J. Phytol. Res. **16**(2) : 175-180, 2003

EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON SEED MICROFLORA, AFLATOXIN CONTAMINATION AND SEED HEALTH OF *DALBERGIA SISSOO ROXB.*

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The effect of relative humidity and temperature on seed microflora of *Dalbergia sissoo* in relation to aflatoxin production and seed health was investigated. The seed microflora exhibited significant changes both qualitatively and quantitatively with regard to humidity, temperature and period of storage. Various species of *Aspergillus* were recorded at different relative humidity and temperature levels. In contrast, species of *Alternaria, Curvularia* disappeared by the end of storage, irrespective of the percentage of humidity and temperature. Among bacteria, *Acinetobacter* spp., *Enterobacter hafniae, Pseudomonas aeruginose* were reported in different humidities and storage periods. Aflatoxin elaboration by *Aspergillus flavus* in *Dalbergia sissoo* was recorded at all relative humidities and temperatures tried.

Keywords : Aflatoxin; *Dalbergia sissoo;* Relative humidity (RH); Seed microflora; Temperture.

Introduction

Seeds absorb some quantity of water under a set relative humidity (RH) and temperture. The quantity of water thus absorbed influences the storage behaviour of seeds and other biological agencies dependent on them. While considerable information on this aspect of physical property of cereals and forage leguminous seeds are available in literature[1-7]. Similar information on tree legumes are very scanty[8,9]. Hence it was considered to study the effect of relative humidity and temperature on seed microflora, aflatoxin contamination and seed health of *Dalbergia sissoo.*

Materials and Methods

500g of perfectly dried pods of *Dalbergia sissoo* were stored for three months at different temperature conditions (10, 17, 28, 37 and 42°C) and at different relative humidities (22, 35, 50, 75 and 95%). The relative humidity was maintained in dessicators[10,11] and stored at room temperature (28°C±1). At the end of 1,2 and 3 months of storage, blotter test methods were employed for the isolation of associated microflora[12]. Germination percentage was determined as per ISTA rules[13]. The percentage incidence of each fungus and bacterium was also calculated.

Changes in biochemical constituents such as total soluble protein[14], total phenol[15], total soluble sugar[16] and total starch content[17] were analysed by standard methods. For aflatoxin, the samples were observed under long-wavelength UV light for bright green yellow fluorescenc[18]. The samples giving positive fluorescence were further extracted for aflatoxin contamination[19]. Quantitative estimation was done by the method of Nabney and Nesbitt[20].

Results and Discussion

Effect of Relative Humidity : Relative humidity exerted significant influence on the seed microflora (Table 1). Field fungi like *Alternaria alternata, Cladosporium cladosporioides, Curvularia clavata, C. lunata* and *Drechslera tetramera* showed higher incidence and appeared in the initial period of storage. These fungi were rapidly succeeded by storage fungi, like species of *Asperigillus (A. flavus, A. fumigatus, A. humicola, A. nidulans).* Incidence of storage fungi increased with the progress of storage period.

Some species of *Aspergillus* were dominant both at low and high RH. Presence of *Aspergillus* at low RH may be due to their xerophillic nature, while their dominance at high RH may be due to their strong competitive sapraphytic ability. These results support the earlier findings that increase in RH promote faster seed deterioration[2,3]. Three bacterial genera namely, *Acinetobacter* sp., *Enterobacter hafniae* and *Pseudomonas aeruginosa* were reported and were predominant at 95 and

Table 1. Effect of relative humidity (RH) on percent incidence of seed microflora and seed germination of *Dalbergia sissoo*.

Microflora	Before storage	Storage in months																	
		1						2						3					
		95%	75%	62%	50%	35%	Control 22%	95%	75%	62%	50%	35%	Control 22%	95%	75%	62%	50%	35%	Control 22%
a. Fungal flora																			
Alternaria alternata	1.2	-	-	-	-	-	-	-	-	-	16.0	-	-	-	3.0	-	-	-	-
Aspergillus candidus	-	-	-	-	-	-	-	-	-	-	-	-	-	20.0	10.0	-	-	1.1	-
A. flavus	-	42.5	31.2	19.5	14.2	19.5	18.2	25.0	6.6	17.6	-	10.5	-	-	-	7.6	-	12.5	31.2
A. fumigatus	-	2.5	6.0	8.5	3.3	-	5.9	31.2	3.0	6.6	48.0	-	18.7	25.0	42.5	-	-	-	4.9
A. humicola	-	-	-	1.2	-	-	-	-	-	-	-	-	-	2.5	-	-	-	1.9	-
A. niger	-	1.2	-	-	-	-	-	-	-	3.0	-	5.1	-	5.0	9.0	9.6	-	4.2	-
A. parasiticus	-	-	-	-	-	4.2	-	10.5	53.3	63.0	8.0	2.1	14.2	10.0	21.2	15.3	3.3	5.9	-
Chaetomium atrosporum	-	-	-	-	-	-	-	4.0	-	3.0	-	-	-	-	-	-	4.0	-	-
C. globosum	-	-	-	-	-	-	-	4.0	-	-	-	-	-	-	-	-	-	-	-
Cladosporium. sp.	2.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.9	-	-	-
Curvulari clavata	1.2	-	-	-	-	-	-	-	-	4.1	-	-	-	-	-	-	4.2	-	-
Curvularia lunata	1.5	-	-	-	-	-	-	-	-	-	8.0	-	-	-	-	-	-	-	-
Drechslera longirostrata	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2
Fusarium roseum	-	-	-	-	-	-	-	-	-	2.1	-	-	-	-	-	-	-	-	-
b. Bacterial flora																			
Acinetobacta sp.	-	7.2	1.5	-	-	-	-	12.0	2.5	-	-	-	-	-	-	-	1.5	16.5	21.5
Enterobacter hafniae	-	7.2	-	-	1.2	-	-	-	4.5	-	-	-	-	15.2	9.5	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	15.1	1.2	-	-	8.6	-	11.2	20.2	-	-	-	-
Seed germination %	67.0	10.2	33.0	50.0	52.0	55.4	60.2	0.0	15.5	41.2	44.0	51.0	58.0	0.0	10.5	33.0	35.2	50.0	55.2
Seed moisture %	11.5	15.0	14.0	13.2	12.0	11.5	11.2	17.2	15.4	14.2	12.5	12.0	11.8	18.0	16.3	16.0	13.5	12.2	12.0

75% RH. The percent incidence of these bacterial genera increased with the incrase in storage period.

The higher incidence of storage fungi and bacteria in seeds stored at higher RH levels coupled with higher moisture content of seeds are responsible for deterioration of stored seeds. These results are in agreement with the observations of Christensen and Kaufmann[21], Kumari and Reddy[22] in fennel seeds and Mazen *et al*[3]. in paddy grain. It was also observed that germination percentage of seeds decreased as the RH and storage period increased.

A negative correlation has been observed between moisture content and germination of seeds. The percentage of germination decreased with increase in moisture content of seed and relative humidity of storage vessel. In the present study, seeds with high moisture content of 13% and above were associated with increased incidence of storage fungi and their activity would have resulted in decreased percentage of seed germination.

Relative humidity influences the biochemical composition in seeds of *D. sissoo* (Table 2). Total protein, total phenol, total soluble sugar increased progressively with the increase in RH, while starch content decreased. The reason for decreased starch and increased sugar content could be due to breakdown of starch into simple sugars by the activity of fungi and bacteria. These results are in agreement with the earlier observations[23,24] and of Kumari and Reddy[22] in fennel seed and Lokesh and Hiremath[2] in *Cajanus cajan* seeds. Aflatoxin contamination by *Aspergillus flavus* in seeds of *D. sisso* was recorded at all the RH tested. Aflatoxin B_1 and B_2 increased quantitatively with the increase in RH. Aflatoxin G_2 was recorded only at 75% RH. These results support the earlier findings of Kumari and Reddy[22]. It is evident from the results that high humidity levels increase the moisture content and enhanced the multiplication of *Aspergilli,* including higher incidence of *Aspergillus flavus* leading to higher production of aflatoxin and degradation of seeds.

Effect of Temperature :- Microflora varied significantly with the temperature of storage environment[25] (Table 3). Field fungi like *Chaetomium globosum, Chetophoma* spp., *Macrophomina phaseolina* and *Weisneriomyces javanicus* were observed during initial period of storage. Present findings and Emayavaramban and Rambadran[26] observations are in agreement

Table 2. Effect of relative humidity on biochemical composition and aflatoxin production in seeds of *Dalbergia sissoo.*

Biochemical constituents	Relative humidity					
	Control 22%	95%	75%	62%	50%	35%
Total soluble protein (mg/g)	59.5	75.4	70.2	65.4	62.2	60.0
Total phenol control (mg/g)	6.0	11.2	9.5	8.2	7.0	6.5
Total soluble sugar (mg/g)	35.2	49.5	45.2	40.2	39.5	37.2
Total starch content (mg/g)	3.2	1.75	1.92	2.10	2.16	2.54
Aflatoxin B_1	0.21	1.2	0.72	0.54	0.50	0.35
(µg/g) B_2	-	0.98	0.54	0.32	0.25	-
G_2	-	-	0.10	-	-	-

Table 3. Effect of storage temperature on percent incidence of seed microflora, seed germinability and seed moisture of *Dalbergia sissoo*.

Microflora	Before storage		Storage in days																													
			30 days										60 days										90 days									
			10°C		17°C		28°C		37°C		42°C		10°C		17°C		28°C		37°C		42°C		10°C		17°C		28°C		37°C		42°C	
	B*	A*	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
a. Fungal flora																																
Alternaria alternata	2.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-
A. flavus	1.2	-	1.2	4.5	3.2	-	3.8	1.6	2.1	1.0	2.4	2.5	2.5	2.0	6.6	5.9	3.3	12.0	10.2	4.2	1.0	4.2	3.9	12.9	15.2	4.1	25.0	4.5	12.2	14.1	7.2	14.5
A. fumigatus	-	-	-	-	-	-	1.8	-		-	1.5	2.9	-	-	4.2	-	-	-	2.1	-	3.6	4.2	4.1	12.6	-	-	5.2	12.6	-	-	14.5	12.5
A. humicola	2.5	1.0	1.5	-	-	-	9.0	2.1	-	-	3.6	5.4	2.1	11.5	-	-	-	-	-	-	5.2	5.6	-	-	5.2	5.2	-	-	-	-	11.5	2.0
A. niger	1.5	-	2.5	2.0	2.5	2.6	5.4	4.2	4.1	-	2.6	2.5	3.5	11.5	-	-	-	-	5.1	16.2	2.6	2.7	-	-	-	-	4.9	14.2	4.1	12.1	1.0	2.5
A. parasiticus	-	-	2.6	1.5	4.0	5.4	-	-	2.5	12.0	1.2	2.5	5.1	-	-	-	6.6	1.5	3.5	10.0	2.5	2.5	-	-	4.9	1.2	-	-	2.5	11.5	2.5	5.0
Chaetomium globosum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macrophomina phaseolina	-	-	-	-	-	-	1.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phoma sp.	4.1	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weisneriomyces javanicus	-	-	-	-	2.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b. Bacterial flora																																
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	2.5	-	-	1.0	-	-	1.5	2.5	-	1.2	-	-	-	-	-	-	-	1.2	-	2.5	5.1	-	-	-
Percentage of seed germination	95.0	97.0	89.0	87.0	92.5	92.5	92.0	93.0	65.2	66.0	62.0	60.0	85.2	82.5	86.2	86.5	91.0	92.0	63.0	64.0	62.0	60.0	84.1	80.2	70.0	76.0	74.2	75.0	66.2	68.0	56.0	59.0
Seed moisture (%)		10.8		11.2		10.9		10.8		10.6		10.3		11.5		11.0		10.8		11.4		10.2		11.7		11.0		10.8		10.1		10.0

B* = Blotter method
A* = Agar plate method

Table 4. Effect of storage temperature on biochemical composition and aflatoxin production in seeds of *Dalbergia sissoo.*

Biochemical constituents	Temperature				
	Control 28°C	10°C	17°C	37°C	42°C
Total soluble protein (mg/g)	65.2	59.2	63.7	68.2	69.7
Total phenol content (mg/g)	6.9	5.2	6.2	7.2	7.6
Total soluble sugar (mg/g)	39.5	36.2	38.5	42.5	43.2
Total starch content (mg/g)	2.0	2.9	2.4	1.7	2.0
Aflatoxin B_1	1.14	0.52	0.25	1.0	8.0
(μ/g) B_2	1.2	0.50	0.72	0.81	
G_1	-	-	-	0.15	

with those of Chrisetensen and Kaufmann[27] who reported that field fungi require higher seed moisture for their incidence in stored seeds. It was observed that *Aspergillus* spp. *A. flavus, A. fumigatus, A. humicola, A. niger* and *A. parasitica* were dominant both at low and high temperature levels and at all storge periods.

Their incidence frequency decreased with increase in storage temperature and decrease in seed moisture. However, their incidence slightly decreased with duration of storage. Because of their capacity to grow at different storage temperature, the *Aspergillus* have dominated during the course of long time storage. Among bacteria only *Pseudomonas aeruginosa* was reported during storage. Its incidence was more during the 90 days of storage. The study indicated that there was no definited correlation between the storage temperature and incidence of *Pseudomonas.*

The seed germination was also influenced by storge temperature. In the present investigation, storge temperature of 10°C recorded higher seed germination. The viability of seed was not affected at the labortory temperature (28°C) during 30, 60, 90 days of storage. While a gradual decrease in seed viability was noticed with increase in temperature which reached maximum at 42°C with the duration of storage. There existed a negative correlation between seed germination and seed moisture content with storage temperature i.e. with the increase in storage temperature there was reduction in the seed germination and moisture content. Our above observation is in agreement with the earlier findings of Mohan[28] in sorghum, Singh[29] in soybean, Sahai[7] in some leguminous taxa.

The biochemical composition of seeds of *D. sissoo* at the end of storage under different temperature conditons are presented (Table 4). It was observed that as the temperature increases, the total protein, total soluble sugar and total phenol showed increase while total starch content showed decrease. These results are in agreement with those of Deo and Gupta[30] in grain seeds. Aflatoxin B_1 and B_2 production were observed at all temperature treid. At temperature 10°C, aflatoxin production was minimum while at 28°C it was maximum. These results are in confirmity with the finding of Trucksess *et al.*[31] in seeds of corn, pinto bean and soybean. This could be correlated with the lower and higher incidence of *Aspergillus flavus* at 10°C and 28°C respectively.

Acknowledgement

We feel indebted and thankful to Head, Department of Botany, University of Rajasthan, Jaipur for his kind gesture and consistent help for this study.

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