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## Biochemical, Physiological and Mycological changes in Gram seeds due to infestation of Pulse beetle during storage

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ABSTRACT

Gram is an important sources of protein. The seed infestation by pulse beetle during storage is a major problem. This paper gives a brief account of certain Physical, Physiological, Biochemical and Mycological changes in qualities of Gram seeds due to infestation of pulse beetle during storage. In the present study, it was found that the percentage of moisture content, total ash, crude fiber, and crude protein significantly increased and crude fat total carbohydrate, total sugar reducing sugar and non-reducing sugar significantly decreased in pulse beetle infested seeds of Gram during storage. Increase in protein content is attributed to insect metabolites like uric acid, which is nitrogenous is nature. The incidence percentage of fungi such as species of Alternaria, Aspergillus, Curvularia, Fusarium Penicilium and Rhizopus were predominant over all other fungi on infested gram seeds and it is increased with increase in pulse beetle infestation during storage. The physical and physiological qualities of Gram seeds i.e. 100-seed weight, germination, seedling vigour and field emergence percentage decreased with increase in infestation of pulse beetle during storage.

Key words: Gram, Pulse beetle, infestation, seed quality and storage.

#### INTRODUCTION

Pulses are the most important source of protein in Indian diet. Storage of pulse seeds is a major problem and it is estimated that about 10% of stored pulse seeds are lost due to biological factors of which insects and rodents alone account of 5%. In severe cases the infestation was observed to be about 90%. Pulse beetles of various species belong to the family Bruchidae are important insect pest attacking variety of pulses in store. Adult female stick their eggs on the pulse seeds and the emerging grubs and bore into the seeds. The grubs remain inside the seed and appearance of a capped exit hole on the seed indicates the pupil stage. After a few days the adult emerges from the seed. About one month is required to complete one generation.

The stored grain insect's pest's infestation also encourages fungus growth by increasing the moisture content of the seeds which decreased the quality and viability of the seeds.

Christensen and Kaufmann (1969) reported that the fungal pathogen associated with stored seed are chiefly responsible for seed deterioration and reduction in germination potential. Apart from this the seedling vigour is also adversely affected. Among the storage fungi species, many were well known toxin producers. The present work was carried out to investigate the post-harvest losses in qualities of Gram seeds due to pulse beetle infestation.

#### **MATERIAL METHODS**

The Freshly threshed Gram seeds were dried upto the safe moisture level  $(10\pm1\%)$  and the experiment conducted in glass bottle of two litre capacity. The glass bottle was then filled with 1,000 grams of Gram cv. Chaffa- 816 seeds. There were four replications. Ten pairs of 2-3 days old pulse beetle (Callosobruchus analis) were released in glass bottles covered with muslin cloth. The set of experiment was kept in well ventilated wire mesh almirah in mesonary building having cemented walls, roof and floor under ambient temperature (18.7 to 46.9°C) and relative humidity (24 to 87%) from March 2015 to Aug. 2015. For determination of physical, physiological, biochemical and mycological changes in stored seeds of Gram were observed at interval of 3 months. The initial observations also taken at the start of experiment. The physical qualities of Gram seeds i.e. seed infestation percentage, moisture content and 100 seeds weight were studied. 100 seed weight was tested in quadruplicated with 100 seeds in each replication. The infested seeds we counted and total damaged seeds were reported in percentage. Moisture percentage was estimated according to International rule for seed testing (Anon. 1985). The physiological qualities of Gram seeds i.e. seed germination, seeding vigour and field emergence were studied. The germination percentage was evaluated on the value for percent normal seedlings (Anon. 1985). The seedling vigour index was worked our following the method of Abdul-Baki and Anderson (1973. For field emergence test, sowing of Gram seeds was done in randomized block design with four replications with inter and intra-row spacing of 1 feet and 6 inches respectively. Observations for field emergence were recorded daily and finally the established seedlings were counted after one month of sowing.

To assess the biochemical qualities of the seeds of Gram i.e. protein, fat, total ash, crude-fibre, reducing and nonreducing sugars according to the standard procedures of A.A.C.C. (Anon., 1962). Values for carbohydrate and total sugar were calculated (Joslyn, 1970) The fungal flora of the seeds were detected by the standard moist blotter and agar medium techniques as prescribed by I.S.T.A. (Anon., 1976) the different types of fungal growth on the seeds were expressed in percentage. The experimental data was statistically scrutinized as per Panse and Sukhatme, 1967.

#### **RESULTS & DISCUSSION**

It was observed from the Table 1 that the moisture content of the seeds increased with increasing the storage periods i.e. 3 months (10.91%) and 6 months (12.26%). A significant increase in moisture content was observed this might be due to the activities of pulse beetles on seeds during storage. Similar observation also reported by Shrivastava et al. (1989). Gadewar et al. (2011) Seed damage is increased with increasing the storage periods of 3 months (25.10%) and 6 months (59.28%) respectively. Charjan et al. (2006) and Gadewar et al. (2011) reported that the infestation of pulse beetles increased with increasing the storage periods. The 100-seed weight of seed decreases with increasing the storage periods. Similar observation also reported by Charjan (1995). Similarly the germination, seedling vigour and field emergence % decreases with increasing the storage periods. In coastal region of Andhra Pradesh percent germinability of Bengal gram was found to decrease from 81% to 65% within 4 months of storage (Vimla and Pushpamma, 1993). Charjan and Tarar (1994) and Gadewar et al. (2011) reported that the germination percentage, seedling vigour and field emergence percentage decreases with increasing storage periods in moth bean and pigeon pea infested by pulse beetles during storage.

Pulse beetle feeds on the cotyledonous portion of the Bengal gram seed leaving the seed coat intact and that is one reason that higher values for crude fibre and total ash have been obtained in infested seed, as seed coat is rich in crude fibre and minerals (Singh *et al.* 1968 and Shrivastava *et al.* 1989). Increase in protein content is attributed to insect metabolites like uric acid, which is nitrogenous in nature (Shrivastava *et al.* 1989). Increase in non-reducing sugars and decreasing in non-reducing sugars has been shown in stored Bengal gram seeds. Similar results have been reported by Khare (1972), Shrivastava *et al.* (1989) Gadewar *et al.* (2011) Charjan and Tarar (1994). The following fungi were found to be associated with stored seeds of Gram. The present pulse

	Seed Quality parameters	Initial	After 3 months	After 6 months
	Physical seed quality			
А	1. Seed moisture (% wb)	10	10.2	12.7
	2. Seed damage (%)	0.00	25.1	52.1
	3. 100-seed weight (gm)	10.12	9.0	7.9
	Physiological seed quality			
В	1. Germination (%)	96	88	49
	2. Seedling Vigour Index (SVI)	4516	3617	2014
	3. Field emergence (%)	88	73	34
	Biochemical seed quality			
	1. Total ash (%)	4.01	5.62	6.67
С	2. Crude fibre (%)	7.9	9.0	8.9
	3. Crude Protein (%)	24.01	24.90	30.0
	4. Crude fat	2.9	3.2	2.1
	5. Total carbohydrate	70.10	69.1	64.0
	6. Total sugar (%)	9.2	8.2	7.1
	7. Reducing sugar (%)	12.7	10.7	2.4
	8. Non-reducing sugar	8.9	7.1	6.1
	Mycological observation			
	1. Alternaria sp. (%)	7.25	5.25	1.75
D	2. Aspergillus sp. (%)	3.25	9.25	4.25
	3. Curvualaria sp. (%)	1.75	5.75	21.00
	4. Fusarium sp. (%)	0.25	6.25	11.75
	5. <i>Penicillium</i> sp. (%)	0.25	4.25	12.25
	6. Rhizopus sp. (%)	0.25	4.25	12.75
	7. Total incidence (%)	14.00	27.00	70.25

Table 1; Effect of pulse beetle infestation on physical, physiological, biochemical and mycological qualities of
Gram during storage.

beetles damaged seeds yielding a particular fungus viz., *Alternaria* sp., *Aspergillus* sp., *Curvualaria* sp., *Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. irrespective of storage periods. In the present study, the incidence percentage of storage fungi increases with increasing seed damages by pulse beetles and storage periods. The results are in conformity with the results of Charjan *et al.* (2006) and Gadewar *et al.* (2011)

Thus from the present study, it can be concluded that infestation of pulse beetle in Gram increases the moisture content which is favorable for multiplication of fungal flora and decreases the 100-seed weight, germinability, seedling vigour and field emergence percentage of seeds during storage. It also observed that the decrease of the crude fat, total carbohydrates and total sugars and increase of total ash, crude fibre and crude protein in infested Gram seeds. Increase in protein content is attributed to insect metabolites like uric acid, which is nitrogenous in nature. The percentage of storage fungi increases with increasing pulse beetles damage and storage period. Among the identified fungi species, many were well known toxin producers. The pulse beetle infested Gram seeds should be avoided for sowing or consumption purposes.

**Conflicts of interest:** The authors stated that no conflicts of interest.

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