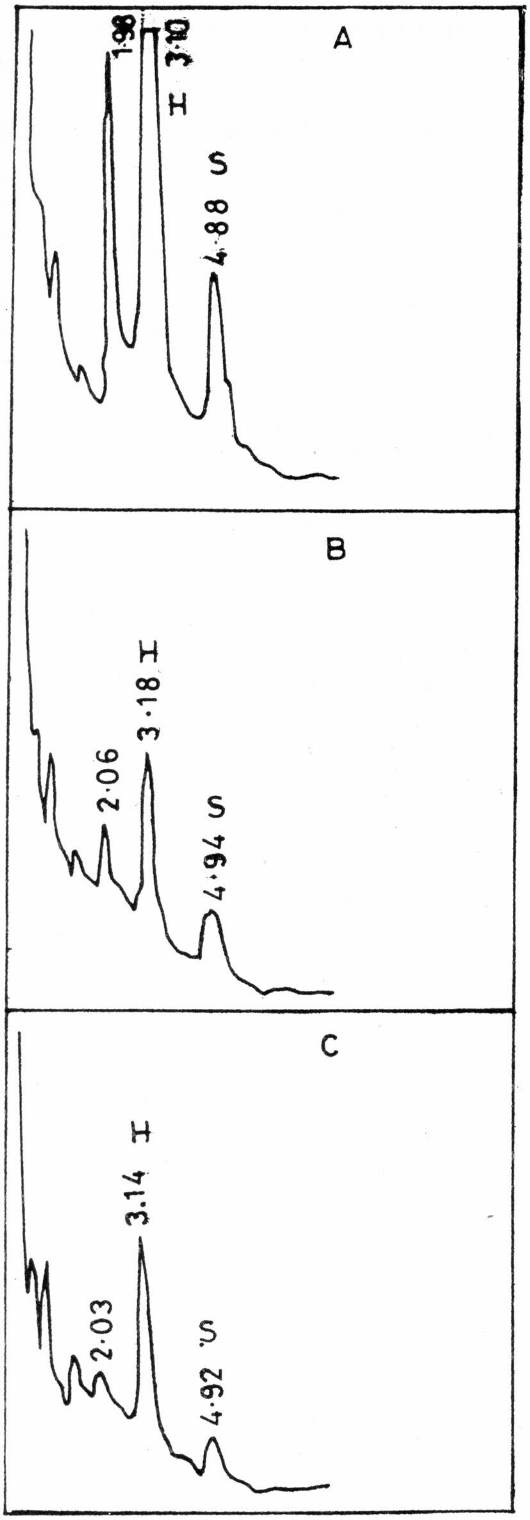

Fig. 2. GC analysis of tropane alkaloid contents of *Datura innoxia* fruits: Healthy (A), Inoculated with *B. zeae* (B) and *F. pallidoroseum* (C) showing maximum deterioration of hyoscyamine (H) and scopolamine (S), respectively

scopolamine were 12.51 mg/g and 3.15 mg/g, respectively in healthy fruits which were considerably reduced in infected fruits. Maximum deterioration of hyoscyamine was caused by *B. zeae* (87.68%), followed by *B. hawaiiensis* (86.01%), *F. pallidoroseum* (82.34%) and *A. alternata* (62.35%). Least deterioration was noticed in *A. solani* (26.37%) and *C. lunata* (22.14%). Scopolamine content was reduced maximum by *F. pallidoroseum* (97.15%), followed by *B. zeae* (67.93%), *B. hawaiiensis* (61.58%), *A. solani* (56.19%), *C. lunata* (45.39%) and minimum by *A. alternata* (34.60%).

The hyoscyamine and scopolamine contents were significantly reduced in the infected fruits of *D. innoxia* as compared with healthy fruits (Fig. 2). Dutta and Roy (1992) have also reported deterioration in strychnine and brucine of *Strychnos nux-vomica* seeds under infestation with *Aspergillus flavus, A. niger* and *Penicillium citrinum.* The reduction in alkaloid contents under infestation has been attributed to several reasons (Wada and Yamazaki, 1954, Niemer *et al.,* 1960). Microbial conversion of alkaloids has also been reported by several workers (Mitscher *et al.,* 1968, Rosazza *et al.,* 1987). The fruits and seeds of *D. innoxia* are used in the manufacture of finished herbal drugs for the amelioration of peoples sufferings. Hence, microbial degradation of *D innoxia* fruits and the consequent deterioration of the tropane alkaloid content is of significant economic importance.

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