



Changes in Blackgram Seed Quality during Storage as Influenced by Various Packaging Materials and Storage Conditions

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Abstract: A storage experiment was conducted to know the influence of various packaging materials and storage conditions on blackgram seed quality. During the course of the investigation, various packaging materials such as cloth, gunny, high density polythene (HDPE) and vacuum packed bags were used and these bags were kept under ambient and cold storage for 18 months. As the storage period progressed, seed infestation with bruchids (*Callosobruchus* spp.) was observed in cloth, gunny and HDPE bags under ambient condition only after 8 months of storage. At the end of the storage period, there was no such bruchids infestation was seen in a vacuum packed bag of ambient condition and in all the packaging materials of the cold condition. Further, at the end of the storage period, seeds of vacuum packed bag under both ambient and cold conditions only have maintained minimum germination percent as described by the Central Seed Certification Board, Government of India. So, this technology can be effectively used to extend the storage period of blackgram without any storage chemicals i.e., in a chemical-free environment. Hence, along with maintaining seed health for a longer time, a threat to the environment can be completely averted.

Keywords: Ambient and cold conditions, Bruchids infestation, Blackgram, Conventional packaging, Vacuum packaging

The significant agricultural production could be impacted due to the variations in periodicity and intensity of climatic events like floods and droughts, temperature and rainfall patterns (Arun et al 2017). Moreover, a substantial amount of food grains is being damaged after harvest due to a lack of adequate storage and processing facilities. FAO estimated worldwide annual losses of about 10 per cent in stored grains (Parfitt et al 2010). Ensuring global food security requires a range of strategies including the diversification and improvement of staple food crops and the reduction of post-harvest loss. In India, due to insufficient and poor storage facilities, lack of knowledge in post-harvest pulse management and storage, the risk of damage due to post harvest storage (25-30 %) is a major concern in pulses (Anonymous 2017). Controlling these major losses along with other post harvest losses viz., threshing, drying and milling losses can meet the demand for pulses and can reduce the import from various countries. Pulses can remain in edible condition for a long time if, properly stored. However, pulses are more difficult to store compared to cereals (Deshpande and Singh 2001) and suffer much greater damage due to insects and microorganisms resulting in deterioration of quality. Since, pulses have high protein content and are prone to very high insect infestation and thus, undergo heavy losses during storage (Lal and Verma 2007). Among the insects of grain legumes, bruchids (*Callosobruchus* spp.) are very serious

pests both in the field and as well as in storage, that can cause considerable economic losses in various pulses such as chickpea, mung, peas, cowpea, lentil and arhar (Srinivasan et al 2008). This insect infestation either originates in the field or in storage and due to their internal feeding behaviour of larvae causes serious loss to the seeds in terms of both quantity and quality (Messina and Jones 2009).

Bruchids are controlled by treating the seeds with carbon disulfide, phosphine or methyl bromide or by dusting with several other insecticides. However, their use by the farmers is criticized worldwide (Williamson et al 2008) as these chemicals are highly toxic and environmentally undesirable and pose a threat to food safety and bio diversity. In addition to chemical control of bruchids, some plant derived extracts viz., plant juice of banana, oil from soybean, maize and neem, hot pepper powder has proven beneficial (Swella and Mushobozy 2007), these plant derived extracts, despite many advantages, have certain limitations such as slow in action, easy degradability (Rozman, 2015), frequent application and importantly affecting non-target organisms to some extent (Trivedi et al 2018). With these limitations of using both synthetic and plant derived chemicals to control bruchids, research on chemical free storage has been conducted with the use of different packaging materials and their storage under different conditions to assess the storability of blackgram seeds.

MATERIAL AND METHODS

A storage experiment was conducted under ambient and cold storage conditions at the University of Agricultural Sciences, Dharwad, Karnataka, India for 18 months (15 November 2019 to 15 May 2021). Average data related to temperature °c and relative humidity (%) that prevailed in the storehouse of ambient condition during the first 15 days of every alternate month were recorded with the help of Anemometer (Table 1), whereas, in the storehouse of cold condition, 5-7°C temperature with 60 ± 2 % relative humidity was maintained throughout the storage period. Freshly harvested healthy seeds of blackgram (DU-1) were collected from farmers' fields and then sun-dried. After sun drying, crops seeds were packed in different packaging viz., cloth bag, gunny bag, HDPE bag and also vacuum packed (wherein the product to be packed is placed in a pouch of suitable material and the air is drawn out from the pack and hermetically sealing it) (Fig. 1). The seeds (3 kgs) were packed in the cloth, gunny and high density polythene (HDPE) bag and replicated 5 times in two storage conditions. However, in the case of vacuum packaging, 1 kg of seeds was packed and 9 bags were packed and replicated 5 times in two storage conditions. Further, stacking of bags was not done, to expose all the bags to prevailing environmental conditions. The characteristics of the polythene bag used for vacuum packaging are presented in Table 2. The machine used for vacuum packaging of different seeds was OLPACK



Fig. 1. Different packages used for storage of blackgram seeds under ambient and cold conditions

Table 1. Average temperature and relative humidity of storehouse of ambient condition

| Months | Temperature (°C) | Relative humidity (%) |
|----------------|------------------|-----------------------|
| January-2020 | 21.3 | 68.6 |
| March-2020 | 24.3 | 54.6 |
| May-2020 | 28.5 | 67.2 |
| July-2020 | 22.8 | 86.9 |
| September-2020 | 22.7 | 85.4 |
| November -2020 | 22.1 | 63.1 |
| January-2021 | 20.9 | 74.1 |
| March-2021 | 24.8 | 51.7 |
| May-2021 | 26.6 | 67.8 |

501/V manufactured by Interprise–Brussels S.A., Bruxtainer Division, Belgium. Seed health parameters such as germination (%), total seedling length (cm), moisture content (%) and α amylase activity were recorded. For the germination test, a number of normal seedlings were counted on the final day (on 7th day) (ISTA, 2013) and the average value was expressed in percent. Total seedling length is the summation of shoot length and root length of seedlings on the final day and the average value is expressed in centimeters. Moisture content was measured as per the procedure described by ISTA (2013) and α amylase activity was also measured (Sadasivam and Manickam 1996). Seeds with initial germination (98.00 %), total seedling length (49.8 cm), moisture content (8.10 %) and α amylase activity (54.77 μ g maltose released/min/ml) were used for packaging. Again, on the 15th of every alternate month, representative samples were drawn from all treatments and all the above-mentioned seed health parameters were recorded for up to 18 months.

RESULTS AND DISCUSSION

Germination: After 2 months of the storage period, there was no significant difference was observed in germination between the treatments (Table 3). As the storage period progressed, there was an infestation of seeds with bruchids under ambient condition in all packaging's except vacuum packed bags. The infestation of seeds with bruchids under ambient conditions was due to the pervious nature of packaging material that allowed the bruchids to penetrate the packaging and multiply. Similar results of seeds infestation

Table 2. Specifications of the LDPE bag used for vacuum packaging

| Characters | Unit | Results | Tested as per (Guidelines of) |
|-------------------------------|---|---------|-------------------------------|
| Thickness (Microns) | Microns | 149.40 | IS: 2508 |
| Water vapor transmission rate | $g/m^2/24$ hrs at 38°C and 90.0 % Relative humidity | 0.95 | ASTM F 1249 |
| Oxygen transmission rate | $cc/(m^2 \times day \times atm)$ | 0.91 | ASTM D 1434-15 |

with insects in pervious packaging was reported by Patel et al (2018). The infestation of seeds with bruchids resulted in significant decrease in germination due to the internal feeding behaviour of larvae hence, those packaging's were discarded and observations were discontinued in which bruchids infestation were seen. Similar results of a decrease in germination due to infestation of bruchids were reported by Miah et al (2013) and Meenatchi et al (2018).

Before seed infestation with bruchids, the rate of seed deterioration was higher in cloth, gunny and HDPE bags which are kept under ambient storage as compared to cold storage and it might be due to the differential rate of seed respiration as they were kept in different environmental conditions. Similar results of a higher rate of seed deterioration in ambient condition as compared to cold storage condition were reported by Vange et al (2015) and Gupta et al (2017). The rate of seed deterioration was much lower in both ambient and cold conditions in vacuum packed seeds as they were kept in modified atmospheric condition, which enables them to maintain more stable relative humidity inside the package and they were packed in a polythene bag having higher thickness, lower water vapor and oxygen transmission rate. In vacuum packed bag, the seeds were placed under low oxygen concentration and due to the low oxygen concentration in the package, there was very little oxidation of the product and thereby, seeds were less deteriorated as compared to other packaging materials.

However, vacuum packed seeds stored in cold condition have performed better as compared to ambient condition and this is because of temperature difference in both conditions as the temperature is also one of the factors which determine the rate of deterioration. Similar results of maintaining the seed viability and vigour for a longer period by vacuum packaging have been reported by Tripathi and Lawande (2014), Khanna et al (2017) and Meena et al (2017). In cold storage condition, as the storage period progressed, there was a decline in viability and vigour of all the seeds in all the pervious packaging due to ageing. As the ageing of seeds is a function of temperature, oxygen and moisture. In cold storage, temperature and moisture are well managed but oxygen has played a major role in seed deterioration. It is well known that, oxygen is an important factor to regulate a series of physiological processes caused by respiration, which was associated with seed deterioration. The results of the investigation are in accordance with the findings of Basavegowda and Arunkumar (2013) and Bhadru et al (2018). Apart from temperature, the main factors of seed deterioration are oxygen and moisture content and maintenance of these constraints will have a greater impact on seed longevity and it can be achieved by vacuum packaging. The beneficial effect of vacuum storage on extending seed longevity has also been reported in sweet corn seeds (Chiu et al 2003). Yeh et al (2005) reported that, the longevity of primed bitter gourd seeds was markedly

Table 3. Influence of packaging and storage conditions on germination (%) at different time intervals of storage in blackgram seeds

| Treatments | | Storage period (Months) | | | | | | | | |
|-----------------|-----------------------------------|-------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| Ambient storage | T ₁ -Cloth bag | 96.8 (79.7) | 93.0 (74.7) | 90.5 (72.0) | * | * | * | * | * | * |
| | T ₂ -Gunny bag | 96.5 (79.2) | 92.5 (74.1) | 90.3 (71.9) | * | * | * | * | * | * |
| | T ₃ -HDPE bag | 97.0 (80.0) | 93.5 (75.2) | 90.8 (72.3) | * | * | * | * | * | * |
| | T ₄ -Vacuum packed bag | 98.0 (81.9) | 98.0 (81.9) | 97.5 (80.9) | 97.5 (80.9) | 97.0 (80.0) | 96.8 (79.7) | 96.5 (79.2) | 96.0 (78.5) | 96.0 (78.5) |
| Cold storage | T ₅ -Cloth bag | 97.5 (80.9) | 95.5 (77.8) | 93.0 (74.7) | 90.3 (71.9) | 85.8 (67.9) | 82.0 (64.9) | 78.8 (62.6) | 76.0 (60.7) | 72.5 (58.4) |
| | T ₆ -Gunny bag | 97.0 (80.0) | 94.8 (76.8) | 93.5 (75.2) | 90.0 (71.6) | 86.3 (68.3) | 81.5 (64.5) | 78.5 (62.4) | 76.3 (60.9) | 73.0 (58.7) |
| | T ₇ -HDPE bag | 96.8 (79.7) | 95.3 (77.5) | 93.3 (75.0) | 89.5 (71.1) | 86.0 (68.0) | 81.8 (64.7) | 79.3 (62.9) | 76.5 (61.0) | 72.2 (58.2) |
| | T ₈ -Vacuum packed bag | 98.0 (81.9) | 98.0 (81.9) | 97.8 (81.5) | 97.5 (80.9) | 97.3 (80.5) | 97.0 (80.0) | 96.8 (79.7) | 96.5 (79.2) | 96.3 (78.9) |
| C. D (p=0.01) | | 1.7 | 1.0 | 1.7 | 1.9 | 2.2 | 1.9 | 1.8 | 2.0 | 1.9 |

Figures in parenthesis are arcsine values

*Observations in cloth, gunny and HDPE bag treatments have been stopped due to bruchids infestation after 8 months of storage period

As per central Seed Certification Board, Department of Agriculture & Co-operation, Ministry of Agriculture, Government of India, the minimum seed germination for black gram is 75 %

decreased after 12 months of storage under ambient oxygen conditions, but seed viability was maintained under partial vacuum storage conditions. Similar results of maintaining seed viability for a longer period by vacuum packaging were reported by Meena et al (2017) and Wang et al (2018). Bruchids last for 29-30 days at 30°C and 70 per cent relative humidity (Raina 1970) and they have higher fecundity (War et al 2017). Many researchers have investigated the origin of the infestation of bruchids and reported that it either starts in the field or in storage. However, in the present study, it originated in the store because no bruchids were detected after two months of storage; if infested in the field, heavy bruchids were seen due to their short life cycle and high fecundity.

Total seedling length: Deterioration in seed quality is associated with a decrease in seedling length with time. In the present investigation, the seedling length was considerably decreased in cloth, gunny and HDPE bags but not in vacuum packed bags irrespective of storage conditions

and seeds (Table 4). Ageing has a damaging effect on enzymes that are necessary for the conversion of food reserve in the embryo to a usable form and ultimately reduced seedling-associated parameters. With the advancement in the storage period, decrease in seedling length was more in cloth, gunny and HDPE bags in cold condition and these treatments were non-significant with each other throughout the storage period. In contrast, a minimal decrease was recorded in vacuum packed bags stored in cold condition as compared to ambient condition and was due to the differential rate of seed deterioration in which seeds are stored. This decreasing trend in seedling length might be attributed to oxidative stress which induced the production of reactive oxygen species that caused lipid peroxidation in terms of malondialdehyde (MDA) as a final product which in turn hinders the cellular metabolism resulting in lower ATP production required for growth and development of the seedling (Blokhina et al 2003). After 18 months of storage, a significant difference was observed

Table 4. Influence of packaging and storage conditions on total seedling length (cm) at different time intervals of storage in blackgram seeds

| Treatments | | Storage period (Months) | | | | | | | | |
|-----------------|-----------------------------------|-------------------------|------|------|------|------|------|------|------|------|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| Ambient storage | T ₁ -Cloth bag | 49.6 | 49.0 | 47.7 | * | * | * | * | * | * |
| | T ₂ -Gunny bag | 49.4 | 49.2 | 48.0 | * | * | * | * | * | * |
| | T ₃ -HDPE bag | 49.6 | 49.1 | 47.7 | * | * | * | * | * | * |
| | T ₄ -Vacuum packed bag | 49.8 | 49.8 | 49.8 | 49.7 | 49.5 | 49.3 | 49.3 | 48.9 | 48.7 |
| Cold storage | T ₅ -Cloth bag | 49.6 | 49.2 | 48.9 | 47.9 | 47.1 | 46.7 | 46.5 | 45.7 | 44.2 |
| | T ₆ -Gunny bag | 49.6 | 49.6 | 48.9 | 47.8 | 47.2 | 47.2 | 46.2 | 46.1 | 44.7 |
| | T ₇ -HDPE bag | 49.7 | 49.5 | 48.9 | 47.9 | 47.6 | 46.8 | 46.5 | 45.9 | 44.6 |
| | T ₈ -Vacuum packed bag | 49.8 | 49.8 | 49.8 | 49.8 | 49.6 | 49.4 | 49.2 | 49.2 | 49.0 |
| | C. D (p=0.01) | 0.4 | 0.8 | 0.5 | 0.7 | 0.7 | 0.5 | 0.8 | 0.9 | 0.7 |

Table 5. Influence of packaging and storage conditions on moisture content (%) at different time intervals of storage in blackgram seeds

| Treatments | | Storage period (Months) | | | | | | | | |
|-----------------|-----------------------------------|-------------------------|------|------|------|------|------|------|------|------|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| Ambient storage | T ₁ -Cloth bag | 9.03 | 8.40 | 9.13 | * | * | * | * | * | * |
| | T ₂ -Gunny bag | 9.08 | 8.38 | 9.00 | * | * | * | * | * | * |
| | T ₃ -HDPE bag | 9.03 | 8.43 | 9.05 | * | * | * | * | * | * |
| | T ₄ -Vacuum packed bag | 8.15 | 8.13 | 8.18 | 8.08 | 8.15 | 8.20 | 8.13 | 8.10 | 8.13 |
| Cold storage | T ₅ -Cloth bag | 8.73 | 8.78 | 8.75 | 8.73 | 8.70 | 8.60 | 8.68 | 8.83 | 8.78 |
| | T ₆ -Gunny bag | 8.70 | 8.68 | 8.63 | 8.68 | 8.78 | 8.70 | 8.70 | 8.75 | 8.75 |
| | T ₇ -HDPE bag | 8.60 | 8.70 | 8.73 | 8.80 | 8.83 | 8.73 | 8.65 | 8.70 | 8.80 |
| | T ₈ -Vacuum packed bag | 8.13 | 8.12 | 8.15 | 8.13 | 8.13 | 8.10 | 8.10 | 8.15 | 8.13 |
| | C. D (p=0.01) | 0.17 | 0.16 | 0.20 | 0.17 | 0.24 | 0.20 | 0.24 | 0.30 | 0.26 |

Table 6. Influence of packaging and storage conditions on α amylase activity (μg maltose released/min/ml) at different time intervals of storage in blackgram seeds

| Treatments | | Storage period (Months) | | | | | | | | |
|-----------------|-----------------------------------|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| Ambient storage | T ₁ -Cloth bag | 54.34 | 51.96 | 50.57 | * | * | * | * | * | * |
| | T ₂ -Gunny bag | 54.30 | 51.68 | 50.41 | * | * | * | * | * | * |
| | T ₃ -HDPE bag | 54.16 | 51.94 | 51.43 | * | * | * | * | * | * |
| | T ₄ -Vacuum packed bag | 54.77 | 54.77 | 54.20 | 54.47 | 54.17 | 54.17 | 53.96 | 53.23 | 53.19 |
| Cold storage | T ₅ -Cloth bag | 54.54 | 53.30 | 51.85 | 50.44 | 47.92 | 46.83 | 43.28 | 42.91 | 42.07 |
| | T ₆ -Gunny bag | 54.21 | 53.17 | 52.30 | 50.73 | 48.47 | 46.90 | 44.04 | 42.80 | 43.69 |
| | T ₇ -HDPE bag | 54.20 | 53.31 | 52.05 | 50.14 | 46.89 | 47.40 | 44.69 | 41.70 | 41.01 |
| | T ₈ -Vacuum packed bag | 54.77 | 54.77 | 54.63 | 54.48 | 54.24 | 54.27 | 53.93 | 53.64 | 53.45 |
| C. D (p=0.01) | | 0.72 | 0.90 | 1.19 | 1.64 | 2.04 | 2.65 | 3.35 | 3.81 | 4.04 |

between the cloth, gunny, HDPE bags and vacuum packed bags whereas, no significant difference was observed between vacuum packed bags stored in ambient and cold storage conditions. Similar results of higher reduction in seedling length due to ageing were revealed by Meena et al (2017) and Autade and Ghuge (2018).

Moisture content: There was a lot of fluctuation in the seed moisture content (Table 5) stored in cloth, gunny and HDPE bags in ambient condition as compared to cold condition. Seeds are hygroscopic in nature hence, they have absorbed moisture when relative humidity was high and they have lost the moisture when humidity was low under ambient condition (Table 2). Similar results of variation in moisture content under ambient condition in pervious packaging materials were reported by Kumar et al (2017) and Shankar et al (2018). Similarly, when seeds were stored in pervious packaging materials (cloth, gunny and HDPE bags) and kept in cold condition, seeds absorbed the moisture initially but thereafter there was no change in moisture content due to the prevalence of similar storage condition throughout the storage period. Similar results of the initial increase in moisture content when stored in pervious packaging materials and kept in relative humidity same as that of cold storage have been reported by Bakhtavar et al (2019). Hygroscopic nature of seeds holds good only when seeds are packed in pervious packaging materials but not in impervious packaging materials like vacuum packed bags as they have very less water vapour and air transmission rate and higher thickness (Table 2). Due to these special characters of polythene bag used for vacuum packaging, there was no variation in moisture content of the seeds throughout the storage period in cold and ambient conditions. Similar results of no variation in moisture content of seeds when vacuum packed have been reported by Deepa et al (2013) and Meena et al (2017).

α Amylase activity: In seeds, amylase is the major amylolytic enzyme found during germination and is involved

in the degradation of endosperm starch. Degradation of starch into soluble sugars is important to support seedling growth and it can be degraded with the help of amylase activity. There was a decrease in α amylase activity as the storage period progressed and this reduction indicates the deterioration of seeds (Table 6). Except at the initial storage period, there was a significant difference in α amylase activity and reduced more in conventional packaging's viz., cloth, gunny and HDPE bags and less in vacuum packed bags under both cold and ambient conditions. After 18 months of storage, a non-significant difference was observed within cloth, gunny and HDPE bags in cold condition and these treatments were significantly differing with vacuum packed bags of both the conditions. No variation in α -amylase activity of vacuum packed seeds is because vacuum treatment enabled seeds to keep low moisture content as oxygen and water were isolated from the storage condition and hence, ageing factor of seeds were controlled in vacuum condition (Wang et al 2018). In general, decreased α amylase activity with an increase in storage period might be due to a reduction in free sugars and amino acids. Further, a direct correlation exists between loss of viability and decline in enzyme activity. Similar results of a decrease in α amylase activity due to ageing have been reported by Bhanuprakash et al (2006). Further, Norastehnia et al (2007) observed the accumulation of aldehyde compounds with the advancement of deterioration especially methyl jasmonate (MeJA) which is a potent inhibitor of alpha-amylase. Higher concentrations of MeJA reduced the concentration of the enzyme protein and consequently inhibition of gibberellin biosynthesis since gibberellin stimulates the alpha-amylase synthesis and its secretion from the embryonic axis into cotyledons.

CONCLUSION

The vacuum packaging technology can be effectively used for the safe storage of blackgram seeds for a longer period without any aid of chemicals.

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