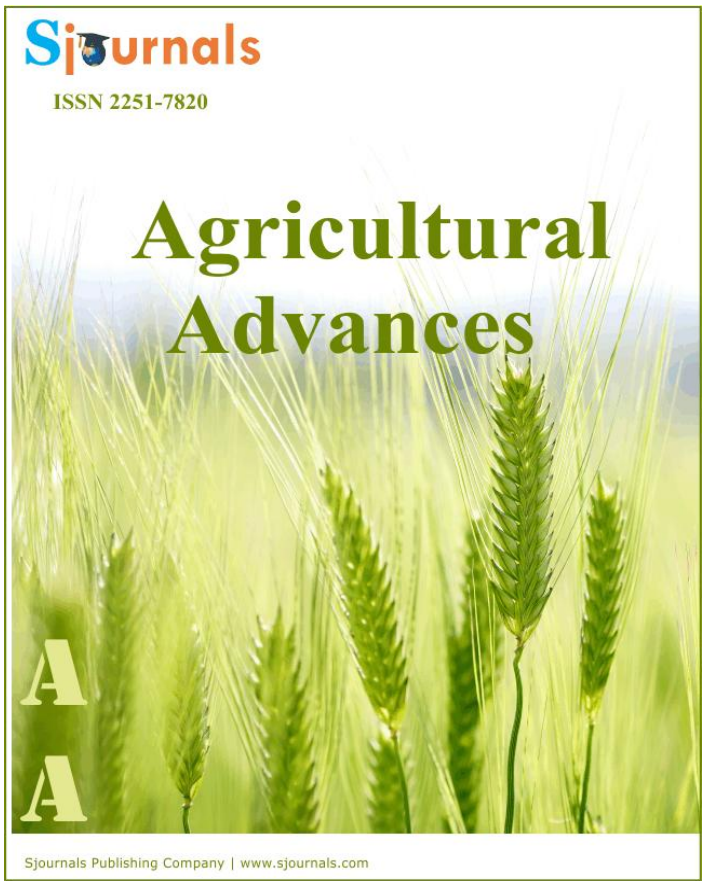
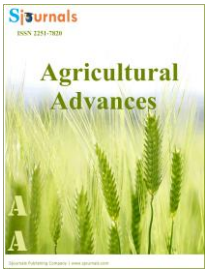


Sjournals
Scientific Journals
Agricultural Advances (2016) 5(12) 375-382
ISSN 2251-7820
doi: 10.14196/aa.v5i12.2356



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Original article

Effect of storage containers and moisture levels on the seed quality of Lentil (*Lens culinaris* L.)

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ARTICLE INFO

Article history,
Received 06 November 2016
Accepted 07 December 2016
Available online 11 December 2016
iThenticate screening 08 November 2016
English editing 05 December 2016
Quality control 09 December 2016

Keywords,
Storage containers
Moisture levels
Germination
Vigour index
Fungal infection
Lentil

ABSTRACT

The effect of moisture levels and storage containers on the quality of lentil seed was investigated in the laboratory of Agronomy Department, Hajee Mohammad Danesh Science and Technology University (HSTU) Dinajpur, Bangladesh during March-April 2016. Three seed containers viz, sealed container, poly bag and gunny bag and three moisture levels i.e. 8.88 %, 12.23 % and 14.10 % were included in the study. Lentil seeds of 8.88% initial moisture level were found in the best condition in respect of germination, shoot and root length, vigour index and the least fungal incidence. The highest germination percent (85.39%), the longest shoot length (17.67 cm) and root length (8.77 cm), and the least fungal incidence (11.67%) were found in sealed container with 8.88% moisture level, while the lowest germination percent (55.99%), the shortest shoot length (11.60 cm) and root length (4.64 cm), and the highest fungal infection (28.00%) were found in the gunny bag of 14.10% moisture level. Among the three containers, sealed container was the best and the gunny bag was the worst storage containers for storage of lentil seed up to 45 days. It is concluded that minimum moisture level and air tight sealed storage container have the greatest benefit for storing seeds and maintaining quality seeds.

1. Introduction

Lentil (*Lens culinaris* L.) is an important grain legume crop of Bangladesh. This pulse crop belongs to the sub family of Papilionaceae under the family Leguminosae. It covers about 32.69% of the total area and shares approximately 34.65% of the total production of pulse in the country (BBS, 2011). Lentil occupies the second position both in areas and production that is extensively used by the people of this country. The protein content of lentil seeds is found to vary from 21.75 to 32.48% (Purseglove, 1968; Dimitrive, 1973). Protein malnutrition is a serious health problem in Bangladesh that has been threatening to cripple the whole nation. Pulses are considered as the "poor men's meat" as these are the cheaper source of protein (Mian, 1976). In Bangladesh capita^{-1}day^{-1} consumption of pulses is only 12 gm whereas the World Health Organization suggests an intake of 45gm of pulses capita^{-1}day^{-1} (BARI, 1998).

Legume like lentil can also improve the physical, chemical and biological properties of soil and can increase soil fertility through biological nitrogen fixation butthe yield of legume is very low as compared to cereals. The average yield of lentil is about 929 kg ha^{-1}, which is very low as compared to the average yield of lentil growing countries of the world (BBS, 2012). Such yield gap of legumes indicates a great opportunity to increase the productivity of lentil at farm level. Several causes are responsible for low yield of lentil of which the use of traditional local cultivar, seed borne infection and low plant density per unit area, infestation and crop management practices constitute the major ones. Moreover, the expansion of lentil cultivation is hindered due to lack of high quality seed and its proper storage.

Lentil is grown during the Rabi season and seeds from harvested crops are stored for at least 8 to 9 months before sowing in the next season. During this period, seeds tend to lose their viability due to prevailing high temperature and high relative humidity. The deterioration of stored seed becomes faster if the seeds are not properly dried and the atmosphere is not controlled (Delouche et al., 1973). Many factors determine the longevity of seeds during storage. These include seed moisture content, temperatures, relative humidity, initial viability, stage of maturity at harvest, storage gas and the initial moisture content of seed entering into the storage (Harrington, 1972). This condition can be achieved by storing the seeds in a climatic region where relative humidity is naturally low by reducing the level of relative humidity to a favorable level by conditioning of storage environment or by storing seeds in moisture proof containers (Delouche, 1968).

Moisture is one of the major factors contributing to the deterioration during storage of durable agricultural products in the tropics and sub-tropics. Above 13% moisture content, seed storage fungi and increased heating due to respiration cause longevity to decline at a faster rate. Once seed moisture reaches 18 to 20%, increased respiration and the activity of microorganisms cause rapid deterioration of the seed. At 30% moisture content, most non-dormant seeds germinate. Seed deterioration increases as moisture content increase (Harrington, 1972). Seed having moisture content of 18% gets heating, is attacked by molds and insects, and can be mechanically damaged. Insects can do damage to the seed and heating can also occur at moisture content ranging from 13-18%. If the crop, whether it is cereal, oilseed or legume, contain viable organism and as such, it continually respires producing heat and moisture, which if present in excessive amount, produce suitable conditions for the growth of other injurious organisms. These, in turn, will cause a loss in both quantity and quality of the crop while in storage.

Therefore, the proper storage of seed is a very important factor for proper production of seed. Thus, successful seed storage is the primary importance to the seed industry. The seed storage relative humidity, temperature and storage container are the main factors affecting seed quality in storage. The sealed plastic container and polythene bags are more effective storage containers than gunny bag or jute bag and earthen pot etc. However, research work regarding the effect of biotic and abiotic factors on lentil seed quality during storage are scarce in Bangladesh. Therefore, a research work has been undertaken to identify the best storage container and to observe the moisture level for the storage of lentil seed.

2. Materials and methods

The experiment was conducted at the Institute of Research Technology (IRT), Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during March to May 2016. The experiment was laid out in completely randomized design (factorial concept) with three storage containers viz. i) Sealed container, ii) Poly bag (Transparent), and iii) Gunny bag, and three moisture levels (ML) viz. i) ML_1(8.88%), ML_2 (12.23%) and iii) ML_3 (14.10%) replicated thrice. Lentil seeds used in this experiment were supplied by the IRT,HSTU, Dinajpur.

Seeds were kept in those three containers and stored at room temperature and relative humidity for 45 days. The sealed container was covered tightly with cap and the poly bag and gunny bag were tight with rope. The containers were kept in the wooden rack in the laboratory. During the storage period seeds samples were taken every 15 days from the containers for determination of moisture content and germination percentage of seeds.

Moisture content was determined by using high constant temperature oven method following International Rules for Seed Testing in the Agronomy laboratory of HSTU. Five gram of seeds from each three containers was taken. After grinding the seeds in grinding mill the weighed ground materials were poured in a small container with cover and kept in an oven maintained at a temperature of 125-130^{o}C for a period of two hours. The moisture content of seeds (wet basis) was determined by the following formula.

$$\% \text{ MC} = \frac{(X_2 - X_3)}{(X_2 - X_1)} \text{ X } 100$$

X_1 = Wt. of container
X_2 = Wt. of container + ground materials before drying
X_3 = Wt. of container + ground materials after drying

Sampling was done randomly for three times from each storage container. First sampling was done at 15 days after storage, second at 30 days after storage and last one at 45 days after storage for testing germinability and health status of the seeds of storage containers. Germination test was conducted using sand as substratum. The sand was sieved to discard particles bigger than 0.8 mm and smaller than 0.05 mm in diameter. Rectangular plastic boxes were used to put the sand. For every test new sand was used. Seed was placed on a uniform layer of moist sand and then covered to a depth of 10 mm with sand, which was left loose. One hundred seeds were planted in each plastic tray and replicated four times. The plastic trays with seed were incubated at room temperature and irrigated at every odd day. After eight days, germination percentage was recorded. The normal seedlings and abnormal seedlings and ungerminated seed were classified according to the prescribed rules given by ISTA (1966).

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated at final count}}{\text{No. of seeds placed for germination}} \text{ X } 100$$

At 10 days after placement for germination, seedlings from each plastic glass were collected as a sampling. Length of shoot and root of individual seedling were recorded manually with scale. The mean lengths (cm) were calculated as per treatment combination. For vigor index or seedling vigor data was recorded on germination up to 10 days of sowing. Then root length and shoot length were measured from seedlings of pots for calculating vigor index. The vigor index (VI) was calculated by using the formula of Baki and Anderson (1973) as shown below:

Vigor index (VI) = Germination (%) X (Mean shoot length + mean root length).

Fungi associated with lentil seeds were detected by using Blotter method as followed by ISTA (1996). In blotter test, three layers of blotter papers (Whatman filter paper No.l) were soaked in sterilized water and placed at the bottom of 9 cm diameter Plastic petridish in which 25 seeds of lentil were placed on the moistened blotting paper at equal distance between seeds and petridish wall in each plate. One hundred seeds were tested for each replication. Petridishes containing seeds were incubated for seven to eight days at 25±2°C under 10-12 hours alternating cycles of Near Ultra Violet (NUV) light and 12-14 hours of darkness. The incubated seeds were inspected individually with the help of a stereo microscope by following the keys outlined by Ramnath et al. (1970) and Khan (1975). The pathogens were detected on the basis of their growth characters. The data were analyzed by partitioning the total variance with the help of computer using MSTAT-C program. The treatment means were compared using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

3. Results

3.1. Effects of different containers and moisture levels on germination of lentil seed

Germination percentage significantly influenced due to moisture level treatments at different storage periods. The maximum values of germination percentage (GP) of the lentil seeds was found at minimum moisture

levels. The highest GP (85.39%) was recorded at the lowest moisture level (8.88%) and the lowest GP (55.99%) was recorded at the highest moisture level (14.10%). Germination percentage gradually decreased with the increasing moisture levels (Table 1).

Table 1
Effects of containers and moisture levels on germination of lentil seeds.

Containers	Moisture levels (ML)		
	Germination percentage		
	ML_1 (8.88%)	ML_2 (12.23%)	ML_3 (14.10%)
Sealed container	85.39 a	80.07 ab	70.99 b
Poly bag	80.55 ab	72.92 b	53.07 c
Gunny bag	78.99 ab	71.52 b	55.99 c
LSD		10.07	
CV%		2.33	

In a column, figures having similar letter(s) do not differ significantly whereas figure s bearing dissimilar letter (s) differ significantly (as per DMRT).

3.2. Effect of containers and moisture levels on shoot length

The analysis of variance (mean squares) presented in the Table 2 indicated that moisture content significantly influenced by the storage containers. Among the three containers, the seeds of sealed plastic container produced the highest shoot length (17.67cm) followed by the seeds stored in poly bag (15.58 cm) with significant variation and the lowest shoot length (11.60 cm) was observed in the seeds stored in gunny bag which was significantly differed from the sealed container and the poly bag (Table 2).

Table 2
Effect of containers and moisture levels on shoot length of lentil seeds.

Containers	Moisture levels		
	Shoot length (cm)		
	ML_1 (8.88%)	ML_2 (12.23%)	ML_3 (14.10%)
Sealed container	17.67 a	16.66 b	15.58 c
Poly bag	15.58 c	14.51 d	13.42 f
Gunny bag	13.85 e	12.75 g	11.60 h
LSD		0.12	
CV%		0.76	

In a column, figures having similar letter(s) do not differ significantly whereas figure s bearing dissimilar letter (s) differ significantly (as per DMRT).

3.3. Effect of different containers and moisture levels on root length

Root length was significantly affected by various types and degrees containers and moisture levels in lentil seed. The highest root length were found (8.77 cm) at plastic container and the lowest (2.82 cm) was found in the gunny bag. At moderate storage level (30 DAS), the highest root length was recorded (7.18 cm) in plastic container and the lowest root length was also found (4.64cm) at 45 days after storage of the gunny bag (Fig. 1). Moisture level and storage condition plays an important role in the lentil seed. The highest root length was found in the sealed container with moisture level (8.8%) and the lowest root length was found in the gunny bag with moisture level (14.1%).

3.4. Effect of different containers and moisture levels on vigor index of lentil seed

Vigor index was significantly influenced by the storage containers and moisture levels (Table 3). The minimum vigor index (948.83) was found in gunny bag with ML_3 of 14.1%, while the maximum vigor index (2286.38) was found in the sealed container with ML_1 of 8.88%.

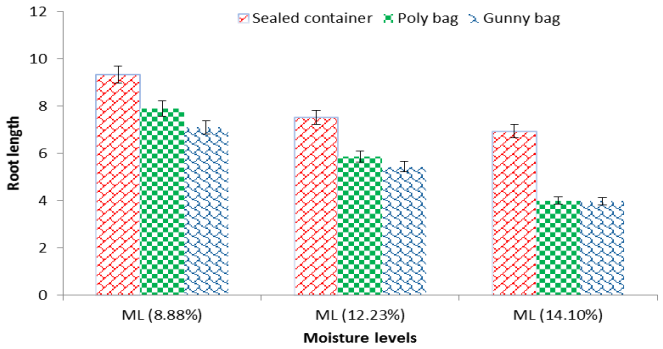

Fig. 1. Effect of different containers and moisture levels on root length of lentil seeds.

Table 3
Effect of different containers and moisture levels on vigor index of lentil seed.

Containers	Moisture levels		
	Vigor index		
	ML_1 (8.88%)	**ML_2 (12.23%)**	**ML_3 (14.10%)**
Sealed container	2286.38 a	1803.69 c	1264.36 e
Poly bag	1965.68 b	1465.73 d	925.14 f
Gunny bag	1831.90 c	1396.79 d	948.83 f
LSD		123.7	
CV%		1.18	

In a column, figures having similar letter(s) do not differ significantly whereas figure s bearing dissimilar letter (s) differ significantly (as per DMRT).

3.5. Biotic factors

No biotic factors like insects and pathogen was observed during the storage period in all the containers, but during investigation like germination analysis, several fungi were observed such as *Ascocyta lentis, Botrytis spp*. and *Fusorium oxysporum.* No insect was found to any storage container. Higher number of fungus was observed in seeds of gunny bag than sealed plastic container and poly bag. Because as seed is highly hygroscopic living materials and it absorbs moisture from the surrounding atmosphere, this higher moisture in the seed may be the main reason for growing of fungus in the seeds of gunny bag.

3.6. Effect of different containers and moisture levels on fungal infection

Interaction between different containers and moisture levels showed significant variation in respect of fungal infection of lentil seed. The fungal infection of lentil seed kept in sealed container, poly bag and gunny bag increased with increasing moisture levels. The highest fungal infection (28) was recorded at 14.10% (ML_3) in the gunny bag while, the minimum fungal infection was found in the sealed container with 8.88% (ML_1) (Table 4).

Table 4
Effect of different containers and moisture levels on fungal infection of lentil seed.

Containers	Moisture levels		
	Fungal infection (%)		
	ML_1 (8.88%)	**ML_2 (12.23%)**	**ML_3 (14.10%)**
Sealed container	11.67 e	15.67 d	17.67 d
Poly bag	17.00 d	22.00 c	24.00 d
Gunny bag	24.89 abc	26.22 ab	28.00 a
LSD		3.22	
CV%		2.33	

In a column, figures having similar letter(s) do not differ significantly whereas figure s bearing dissimilar letter (s) differ significantly (as per DMRT).

4. Discussion

The highest GP of stored seeds was found in sealed containers, which was significantly different from other container treatments. However, the germination rate of the containers was decreased over the storage period of seeds and the highest decreasing rate was observed in the gunny bag which showed significantly the lowest GP from all other container treatments. This is due to the fact that seeds stored in different containers gradually deteriorated and seed deterioration is natural phenomena with the passing of time. Seed deterioration processes, however, depend on a large number of genetic and environmental factors. As seed is highly hygroscopic living materials and it absorbs moisture from the surrounding atmosphere. This higher moisture in the seed may be the main reason of quick germination deterioration in the seeds of gunny bag. Significantly the highest GP of seeds in the sealed container indicates that the viability of the seeds in this container remains higher. The lower moisture absorption by seeds stored in the sealed container probably helped to maintain the seed quality during the storage period. The results are in agreement with the findings of Ching et al. (1960) who observed that seed moisture plays a vital role in keeping the seed viability and seed contamination by storage fungi. According to their findings, it was reported that increase of seed moisture may be higher in permeable containers. In the present study, seed germination decreased with increasing storage period was recorded in almost all the tested containers. Earlier reports (Christensen, 1970; Mian and Fakir, 1989; Kaur et al., 1990) also are in agreement with the present findings. Mendoza and Molina (1980) also reported that seed-borne pathogens are also responsible for decreasing germination and seedling abnormalities.

It was observed that there was significant difference in the emergence of normal seedlings. The shoot and root lengths were also influenced by the different storage containers and conditions. In case of containers, the highest shoot length (17.67cm) was produced in the sealed containers and the lowest (13.85 cm) in the gunny bag with the minimum storage level (8.88%), shoot length of normal seedlings decreased (22%) indicating substantial loss in seed viability due to storage conditions (Table 2). Similar observation was also reported by Kaur et al. (1990). With increasing moisture levels from 8.88 to 14.10%, the shoot length also decreased 11.83, 13.86 and 16.24% in sealed container, poly and gunny bags, respectively. With the passing of time, seeds absorbed moisture and deteriorated gradually resulting decreased shoot length. Similar findings have been reported by earlier studies (Ching, 1960; Kaur et al., 1990). The highest root length was also observed in sealed container under the conditions while gunny bag showed the least effective performance and root length decreased with increasing moisture levels from 8.88 to 14.10%. Seeds absorb moisture from atmosphere (where relative humidity is higher than the seed moisture content) with time due to its highly hygroscopic nature, and the ranking of absorption is gunny bag > poly bag > sealed container due to gunny bag is not air tight. Higher moisture absorption in the seed may be one of the reasons of quick deterioration due to fungal infection, resulting reduced root length. However, higher moisture resulted in root length, consistent with the findings of Mali et al. (1983).

Seedling vigour index (VI) was decreased remarkably due to storage containers and moisture levels. The highest and the lowest VI were observed in the sealed and gunny bag, respectively (Table 3). Seed absorbs moisture from the surrounding atmosphere due to nature of its highly hygroscopic affinity. The gunny bag absorbed the maximum moisture among the three containers and higher moisture in the seed may be the main reason of quick deterioration of VI in the seeds of gunny bag. Copeland (1967) reported that seedling vigor (growth rate) decline rapidly with the length of seed storage. Vigor index decreasing gradually from 8.88 to 14.10% moisture level might be due to the low vigor of seeds for long storage periods and fungal infestation. The increasing rate was higher in seeds of gunny bag with ML_1 seed, because it was due to high moisture and fungal activities (Mali et al., 1983). The moisture accelerates the respiration rate of seed and microorganism. A higher moisture level may produce heat rapidly enough to deteriorate seed quality (Brandenburg et al., 1961). Moreover, high moisture initiates incomplete physiological process of seed germination resulting in loss of viability (Harrington, 1972). It is interred that the increase rate of deterioration is agreed with the increase of moisture level in seed (Agrawal, 2003). Moisture content was greatly influenced by the storage containers especially in gunny bag possibly due to the fact that gunny bag absorbed moisture through its pore space from the atmosphere and it is highly correlated with the relative humidity.

Different containers and moisture levels showed remarkable variation of fungal infection of lentil seed. The highest and the lowest percentages of fungal infection were recorded in the gunny bag and sealed container, respectively (Table 4). Fungal infection increased with increasing moisture levels and the highest fungal infection (28) was recorded at the highest ML while, the minimum fungal infection was found at the lowest ML (Table 4).

Results from the present experiment revealed that the occurrence of fungal flora is influenced by seed containers where in the seeds are placed and stored, storage duration and storage condition, that is, location of storage. The incidence of occurrence of different storage fungi increased gradually with the storing period of seeds almost in all the containers. The occurrence of seed borne inocula was different in different containers and the highest occurrence was observed in the gunny bag and the lowest in the sealed container. The association of field fungi in stored seeds and the reduction of their incidence in storage have also been observed by Mian and Fakir (1989). *Aspergillus niger* was found to be the most predominant fungus in lentil seeds. The seeds under experiment were collected from Kharif-1 season immediately after harvest by retailer. The weather condition especially humidity was high in that season. So the seeds were initially infested with the field as well as seed-borne fungi *Aspergillus niger.* This may be the cause of presence of higher number of *Aspergillus niger*. Here it is also found that seed moisture content influenced the lentil seed-borne pathogens during storage. Moreover, Sutherland (1981) reported that lentil seed-borne fungi were dominant at six months storage compared to that of three months of storage when kept in all the four selected containers. He also observed that gunny bag seeds contained higher percent of moisture (18 and 20%) than the other containers and cause higher amount of seed borne infection (11.2 and 15.4%).

5. Conclusion

Lentil seed kept in sealed plastic container gave the highest germination percentage, seedling growth (shoot and root length), vigour index and the least fungal infection, and gunny bag was found to be the worst but poly bag performed intermediate result among the three storage containers. Less moisture containing (8.88%) seeds performed the best result than ML_2 (12.23%) and ML_3 (14.10%) seeds. Therefore, it can be concluded that seeds should be stored in sealed container with minimum moisture level.

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How to cite this article: Hasan, K., El Sabagh, A., Islam, M.S., 2016. Effect of storage containers and moisture levels on the seed quality of Lentil (*Lens culinaris* L.). Agricultural Advances, 5(12), 375-382.