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ORIGINAL ARTICLE

Effects of Storage Relative Humidity and Moisture Content on Seed Viability, Seed Health of *Vigna mungo* During Storage Periods

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ABSTRACT

The study was conduct to investigation the effect of environmental factors like relative humidity (RH) and moisture content (MC) on seed germination, seed micro flora of Vigna mugo in relation to aflatoxin production and seed health. The incidence of storage fungi increased with the increase percentage of relative humidity (35%, 50%, 62%, 75% and 95%) and increase percentage of moisture content (MC) (10%, 12%, 14%, 16% and 18%) during storage periods (30, 60 and 90 days). Various species of Aspergillus were recorded at different relative humidity and moisture levels. The maximum seed germination percentage was recorded in seeds with storage at low moisture content and low relative humidity. There was decline in seed germination percentage after 90 days storage at high moisture content and high relative humidity. The biochemical composition of Vigna mungo seeds during storage changed significantly at different relative humidity and temperature. Aflatoxin production was higher at 18% moisture content and at 95% relative humidity as compared to other moisture content and relative humidity regimes. The storage life of Vigna mungo seeds can be increased by lowering relative humidity and moisture content during storage.

***Key words**: Relative Humidity (RH), moisture content (MC), Seed Microflora, Aflatoxin and Vigna mungo.*

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INTRODUCTION

Mostly 90% of plants grow by their seeds only thus seed storage is very important operation that decides the success of seeds in next generation. Quality of seed characterized by higher viability and vigor is one of the most important basic needs for higher productivity (Yaklich, *et al*., 1979). The quantity of water thus absorbed influences the storage behavior 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 (Arulnadhy and Senanayake 1991; Lokesh and Hiremath 1993; Mazen, *et al.,* 1996; Fabrizius, *et al.,* 1996; Ray 1998; Wicklow, *et al.,* 1988; Sahai 1999) Pulses are mesobiotic in storage behavior, which can only be stored up to a storage period of three years (Ewart, 1908). Higher lipoprotein content of pulses gain moisture on storage undergo rapid lipid per oxidation (Agrawal and Dadlani, 1995) that leads to degradation of seed quality and finally ends with death of seed. Seed, like any other biological materials deteriorate with ageing and the rate of which depended on many factors such as dormancy (Parameshwari, 1999), chemical composition (Maranville and Clegg, 1977), storage temperature, micro flora (Chacko and Singh, 1971), seed

moisture and relative humidity (Justice and Basu, 1978, Bhushan, *et al.,* 2013a &b). Hence it was considered to study the effect of relative humidity and temperature on seed microflora, aflatoxin contamination and seed health of *Vigna mungo*.

MATERIAL AND METHODS

Freshly harvested and properly sun dried *Vigna mungo* seeds were collected from Bareilly. 500g seeds of *Vigna mungo* were stored for three months. After determining the initial moisture content, the grains were adjusted to the required MC by adding the required amount of distilled water and leaving over night. The MC was then checked by oven drying and readjusted, if necessary, until the desired MC was achieved. The different grains MCs tested in our experiments were 10,12,14,16 and 18 percent. The grains were maintained at these MCs in sealed polythene bags and kept in separate

The relative humidity (35%, 50%, 62%, 75% and 95%) was maintained in dessicators (Sacheti 1996; Solomon) and stored at room temp (28C+1). At the end of 1, 2 and 3 months of storage, Agar Plate methods were employed for the isolation of associated microflora (ISTA. 1993). Germination percentage was determined as per ISTA rules (ISTA. 1976). The percentage incidence of each fungus was also calculated. Changes in biochemical concentration such as total soluble protein (Lowry, *et al.,* 1951) phenol (Bray and Thorpy 1954), sugar (Dubois, *et al.,* 1951) and starch (Mccready, *et al.,* 1950) content were analyzed by standard methods, for aflatoxin the samples were observed under long wavelength UV light for bright green yellow fluorescence (Fennell, *et al.,* 1975). The samples giving positive fluorescence were further extracted for aflatoxin contamination (Thomas, *et al.*, 1975). Quantitative estimation was done by the method of Nabney and Nesbitt 1965.

RESULT AND DISCUSSION

Effect of Relative Humidity:

Relative humidity exerted significant influence on the seed microflora (Table 1). Five fungal species (*Alternaria alerneta, A. niger, Curvularia clavata, Rhizopus* sp, *Mucor* sp.) were isolated before storage. Only *Penicillium citrinum* was isolated from agar plate method only at 75% Rh and 95% Rh on 90 days storage. These results supported the earlier findings that increase in RH promote faster seed deterioration (Jamaluddin, *et al.,* 1977). The higher incidence of storage fungi in seed stored at higher RH level coupled with higher moisture content of seeds are responsible for deterioration of stored seeds. Similarly results have been observed by (Christensen and Kaufmann 1965), (Kumari and Reddy 1993) in fennel seeds, (Mazen, *et al*., 1996) in paddy grain and (Gupta, *et al., 2003)* in *D. sissoo* seeds, (Bhushan, *et al*., 2013a & b) in seeds of *Pennisetum typhoides* and *Sorghum vulgare*. It was also observed that germination percentage 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, average percentage incidence of seed germination was maximum 88.5% at 35% RH followed by 82.2% at 50% RH, 57.3% at 62% RH, 50.1% at 75% RH and 47.2% at 95% RH and average percentage incidence of seed moisture content were maximum 51.63% at 95% RH. 48.66% at 75% RH, 41.29% at 62% RH, 15.77% at 50% RH and 9.32% at 35% RH were obtained after 90 days of storage.

Relative humidity influences the biochemical composition in seeds of *Vigna mungo* (Table 3). 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. These results are in agreement with the earlier observation (Jamaluddin, *et al*., 1977; Vidhyasekaran and Kandaswamy 1972; Gupta, *et al.,* 2003; Gupta, *et al.,* 2004)

and (Kumari and Reddy 1993) in fennel seed and (Lokesh and Hiremath 1993) in *Cajanus cajan* seeds. Aflatoxin contamination by *Aspergillus flavus* in seeds of *Vigna mungo* was recorded at all the RH tested. Aflatoxin B_1 and B_2 increased quantitatively with the increase in RH. Aflatoxin G_1 was recorded at 75%, 95% RH and Aflatoxin G_2 only at 95% RH. These results support earlier findings of (Gupta and Rao 2000), (Gupta, *et al., 2003 & 04)* (Bhushan, *et al*., 2013a & b). It was evident from the multiplication of *Aspergillus,* including higher incidence of *Aspergillus flavus* leading to higher production of aflatoxin and degradation of seeds.

Table 1: Effect of Relative humidity on Percent Incidence of Seed Microflora and Seed Germination of *Vigna mungo*

Percent incidence of Fungi	Before Storage	30 Days storage in different RH					60 Days storage in different RH					90 Days storage in different RH				
		35	50	62	75	95	35	50	62	75	95	35	50	62	75	95
Alternaria alerneta	1.4	1.9	3.6	4.2	5.9	6.6	1.2	-	-	-	-	-	-	-	-	-
Aspergillus flavus	-	-	-	1.4	3.0	4.2	2.6	4.5	7.9	9.4	10.6	5.6	8.4	9.0	12.7	20.7
A.niger	0.3	1.2	3.2	6.3	6.8	7.4	4.9	7.4	9.2	13.6	15.7	6.8	8.5	12.6	18.9	24.6
A.fumigates	-	-	1.5	2.8	3.8	4.9	4.9	8.5	9.4	12.0	14.8	8.4	9.4	10.4	18.4	25.3
A.versicolor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium oxysporum	-	4.9	6.2	6.8	7.4	5.3	6.3	5.3	-	-	-	2.1	-	-	-	-
Penicillium crysogenum	-	-	-	2.4	5.6	8.1	-	-	6.7	8.4	11.3	-	-	6.9	10.4	14.7
Penicillium citrinum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.8	4.1
Curvularia clavata	2.8	3.5	4.1	5.8	4.2	1.4	4.6	7.9	4.6	-	-	1.9	-	-	-	-
Rhizopus sp.	6.9	8.5	9.4	10.5	8.5	4.9	9.5	10.4	-	-	-	2.2	4.2	-	-	-
Mucor sp.	1.8	2.6	4.8	6.7	3.8	1.0	-	-	-	-	-	-	-	-	-	-
Seed Germination%	97.6	96.3	90.2	84.0	75.8	65.4	93.8	85.4	70.3	63.5	54.9	88.5	82.2	57.3	50.1	47.2
Moisture Content	13.69	14.2	14.76	15.2	15.8	17.56	14.8	15.6	15.9	16.32	18.13	15.2	16.8	17.32	18.4	19.0

Table 2: Effect of Moisture Content on Percent Incidence of Seed Microflora and Seed Germination of *Vigna mungo*

Fungi	Before storage	30 Days storage in different MC					60 Days storage in different MC					90 Days storage in different MC				
		10	12	14	16	18	10	12	14	16	18	10	12	14	16	18
Alternaria alerneta	1.4	2.1	4.5	5.9	4.2	2.1	2.4	5.6	2.4	-	-	2.8	1.5	-	-	-
Aspergillus flavus	-	-	-	3.5	6.8	8.4	-	3.6	6.5	8.5	10.4	-	4.9	8.4	17.8	26.5
A.niger	0.3	1.8	4.8	6.9	9.4	10.4	2.5	8.4	8.6	13.4	19.7	3.0	8.5	12.4	18.4	24.2
A.fumigates	-	-	2.3	3.8	4.9	8.4	-	5.9	7.9	10.7	16.9	-	6.9	9.5	16.3	20.0
A.versicolor	-	-	-	-	-	-	-	-	-	1.9	4.2	-	-	-	3.8	6.4
Fusarium oxysporum	-	-	1.0	2.8	5.4	8.2	-	1.4	1.8	3.2	4.9	-	-	-	-	-
Penicillium crysogenum	-	-	1.8	4.8	8.5	10.4	-	4.5	5.3	10.6	12.6	-	7.4	9.4	14.5	20.5
Penicillium citrinum	-	-	1.4	4.9	8.6	10.3	-	4.2	7.8	10.0	16.4	-	5.3	9.4	16.4	22.5
Curvularia clavata	2.8	3.7	-	-	-	-	3.5	-	-	-	-	2.5	-	-	-	-
Rhizopus sp.	6.9	7.4	7.6	4.0	3.1	0.3	6.2	-	-	-	-	6.3	-	-	-	-
Mucor sp.	1.8	2.2	4.5	3.8	1.0	0.7	4.0	-	-	-	-	5.8	-	-	-	-
Seed Germination%	97.6	94.8	86.8	70.5	60.4	50.4	92.0	80.3	65.3	56.8	46.4	89.4	76.9	53.6	48.3	38.0
Moisture Content	13.69	14.1	14.5	15.8	16.4	16.9	14.6	14.89	16.29	16.99	17.05	14.73	15.24	17.07	18.6	20.32

Effect of Moisture Content:

The total numbers of eleven fungal species belonging to seven genera were isolated using agar plate methods (Table 2). *Fusarium oxysporum* was present only in 14, 16, 18% MC in 30, 60 day stored seeds and abse4nt in 90 day stored seeds. *A. versicolor* was isolated in 16, 18% MC in 60 and 90 days stored seeds sample. Table 1 reveals that the microflora varied significantly with the moisture contents of the storage environment. It was observed that initially (before storage) five fungal species were isolated. After 30, 60 and

90 days of storage period eleven fungal species were isolated but *A.niger* found to be associated with seeds at all moisture content levels. *Curvularia clavata* appeared only at 10% MC during 30-90 days of stored seeds. These results supported the earlier finding which showed that increase in MC promotes the seeds deterioration (Jayaraman and Kalyansundaram, 1989) in seeds of rice; (Mazen, *et al.,* 1993) in seeds of cotton; (Bielecka, *et al.,* 1995) in rape seeds; (Paderes, *et al.,* 1997) in paddy seeds. The field fungi were noticed mainly in low seed moisture. It is suspected that higher seed moisture would have enhanced the rapid multiplication of the more aspergilli and thus would have supported the growth of storage fungi. Further, a direct correlation between reduced seed germination and higher incidence of aspergilli has been observed. Important factors that determine the viability of seeds in storage is seed moisture content (Ananthi, *et al.,* 2017). The results are in agreement with the observations of (Christensen 1973), (Gupta, *et al., 2003 & 04)* and (Bhushan, *et al.,* 2013a & b).

The moisture content influences the biochemical composition in seeds of *Vigna mungo* (Table 2). Total protein, total phenol, total soluble sugar increased progressively with the increase in MC, while starch content decreased. The reason for decreased starch and increased sugar content could be due to bacteria and fungi. These results are in agreement with the earlier observation (Lokesh and Hiremath 1993; Vidhyasekaran and Kandaswamy 1972; Kumari and Reddy 1993; Jamaluddine, *et al.,* 1985). Aflatoxin contamination by *Aspergillus flavus* in seeds of *Vigna mungo* was recorded at all the MC tested. Aflatoxins B1 and B2 increased quantitatively with the increase in MC. Aflatoxins G1 was recorded at 14, 16, 18 % MCs and aflatoxin G2 at only 18 % MCs. Our above observation is in agreement with the earlier finding of (Ragab and Syiad 1998) in food grains.

Table 3: Effect of Relative humidity and Moisture Content on Biochemical Composition and Aflatoxin Production in Seeds of *Vigna mungo*

Experinent		Biochemical Constituents (mg/g)				Aflatoxin(µg/g)			
		Total Soluble Protein	Total Phenol Content	Total Soluble Sugar	Total Starch Content	B_1	B_2	G_1	G_2
Before storage	-	4.24±0.03	0.56±0.02	2.37±0.05	3.68±0.01	-	-	-	-
Relative humidity (%)	35	4.0±0.01	.052±0.03	2.52±0.03	3.58±0.03	-	-	-	-
	50	3.43±0.02	.047±0.01	3.36±0.02	3.45±0.01	0.72±0.01	0.38±0.01	-	-
	62	3.19±0.05	.034±0.05	3.68±0.04	3.02±0.02	0.98±0.05	0.42±0.05	-	-
	75	2.88±0.01	.067±0.01	3.83±0.02	2.87±0.04	1.04±0.03	0.53±0.05	0.21±0.02	-
	95	2.19±0.03	.089±0.05	4.38±0.01	2.01±0.05	1.20±0.04	0.84±0.02	0.22±0.04	0.12±0.03
Moisture Content (%)	10	4.12±0.01	.055±0.62	2.62±0.03	3.48±0.02	0.29±0.01	0.02±0.01	-	-
	12	3.70±0.02	.048±0.05	3.40±0.04	3.08±0.05	0.38±0.82	0.17±0.05	-	-
	14	3.20±0.01	.037±0.02	3.64±0.02	2.75±0.01	0.83±0.64	0.62±0.03	0.29±0.01	-
	16	2.86±0.02	.061±0.01	4.09±0.05	2.30±0.03	0.95±0.02	0.42±0.02	0.17±0.05	-
	18	2.00±0.02	.086±0.04	4.53±0.01	1.82±0.01	1.09±0.01	0.71±0.04	0.49±0.02	0.14±0.05

CONCLUSION

In general, the study clearly indicated that when the seeds were stored at higher relative humidity and moisture content, the activity of aspergilli was found to be more which release toxic metabolites into seeds. Presence of these toxic substances in the seeds, affect quality of the stored grain, adversely making it unfit for consumption.

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