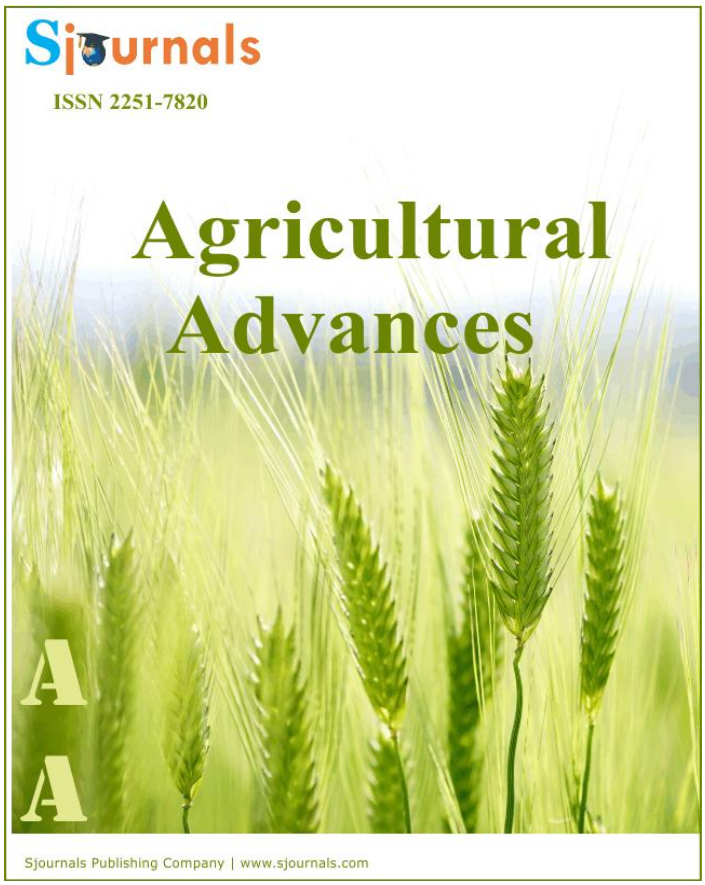

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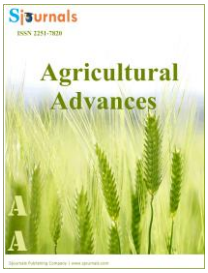

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Original article

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

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ABSTRACT

To evaluate the effect of moisture levels (ML) and storage periods (SP) on the seed quality of lentil was investigated in the laboratory of Agronomy Department, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during March-April 2016. Three moisture levels i.e. ML_1 (8.88%), ML_2 (12.23%) and ML_3 (14.10%), and three storage periods viz, i) 15 DAS, ii) 30 DAS and iii) 45 DAS were included in the study. Lentil seeds of ML_1 were found in good condition in respect of germination percentage (GP), better shoot and root length, vigor index and poor fungal incidence. The highest GP (84.69%) was found at ML_1 (the initial moisture level), with shortened SP (15 DAS) while the lowest GP (52.84%) was found at ML_3 of 14.10% moisture level with the longest SP of 45 DAS. The maximum shoot length (17.40cm) was recorded at ML_1 seed with 15 DAS while the minimum (12.72cm) was found at ML_3 with 45 DAS. The seeds in less moisture content with 15 DAS SP produced the highest root length (9.24 cm) and vigor index (2284) and the lowest root length (3.74 cm) and vigor index (813) was obtained in seeds with ML3 at the SP of 45 DAS, respectively. Fungal incidence was found less (14.0%) in lentil seeds with the initial ML (ML_1) and it increased 18.33% at ML_3 with 15 DAS. It also increased with the increasing ML from 8.88 to 14.10% and storage periods from 15 to 45 DAS.

The least ML with shortened SP was the best and the highest ML with the longest SP was the worst in respect of all the parameters. Therefore, optimum moisture level and ideal storage condition have the greatest benefit on the quality of lentil seeds.

1. Introduction

Lentil (*Lens culinaris* L.) is one of the most important legumes among pulses of Bangladesh. It contributes about 34.65% of the total production of pulse in our country (BBS, 2011). Lentil is widely used by the people of this country and ranks the second position both in areas and production. The protein content is found to vary from 21.75 to 32.48% (Purseglove, 1968; Dimitrive, 1973). Bangladesh faced seriously in protein malnutrition that has been threatening to gammy 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 suggest an intake of 45gm of pulses capita^{-1} day^{-1} (BARI, 2008). Lentil enhances the soil fertility and productivity through biological nitrogen fixation. The total yield of legume is very lower as compared to cereals. Further, yield of legumes in farmers' field is usually less than 1 ton ha^{-1} against the potential yield of 2 to 4 t ha^{-1} suggesting a large yield gap. So, it is a great scope to increase the total production of lentil at farm level. Several causes are responsible for low yield of lentil of which the use of traditional local cultivar, uses of low quality seeds, seed borne infection, insects and disease infestation, fluctuations of temperature and relative humidity during storage, and crop management practices constitute the major ones. Insufficient amount of quality seed and its proper storing procedures/conditions, the lentil cultivation is interrupted. During the Rabi season (November to March) lentil is grown well and seeds harvested from crops are stored for at least 8 to 9 months before sowing in the next season. Seeds tend to lose their viability at this time 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). The longevity of seed is determined by many factors 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, infestation of seed storage fungi increases and also increases respiration due to high temperature causing to decline longevity at a faster rate. Once seed moisture reaches 18 to 20%, increased respiration and the activity of microorganisms cause rapid deterioration of the seed. The most non-dormant seeds germinate at 30% moisture content. Seed deterioration increases as moisture content increase (Harrington, 1972).

The molds and insects severely attacked the seed having 18% moisture content and can be damaged mechanically. 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, proper storage of seed is a very important factor for crop production. Thus, the primary importance to the seed industry is successful storage. The main factors affecting seed quality during storage are the seed relative humidity, temperature and storage conditions. However, research work regarding on the effect of storage periods and moisture levels on the seed quality of lentil are scarce in Bangladesh. Therefore, a research work has been undertaken to identify the optimum moisture level for storage and to observe the optimum storage period for lentil seed.

2. Materials and methods

The experiment was conducted at Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, during March to May 2016. The experiment was carried out in two factors completely randomized design

(CRD) with three replications. The experiment consisted of three moisture levels viz. i) ML_1 (8.88%), ML_2 (12.23%) and ML_3 (14.10%) and three storage periods viz. i) 15, ii) 30, and iii) 45 DAS (days after storage). The seeds of lentil were supplied by the Institute of Research and Technology (IRT) of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for this experiment. Seeds were stored at room temperature and relative humidity (RH) with three moisture levels for one and a half months. During the storage period, seeds samples were taken every 15 days from the containers for determination of moisture content of seeds and germination percentage. To determine the moisture content, the high constant temperature oven method was used following ISTA rules for seed testing in the laboratory of Agronomy, HSTU. The seeds of about five gram were taken from each 3 containers. The seeds in grinding mill was weighed after grinding ground materials were poured in a small container with cover and kept at a temperature of 125-130^{o}C in an oven maintained for a period of 2 hours. The seeds moisture content of (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 3 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. The seeds were preserved in the containers and closed with the lids. The samples were enclosed in three different containers viz. sealed plastic container, polythene bag and gunny bag with proper labeling at 25±2°C until used for subsequent studies. About 300-400g seeds were used per container as required filling up the containers based on the size. The lids of the containers were placed firmly to make it air tight as per as possible. The containers were kept in the wooden rack in the laboratory.

The sand was used as substratum during the germination test. The sand was finely sieved to remove particles bigger than 0.8 mm and smaller than 0.05 mm in diameter. The rectangular plastic boxes were used to put the sand. The new sand was used for every test. A uniform layer of moist sand was selected for seed germination and then covered with sand to a depth of 10 mm, which was left loose. In each plastic tray one hundred seeds were placed and replicated with fourth. At room temperature the plastic trays with seed were incubated and irrigated at every odd day. After 8 days, germination percentage was recorded. The normal seedlings and abnormal seedlings and ungerminated seed were classified according to the prescribed rules given by ISTA.

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

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 everyday on germination up to 10 days of sowing for calculating vigor index. Then root length and shoot length were measured from seedlings of the pots. 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 twenty five (25) seeds of lentil were placed on the moistened blotting paper at equal distance between seeds and petridish wall in each plate. One hundred (100) 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 of 25x magnification following

the keys outlined by Ramnath et al. (1970) and Khan (1975). The pathogens were detected on the basis of their growth character.

2.1. Data analysis

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 and discussion

3.1. Germination percentage

Germination is the most important function of a seed as an indicator of its viability and worth as seed. The germination capacity of lentil seeds was observed in this study. Germination percentage (GP) at the different storage period was presented in Fig. 1. The germination of lentil seeds after one and half month storage at all the moisture levels ranged from 52.84 to 84.69%. The highest GP (84.69%) was recorded at the moisture level of 8.88% while the minimum germination (52.84%) was recorded at the moisture level of 14.10% in 15 and 45 days after storage (DAS), respectively.

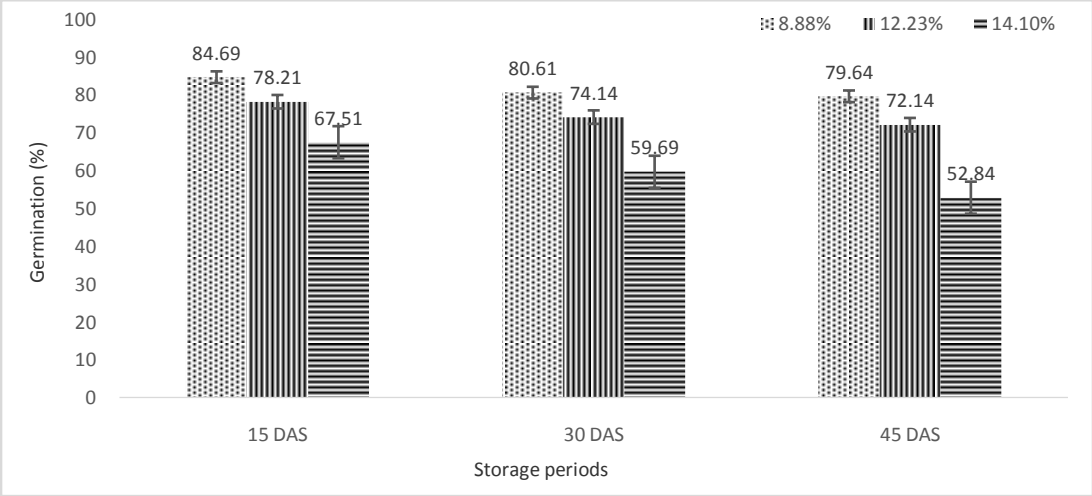

Fig. 1. Effect of moisture levels and storage conditions seed on germination percentage.

3.2. Shoot length

Various moisture levels and storage periods treatments significantly influenced the shoot length of mungbean. The maximum shoot length (17.40cm) was recorded in initial moisture level (8.88%) seed at 15 DAS while the minimum (12.72cm) was found in the highest moisture level (14.10%) at 45 DAS (Table 1).

Table 1
Effect of different moisture levels and storage conditions on shoot length.

Moisture levels	Storage conditions		
	Shoot length (cm)		
	15 DAS	30 DAS	45 DAS
ML_1 (8.88%)	17.40a	15.29bc	12.20e
ML_2 (12.23%)	16.44ab	14.17cd	13.27de
ML_3 (14.10%)	16.05ab	14.04cde	12.72de
LSD		1.88	
CV%		0.79	

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. Root length

Root length varied significantly due to moisture level treatments at different storage periods. The minimum moisture level (8.88%) treatment produced significantly higher root length than the moisture levelsof 12.23 and 14.1% in all the sampling date from 15 to 45 DAS. The highest root length (9.24 cm) was recorded in the lowest moisture levels (8.88%) seeds at 15 DAS and the lowest root length (3.74 cm) was recorded in the highest moisture leveled (14.1%) seeds at 45 DAS (Table 2).

Table 2
Effect of different moisture levels and storage conditions on root length.

Moisture levels	Storage conditions		
	Root length (cm)		
	15 DAS	**30 DAS**	**45 DAS**
ML_1 (8.88%)	9.24 a	8.15 b	6.93 c
ML_2 (12.23%)	7.52 bc	6.00 d	4.95 ef
ML_3 (14.10%)	5.30 de	4.47 fg	3.74 g
LSD		0.79	
CV%		3.55	

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

3.4. Seedling vigor

The different moisture levels and storage periods significantly influenced the vigor index or seedling vigor. Vigor index was decreased with the increase of moisture levels and storage periods. The highest vigor index (2283.97) was obtained from 8.88% moisture level, while the lowest vigor index (813) was recorded in 14.10% moisture in 15 and 45 days after storage, respectively.

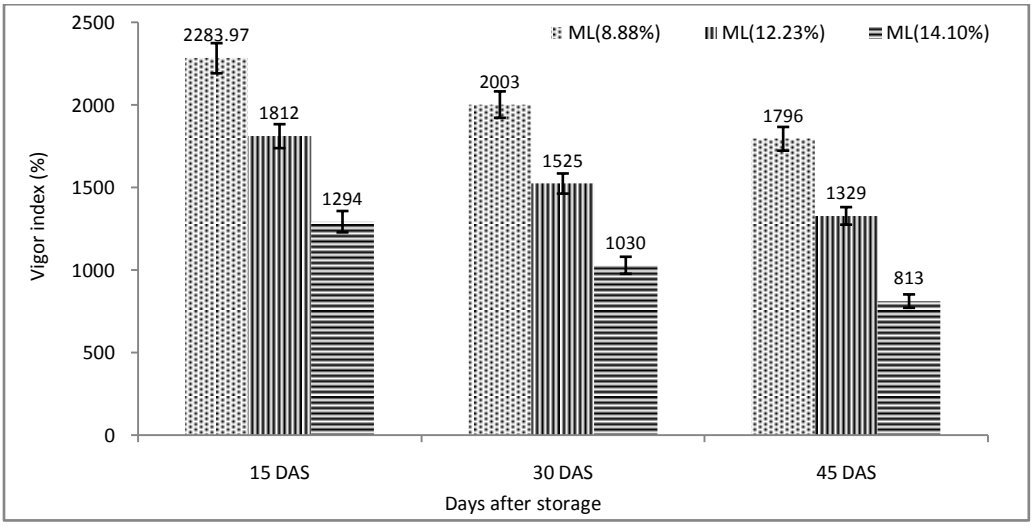

Fig. 2. Effect of different moisture levels and storage conditions on vigor index of lentil seed.

3.5. Germination rate and abnormal seedling

Among the different storage periods and moisture levels, the average germination rate of seeds was found highest at 15 DAS followed by 30 and 45 DAS and at ML_1 (8.88%) followed by ML_2 (12.23%) and ML_3 (14.10%), respectively. The analysis of variance indicated that the percentage of abnormal seedlings did not influence significantly by the storage containers and seed moisture levels. At the end of 45 DAS the abnormal seedling of lentil seed were increased in all treatment combinations (data now shown).

3.6. Fungal infection of seed

The fungal infection of seed increased with the increasing storage periods from 15 to 45 DAS and moisture levels 8.88 to 14.10%. The lowest fungal infection (14%) was observed with the lowest ML of 8.88% at 15 DAS and the highest fungal infection (28.33%) was observed with the highest ML of 14.1% at 45 DAS (Table 3).

Table 3
Effect of moisture levels and storage periods on fungal infection of lentil seed.

Moisture levels	Storage periods		
	Fungal infection (%)		
	15 DAS	30 DAS	45 DAS
ML_1 (8.88%)	14.00 g	17.33 ef	22.22 cd
ML_2 (12.23%)	16.33 f	20.89 d	26.67 b
ML_3 (14.10%)	18.33 e	23.00 c	28.33 a
LSD		1.65	
CV%		2.33	

Values having same letter(s) do not differ significantly by DMRT at P ≤ 5% level.

The highest GP of stored seeds was found at ML (8.88%) in the 15 days after storage conditions for the two storage duration which were significantly different from other treatments. The highest decreasing rate of GP was observed at ML_3 (14.10%) which was significantly different from all other treatments. It was observed that there was significant difference in emergence of normal seedlings influenced by storing periods. In case of storage periods, the GP gradually decreased with increasing storage periods and the highest percentage of normal seedlings was produced 15 DAS and the lowest at 45 DAS in all moisture levels with the increase in storage duration, percentage of normal seedlings decreased whereas production of abnormal seedlings and the number of dead seeds increased indicating substantial loss in seed viability. Similar observation was also reported by Kaur et al. (1990). Storage conditions along with pathogenic presence of inocula also responsible for causing loss in seed germination and also eventually causing disease to the emerged seedlings. As seed is highly hygroscopic living material. It absorbs moisture from air if it is stored in natural environment where relative humidity is higher than the seed moisture content. For this reason, seeds absorbed moisture from the ambient air and tended to equilibrium with relative humidity resulting germination percentage decreased.

Moisture levels and storage periods remarkably influenced the shoot and root length of lentil seed. The decreasing trends of shoot and root length were observed with increasing moisture levels from 8.88 to 14.10% and increasing storage duration from 15 to 45 DAS (Table 1 and 2). Seed deterioration is natural phenomena and life span of seeds decrease with the passing of time. Seed deterioration processes however depend on a large number of genetically 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 deterioration of GP as well as shoot and root length in the seeds of gunny bag. The results are in agreement with the findings of Kaur et al. (1990) who stated that seeds absorbed moisture from atmosphere over time and increased the infestation of insects and diseases resulting decreased GP as well as shoot and root length.

Higher moisture level in seed during storage is one of the main reasons for loses of viability and vigor sooner. Vigor index decreasing gradually with time (up to 45 DAS) in all moisture levels from 8.88 to 14.10%, might be due to the low vigor of seeds for long storage periods and fungal more infestation for high moisture content. 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 and germination rate (Harrington, 1972). It is interred that the increase rate of deterioration is agreed with the increase of moisture level in seed (Agrawal, 2003). Copeland (1967) reported that seedling vigor (growth rate) decline rapidly with the length of seed storage.

Results from the present experiment revealed that the occurrence of fungal flora is influenced by moisture levels where in the seeds are stored up to 45 DAS. The incidence of occurrence of different storage fungi increased gradually with the storing periods of seed. The lower moisture absorption by seeds stored in less time after storage helped to maintain the seed quality i.e. less infestation (14.0%) during the storage period (Table 3). The present

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. Moisture content in seeds and storage periods are responsible for the infestation of fungus diseases (Christensen, 1970; Mian and Fakir, 1989). Mendoza and Molina (1980) also reported that seed-borne pathogens are also responsible for seedling abnormalities.

Incidence of most of the field fungi was found to reduce with the increase in storage period, whereas there was no reduction on the occurrence of the storage fungi. The association of field fungi in stored seeds and the reduction of their incidence with lowering seed moisture content have also been observed by Mian and Fakir (1989). The seeds under experiment were collected from Kharif-1 season immediately after harvest by the 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.

Here it is also found that seed moisture content influenced the lentil seed-borne pathogens during storage. Moreover, lentil seed-borne fungi were dominant at six months storage compared to that of three months of storage when kept in different like poly-bag, gunny-bag, earthen pots, tin containers etc. This phenomenon is supported by Sutherland (1981) who 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%). Moisture content was greatly influenced by the storage containers especially in gunny bag possibly due to the fact that gunny bag absorbed more moisture through its pore space from the atmosphere and it is highly correlated with the relative humidity.

4. Conclusion

Germination percentage and growth rate of stored seed at ML_1 was significantly higher than the seeds stored at ML_3 with 15, 30 and 45 DAS and the GP decreased with increasing storage periods. Seeds preserved in 8.88% moisture level along with 15 DAS performed the highest vigor index, root length and less fungal infections. Therefore, it can be concluded that seeds should be stored in minimum moisture level for short duration of storage periods.

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