Journal of Plant Process and Function, Vol. 7, No. 25, Year 2018

Evaluation of the germination performance and biochemical indices of faba bean (*Vicia faba* L.) seeds stored at different temperatures and moisture contents

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(Received: 08/07/2017 - Accepted: 10/03/2018)

Abstract

This study was performed to investigate the effect of storage conditions on seed germination performance of faba bean (cv. Shami). For this purpose, a three factor factorial experiment was conducted in laboratory of Seed Science and Technology, Faculty of Agriculture, Yasouj University, in 2013-2014, based on CRD design with five replications. The experimental factors were storage temperature (15, 25, 35 and 45 °C), seed moisture content (6, 10, 14, 18 and 22 percent) and storage duration (0, 30, 60, 90, 120, 150, 180, 210, 240 and 270 days). Also, in order to study some biochemical characteristics, 5 storage durations (0, 30, 90, 180 and 270 days of storage) were selected. Results showed that during storage, germination percentage, seedling length, seedling dry weight and seedling vigor index responded to temperature and seed moisture content (SMC), that is by increasing the moisture content of seeds at each temperature, germination percentage reduced (5-100%) whereas electrolyte leakage (60 to 370%) increased. At temperatures of 15 and 25 °C at moisture contents of 6, 10 and 14%, the seed deterioration rate was low whereas it was fast (more than twice) at moisture levels of 18 and 22%. However, at 35 and 45 °C, the rate of seed deterioration, increased storage moisture content of 10 %, so that at SMC of 22 %, germination and seedling growth dropped to the lowest levels (zero) after 30 days of storage. Soluble protein content and catalase activity also decreased during storage period. Reduction of antioxidant enzymes activity and increase of free radical damage were found as main reasons for the decline of faba bean seed viability during storage at high temperatures and high seed moisture content. Seed moisture contents of 6, 10 and 14% at temperatures of 15 °C and 25 °C are recommended for long-term storage of faba bean seeds.

Keywords: **Biochemical characteristics, Faba Bean, Seed deterioration, Seed moisture content, Storage temperature**

Introduction

While the world population is increasing daily, the issue of food shortages and hunger are becoming a serious problem for mankind. Plant protein sources can help to solve malnutrition and protein deficiency. Faba bean, *Vicia faba* is a protein plant used as fresh and dry. This plant is one of the old world plants originating from Europe and Western Asia. Bean is a rainfed cool season crop that grows in areas with adequate rainfall, thus, it has the possibility to develop in different countries. It has become one of the strategic crops due to its income for farmers. Also, it is important for soil fertility, human nutrition (i.e. as a good source of vegetarian protein), animal feeding and industrial purposes (Sawant *et al*., 2012).

Seed deterioration or aging is defined as the process of loss of seed quality with time which reduces the ability of seeds to germinate and produce a complete seedling. Improper storage condition is a cause of deterioration and aging of seeds. These conditions lead to biochemical and physiological changes whose effects include the reduction of seed germination and seedling growth, the increase in sensitivity to environmental stresses and sometimes a reduction of crop yield (Tekrony *et al*., 1989). Deterioration during storage is not only a function of time, but also related to temperature and seed moisture content, therefore, by increasing these factors, deterioration is increased. (Bewley *et al*., 2013). Relative humidity and temperature affect the content, amount and rate of seed moisture absorption in protein seeds which is higher than in starchy and oily seeds.

Seed storage can be challenging given the inherent nature of seeds. Seed storage of *V. faba* has been previously studied, but debate on the subject has not been solved yet, In a research on faba bean, Nasar-Abbas *et al.*, (2009) found that the initial beige testa color changed to light brown, dark reddish-brown or almost black depending on storage conditions. The higher the temperature and SMC the faster the rate of change in color. Ansari and Sharif-zadeh (2012) studied the behavior of mountain rye plants stored under different storage conditions (moisture content levels of 8 to 12 % and temperatures of 5 to 35°C) and reported that increasing the storage duration reduced the germination indices where the reduction was more

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severe in higher moisture content and temperatures. In another study on oat, Xia *et al*., (2015) stated that seed germination decreased with the increase in duration of storage and moisture content. Balouchi *et al*, (2017) stored the *Linum usitatissimum* seeds at 5, 9, 13 and 17% moisture content and 15, 25, 35 and 45°C for 0, 30, 60, 90, 120, 150 and 180 days and observed that seed longevity increased as moisture content and storage temperature decreased.

Reducing the integrity of the plasma membrane, changing the molecular structure of the nucleic acids and reducing the activity of the enzymes are the most important changes that occur during deterioration. Various biochemical and metabolic changes occur during the aging process. The accumulation of reactive oxygen species (ROSs) such as hydrogen peroxide (H_2O_2), superoxide (O2) and hydroxyl (OH) radicals, which are commonly regarded as toxic molecules, cause lipid peroxidation, deactivation of enzymes, damage to nucleic acids and destruction of cell membranes (Kibinza *et al*., 2011). Kong *et al*., (2014) reported that the oat seed was damaged after 2 days at 45 °C and the moisture content of more than 22 percent due to lipid peroxidation and the mitochondria damage.

Since faba bean is an indeterminate plant harvested with different quality and also cultivated in areas where the incidence of high temperature and humidity during storage may affect seed quality, the storage conditions of faba bean seeds are critically important. In this regard, this study examined the effects of temperature and moisture content on some seed germination performance of faba bean cultivar Shami during different periods of storage.

Material and Methods

This study was performed in Laboratory of Seed Science and Technology, Faculty of Agriculture, University of Yasouj, during 2014 and 2015. Seeds of faba bean (*Vicia faba* L. cv. Shami) were prepared from Shushtar, Khuzestan province, Iran in 2014.

The research was performed as a three-factor experiment in a completely randomized design with five replications. The experimental factors were storage temperature at four levels (15, 25, 35 and 45 °C), seed moisture content at five levels (6, 10, 14, 18 and 22 percent) and the storage duration in 10 levels (zero, 30, 60, 90, 120, 150, 180, 210, 240 and 270 days). To study the biochemical characteristics of treatments, 5 storage durations were selected (0, 30, 90, 180 and 270 days of storage). Desired seed moisture content was determined, using the following equation:

$$W2 = W1\frac{(A-B)}{(100-A)}$$

where B is the percentage of initial seed moisture content, A represents the percentage of desired moisture content, W1 the initial weight of seed (g) and W2 distilled water weight (g) (Hampton and TecKrony, 1995). After that, seeds were placed in aluminum foil packets and water was added when needed. Seeds were packed to prevent the moisture exchange with the outside and were kept for 24 hours at 15 °C to obtain the identical moisture content. During storage, with the interval of one month, for each treatment sampling was performed in 5 replicates each including 20 seeds and germination was also conducted. Germination test was performed on pleated paper (PP) in boxes (19×10×2.5 cm) at 25 °C for 10 days under dark condition (ISTA, 2010). 10 ml distilled water was added to ensure the moisture needed for seed germination. At the end of the experiment, seed germination percentage (Equation 1), seedling length, seedling dry weight, seedling vigor index (Equation 2), electrolyte leakage (Equation 3), soluble protein content of the embryo, catalase, peroxidase and polyphenol oxidase activity were measured.

Equation 1: Percentage of germination = (number of germinated seeds/total number of seeds) ×100

Equation 2 (Reddy and Khan, 2001): Seedling vigor index = (standard germination percentage×average seedling length) /100

Equation 3 (ISTA, 2010):

Electrolyte leakage (µs. $cm^{-1}.g^{-1}$ seed weight) = (leachate solution electrical conductivity - distilled water electrical conductivity) / seed sample weight (gram)

The soluble protein content of the embryo was measured using Bradford (1976) method.

The catalase activity was measured using Cakmak and Horst (1991) method with a little modification. To measure the enzyme activity, 30 ml of 100 mM H_2O_2 and 100 µl of protein extract were added to 8.2 mL of 25 mM sodium phosphate buffer with pH 6.8, and the absorption was measured in 1 minute using spectrophotometer at a wavelength of 240 nm. Enzyme activity was reported in mmol per gram of fresh weight of the embryo. Catalase extinction coefficient was Ɛ = 0.0394 $mMol^{-1}cm^{-1}$.

The peroxidase activity was measured using Ghanati *et al*., (2002) method with a little modification. To measure the enzyme activity, 100 µl of protein extract were added to 2 ml of 100 mM potassium phosphate buffer (pH 6.1), 0.5 ml guayacol 28 mM and 0.5 ml H_2O_2 30mM and the absorption was measured in 1 minute using spectrophotometer at a wavelength of 470 nm. Enzyme activity was reported in mmol per gram of fresh weight of the embryo. Peroxidase extinction coefficient was 0.0255 $mMol^{-1}cm^{-1}$.

The polyphenol oxidase activity was measured using Ghanati *et al*., (2002) method with a little modification. To measure the enzyme activity, 100 µl of protein extract were added to 0.5 ml H_2O_2 30mM, 0.5 ml of methyl Katkol 0.02 mM in 1.9 ml phosphate potassium buffer (pH 6.1) and the absorption was measured in 1 minute using spectrophotometer at a wavelength of 470 nm. Enzyme activity was reported in mmol per gram of fresh weight of the embryo. Polyphenol oxidase extinction coefficient was 0.0062 $mMol^{-1}cm^{-1}$.

Statistical analysis of experimental data was performed using SAS software version 9.2. to diagram plots, Excel software was used. Considering the significant interaction effects, treatments were fitted using L.S.Means procedure of SAS statistical software and the means were compared using LSD test at 5% statistical level.

Results and Discussion

Analysis of variance showed that the main effects of experimental factors including storage temperature, seed moisture content (SMC) and storage duration, as well as all dual and triple interactions, were significant ($P \leq 0.01$) on germination percentage, seedling length, seedling dry weight, seedling vigor index and electrolyte leakage (Table 1).

The results showed that germination decreased with increasing storage duration; this reduction was different at different temperatures and seed moisture content (Figure 1). The seeds stored at 15 ℃ with SMC of 6, 10 and 14 % had higher storage durability, in these moisture contents, germination was 94%, that decreased to less than 20% after 270 days of storage. However, at the same temperature and in moisture content of 22%, germination of seeds decreased to less than 50% and 10% after 150 and 270 days of storage, respectively.

The seeds stored at 25 ℃ showed a similar trend to those stored at 15 ℃, except that the decrease in seed germination at seed moisture content of 14% was intense after 120 days (decreased by 23%). However, at the temperatures of 35 and 45 ℃, decrease in germination was more severe than the other two temperatures. Sawant *et al*., (2012) reported that for wheat with increase in seed storage under unsuitable temperature and moisture treatments, germination percentage decreased. The main mechanisms for the reduced germination as a result of seed deterioration are not known, however some studies have shown that lipid peroxidation caused by oxidative damage can lead to inactivation and/or depletion of key enzymes of recipient protein transport or ion channels, as well as impairment of RNA and DNA synthesis (Murthy *et al*., 2003; Lehner *et al*., 2008).

Mean comparison of all storage conditions showed that by increasing the storage duration, seedling length decreased (Figure 2). Hence, in the seeds stored with seed moisture content of 6% and a temperature of 15 ℃, seedling length was 11.44 cm at the beginning of the storage, and then it decreased to 5.89 cm after 270 days. At the temperature of 25 ℃ and in seed moisture contents of 18% and 22%, seedling length was reduced and reached the lowest amount, after 180 and 90 days of storage, respectively. However, the rate of decline in seedling length accelerated with increasing incubation temperature; at the same seed moisture contents (18 and 22%) and in the seeds stored at 35 ℃ of 45 ℃ (Figure 2) this index decreased to zero in 60 days of storage. The presence of small and weak seedlings at the end of the experiment indicates the positive correlation between germination and seedling length thus the seeds, which germinate later, will have shorter seedlings length that can be due to a decreased ability to move the food supply.

The comparison of seedling dry weight showed that at the beginning of storage at 15 ℃ and moisture content of 6%, the seedling dry weight was 0.89 g that reduced by 38 % (0.56 g) after 270 days of storage. Meanwhile, during the same duration of storage at this temperature, seedling dry weight was reduced to 62% in the seed stored with moisture content of 22%. At 25 ℃, and seed moisture contents of 6, 10 and 14%, seedling dry weight were decreased with a constant gradient, whereas, at 18% and 22% this slope was quick. Like other traits, the rate of decrease in seedling dry weight in 35 ℃ and 45 ℃ was greater compared to the other two temperatures (Figure 3).

Chavoshinasab *et al*., (2010) has stated that with the increase in storage duration, temperature, seed moisture content and seedling dry weight significantly decreased. It has been stated that seedling dry weight loss during seed storage could be due to reduction in remobilization of the stored materials caused by the reduction of hydrolyzing-remobilizing enzyme activities (Mohammadi *et al*., 2011). Also, the inability of the embryo in using the mobilized materials leads to the enzyme activity loss, and the consumption of these reserves due to increase in the protease activity is also another cause of loss of storage materials in the embryo (Bewely and Black, 1982).

The seedling vigor index also decreased by increasing seed moisture content during storage at all incubation temperatures (Figure 4). At the temperature of 15 ℃ and seed moisture content of 6%, seedling vigor index was 10.75 at the beginning of the storage which reduced to 4.68 after 270 days of storage. At 25 °C and moisture content of 6, 10 and 14%, this index decreased with a steep slope and increased with increasing seed moisture content to 18% and 22%. However, the amount of this reduction at of 35 ˚C and 45 ℃ was more than the two previous temperatures, with a greater effect at 45 °C (Figure 4).

The seeds with higher vigor and high germination percentage are able to produce stronger seedlings that can tolerate environmental stresses. Alivand (2012) examined five oilseed species including rapeseed, sesame, soybean, sunflower and safflower seeds, reporting that with increasing seed longevity in different storage conditions, seedling vigor index, seedling length and seedling dry weight significantly decreased. An increase in electrolyte leakage due to the disorganization of intracellular membranes structures and the disorder of intracellular biochemical processes can be a possible reason for such a reaction; the negative and significant correlation between the seedling vigor index and electrolyte leakage of seed could explain such a reaction ($r = 0.76^{**}$). Also, it has been reported in maize (Mansouri-Far *et al.*, 2015), *Ricinus communis* (Soltani et al, 2017) and *Linum usitatissimum* (Bakhit *et al*, 2017), that seed vigour reduction is associated with the

Table 1. Analysis variance of the effect of storage temperature, seed moisture content and storage duration on seed germination and some seedling growth characteristics of faba bean (cv. Shami)

		Mean Square				
Source of variation	df	Germination Percentage	Seedling dry Weight	Seedling Vigor Index	Seedling Length	Electrolyte Leakage
Storage temperature (A)	3	124629.2**	9.47**	729.31**	1042.31**	21088.3**
Seed moisture content (B)	4	120114.3**	4.24**	775.54**	945.88**	9957.5**
Storage duration (C)	9	32525.7**	3.50**	727.63**	726.78**	4332**
A*B	12	10976.9**	0.31**	42.55**	89.37**	1602.2**
A*C	27	1787.7 **	0.17**	17.05**	24.26**	452.19**
B*C	36	1653.7**	0.07**	13.81**	16.77**	183.94**
A*B*C	108	1055.9**	0.04**	4.19**	10.41**	59.77**
Error	800	59.18	0.004	0.35	0.18	11.59
C.V (%)	-	15.34	17.92	14.66	8.18	13.13

****Significant at P≤0.01**

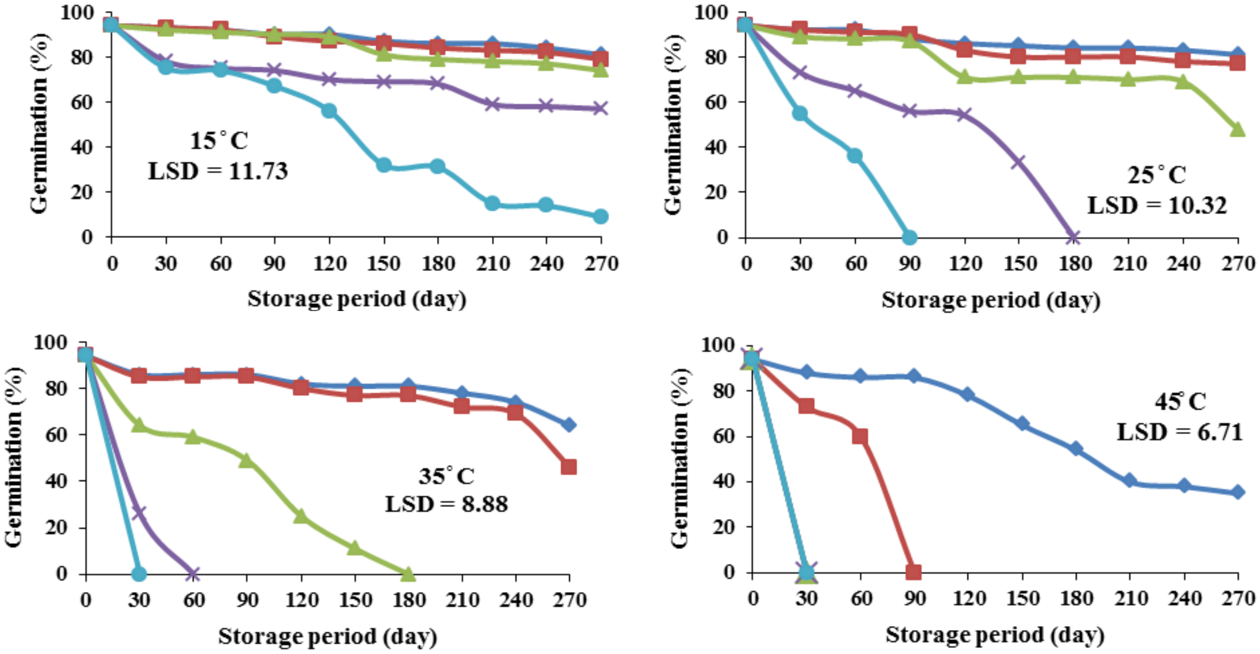

Fig 1. Germination percentage of Faba bean seed, stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ●, at different temperatures. Germinate later, will have shorter seedlings length that can be due to a decreased ability to move the food supply.

reduction of the SOD, CAT and POD activity.

Measuring the electrical conductivity of seeds can be one of the important parameters in determining the seed vigor. In contrast to the observed results for other traits, by increasing the storage periods, the leakage increased (Figure 5). This increase was lower at lower temperatures and seed moisture contents. So that at the temperature of 15°C, the average leakage was 25 ($\mu s.cm^{-1}. g^{-1}$) after 270 days of storage, whereas, at 45°C this average was around 50 ($\mu s.cm^{-1}.g^{-1}$). Increasing seed moisture content had a similar effect on this trait. The increase in the leakage from the seeds stored at 35 °C and 45 °C was more severe than that of the two other storage temperatures. In line with this result of Mohammadi *et al.*, (2008) he reported that the electrolyte leakage increased with increasing deterioration of soybean seed. Also, Goel *et al.*, (2003) reported that one of the main causes of seed deterioration is impairing the membrane integrity, which enhances electrolyte leakage from the cell. While the content of moisture increases during the germination process, active oxygen species are produced by mitochondria respiratory or glyoxysomes activity. The increase in production and release of reactive oxygen species lead to increase in the membrane lipids and protein peroxidation that is followed by cell membrane destruction (Goel and Sheoran, 2003).

The results of analysis of variance showed that the effects of the storage temperature, seed moisture content and storage duration as well as all double and triple interactions between experiment factors, were significant for biochemical indices at the probability level of 1% (Table 2).

By increasing the storage duration at all temperatures embryo soluble protein content decreased. At all storage temperatures, the stored seeds in the

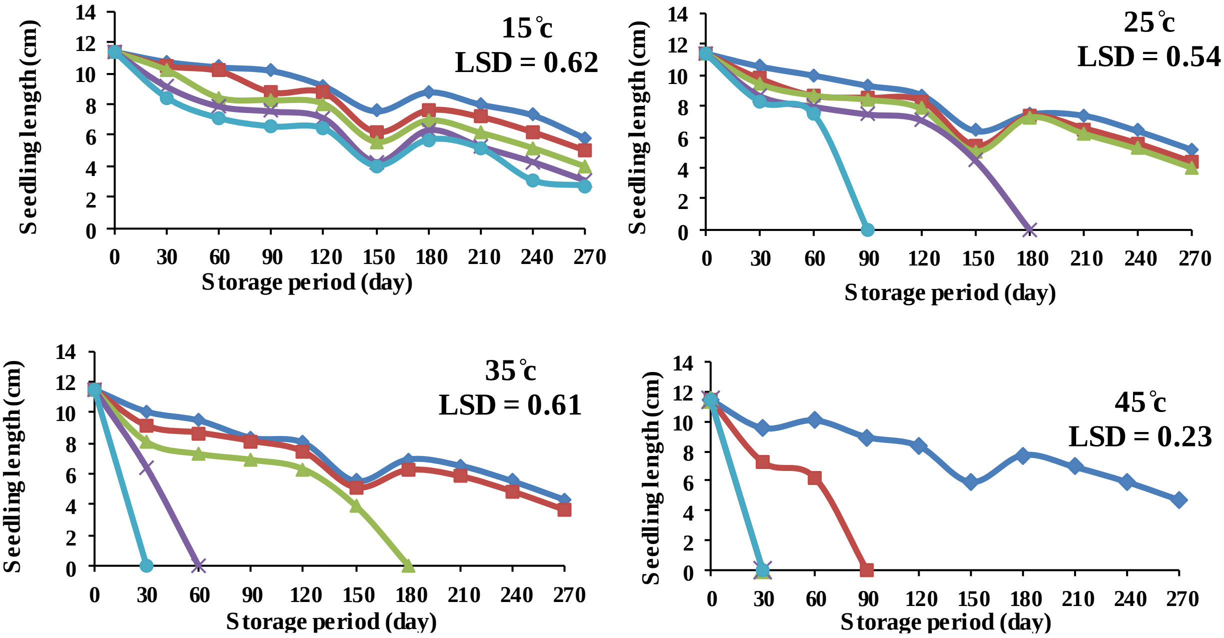

Fig 2 -seedling length of Faba bean seed, stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ● at different temperatures.

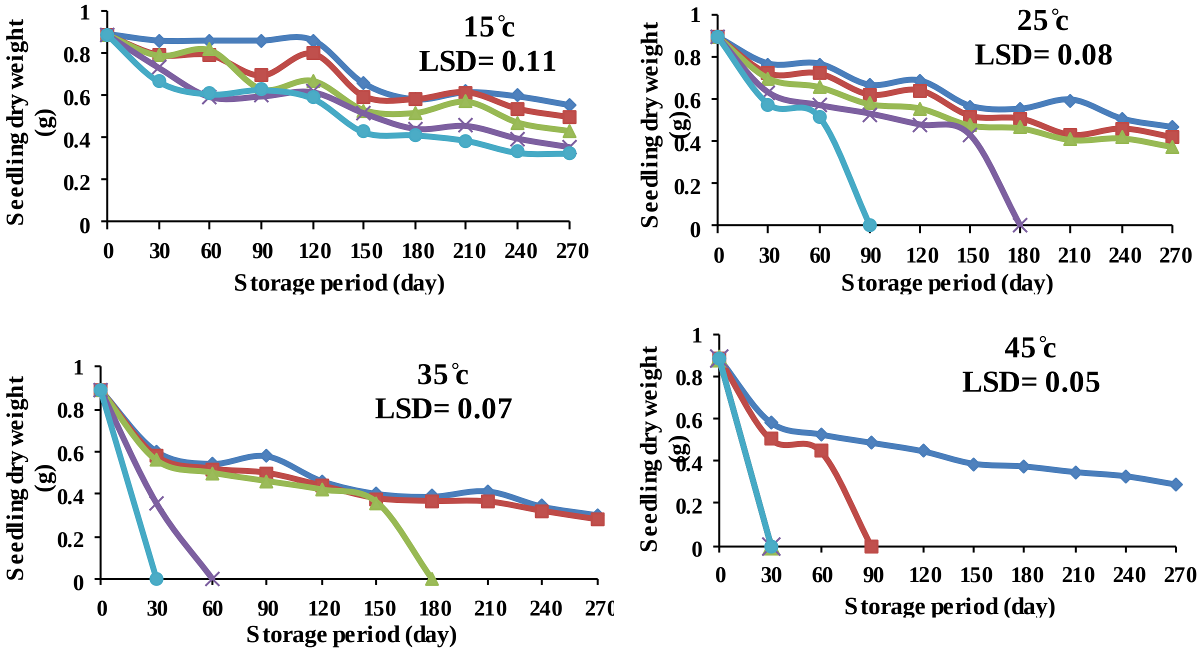

Fig 3 -seedling temperatures.day weight of Faba bean seed, stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ● at different

moisture contents of 6 and 10% had higher levels of protein content than those of 14, 18 and 22%, and were less affected by storage duration (especially at 15 and 25 ° C). The most reduction rate was observed in seed moisture contents of 18 and 22%. This index was 141.12 (mg.g^{-1} of embryo) at the beginning of storage at 15 ℃ and seed moisture content of 22% that decreased to 55.40 (mg.g^{-1} of embryo) after 270 days of storage. Reducing the amount of protein in seeds stored at 25 ℃ showed a similar trend with those stored at 15 ℃. At the temperatures of 35 and 45 ℃, the loss of protein content began from the beginning of storage. However, in the seeds stored at 45 ℃, soluble protein content reduced more quickly compared to other temperatures (Figure 6).

Yao *et al*., (2012) reported that in several varieties of peas, the soluble protein content decreased because of aging treatment. It has been stated that during seed deterioration, the great affinity of active oxygen and other generated aldehydes for vital biomolecules such as

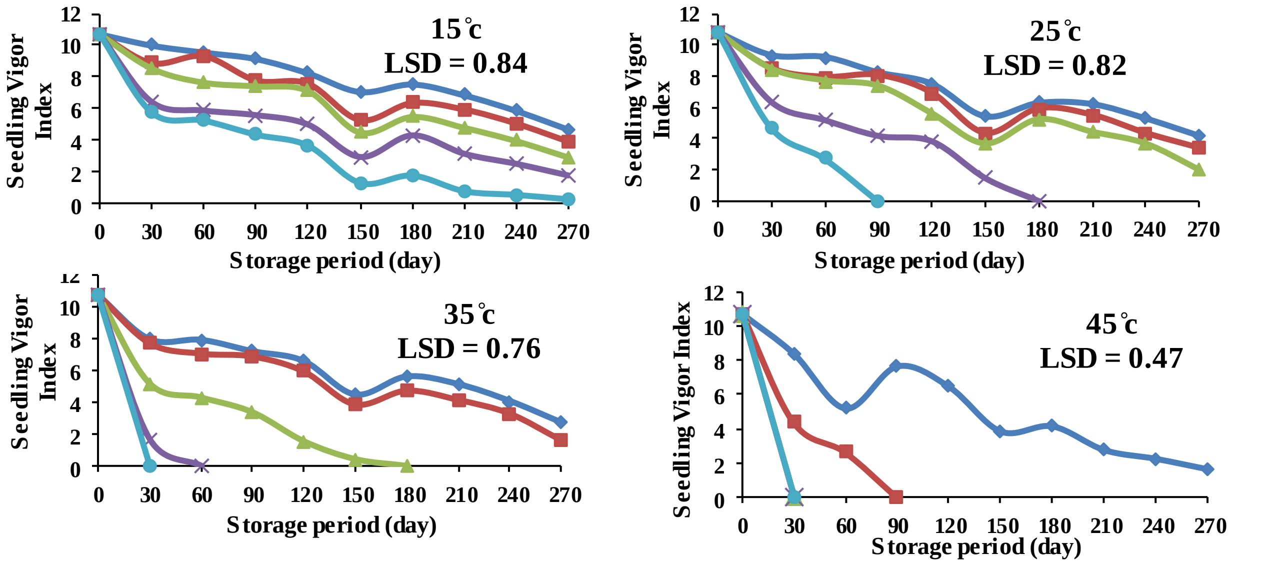

Fig 4. Seedling vigor index of Faba bean seed, stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ● at different temperatures.

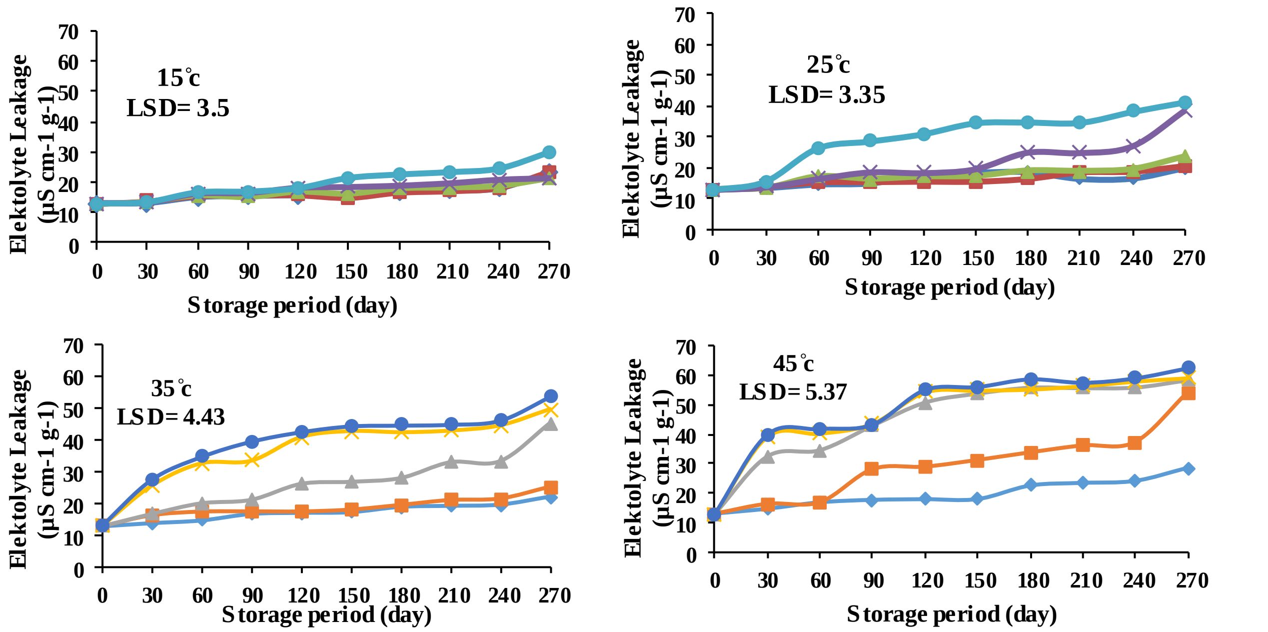

Fig 5. Electrolyte leakage in Faba bean seeds stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ●, during storage in different temperatures

proteins causes denaturation of them that finally exacerbates protein breakdown by protease enzymes (Kapur *et al*., 2010). In addition, the reduction in basic amino acids due to ROS attack has been mentioned as another reason for reducing the amount of protein during the aging process (Jin *et al*., 2006).

By increasing of storage duration, catalase activity decreased (Figure 7). The activity of this enzyme at the beginning of the storage at the temperature of 15 °C and moisture content of 6% was 145 (mmol. min^{-1} g^{-1} fresh weight of the embryo), which decreased by 33% (98.7 mmol. min^{-1} g^{-1} fresh weight of the embryo) after 270 days of storage. In addition, during the same storage time and at 22% moisture content, enzyme activity decreased significantly (less than 30% of the initial value). Also, the loss of catalase activity at 25 ℃ and moisture contents of 14, 18 and 22%, s reduced to less than half of the initial value after 30 days of storage. The catalase activity sharply decreased during storage at 35 ℃ and 45 ℃. However, in seeds stored at the temperature 45 ℃, the catalase activity loss was more severe than previous temperatures.

According to these observations, findings of Siadat *et al*., (2012) on corn plant, as well as the study of Ansari and Sharifzadeh (2012) on *Secale montanum*, showed that the activity of antioxidant enzymes was in

Table 2. Analysis variance of the effect of storage temperature, seed moisture content and storage duration on some biochemical characteristics of faba bean (cv. Shami) seeds.

		Mean Square			
Source of variation	df	Embryo Soluble Protein	Catalase	Peroxidase	Polyphenol Oxidase
Storage temperature (A)	3	18836.9 **	32166.1**	6430505**	64845**
Seed moisture content (B)	4	18803**	27807.3**	254479**	46206**
Storage duration (C)	4	73911.2**	80931.1**	159471**	22673**
A*B	12	1651.6**	1251.6**	23998**	5514**
A*C	12	1219.6 **	2355.4**	59140**	6575**
B*C	16	1361.4**	2233.4**	36958**	6084**
A*B*C	48	199.86**	390.2**	4448**	904.6**
Error	200	13.79	254.94	505	309.46
C.V (%)	-	4.13	19.16	10.3	22.19

****Significant at P≤0.01**

less than 50% and 10% after 150 and 270 days of storage, respectively.

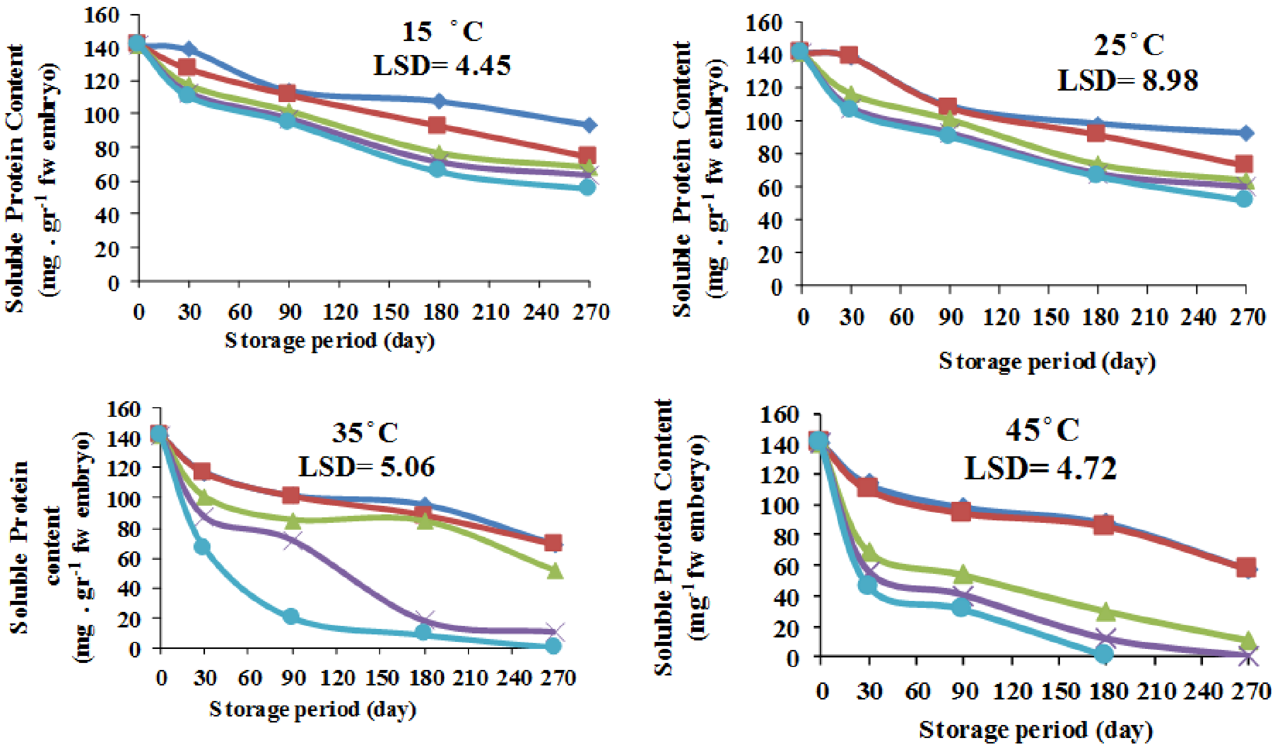

Fig 6. Embryo soluble protein content of Faba bean seed, stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ● at different temperatures.

aged seeds due to the increase in free radicals. In addition, in a study conducted by Goel *et al*., (2003) on cotton seeds under accelerated aging conditions, it was found that the ability of germination decreased with increasing concentrations of hydrogen peroxide and reducing the activity of antioxidant enzymes such as peroxidase, catalase and superoxide dismutase. Moreover, reduction of the activity of antioxidant enzymes such as catalase during storage can be attributed to protein degradation by cell proteases (Demirkaya, 2013). However, changes in antioxidant enzymes, especially catalase, can also be considered as one the most important event in aged seeds (Ansari *et al*., 2013). Therefore, it can be concluded that antioxidant enzymes affect seed germination after aging and seeds with higher enzyme activity have higher germination percentage.

Observation of the average peroxidase activity (POD) of stored seeds in different seed moisture (SMC) temperatures at 15 ºC and 25 ºC showed that peroxidase activity increased during the storage period up to 180 days and then decreased (Fig. 8). At 15 ºC and SMC of 6 %, the POD activity was 166.96 (mMol min^{-1} per gram fresh weight of the embryo) at the beginning of storage which then increased up to 516.56 and decreased to 285.60 after 180 and 270 days of storage, respectively. Also, at the end of storage at this temperature, the value of this index at 22% SMC fell by more than 60% compared to a 6% SMC. Similar to what was observed at temperatures of 15 °C and 25 °C, the

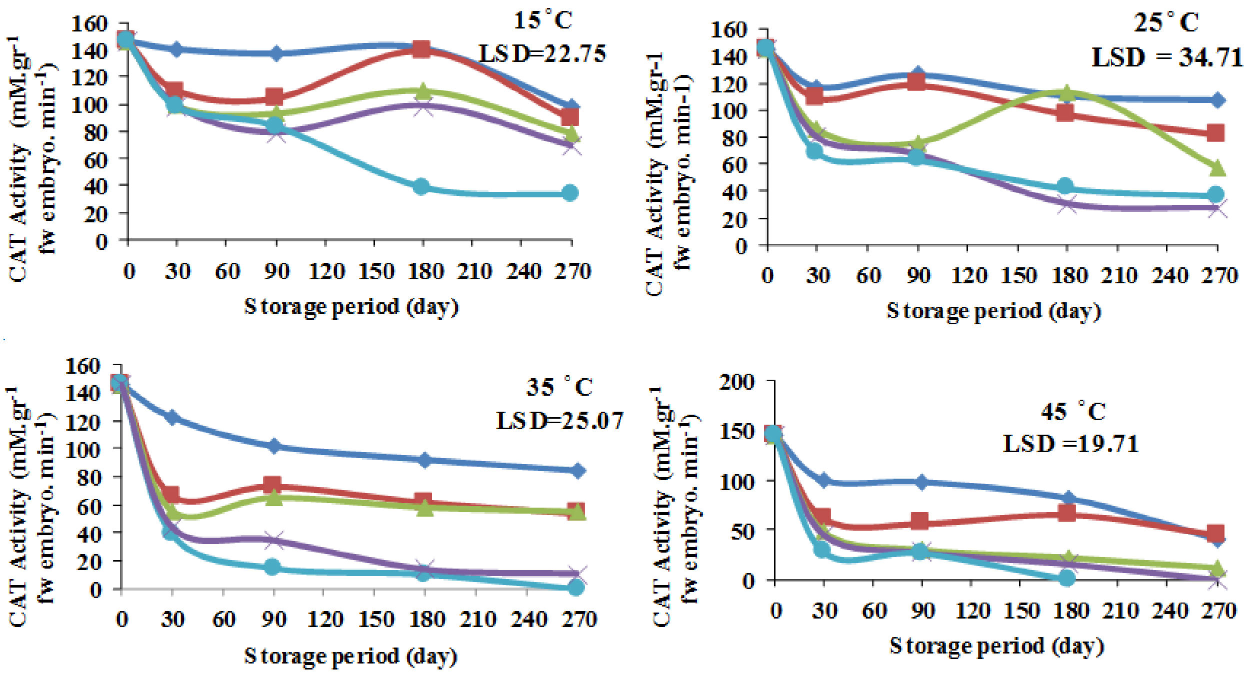

Fig 7. Catalase activity of Faba bean seed, stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ● at different temperatures.

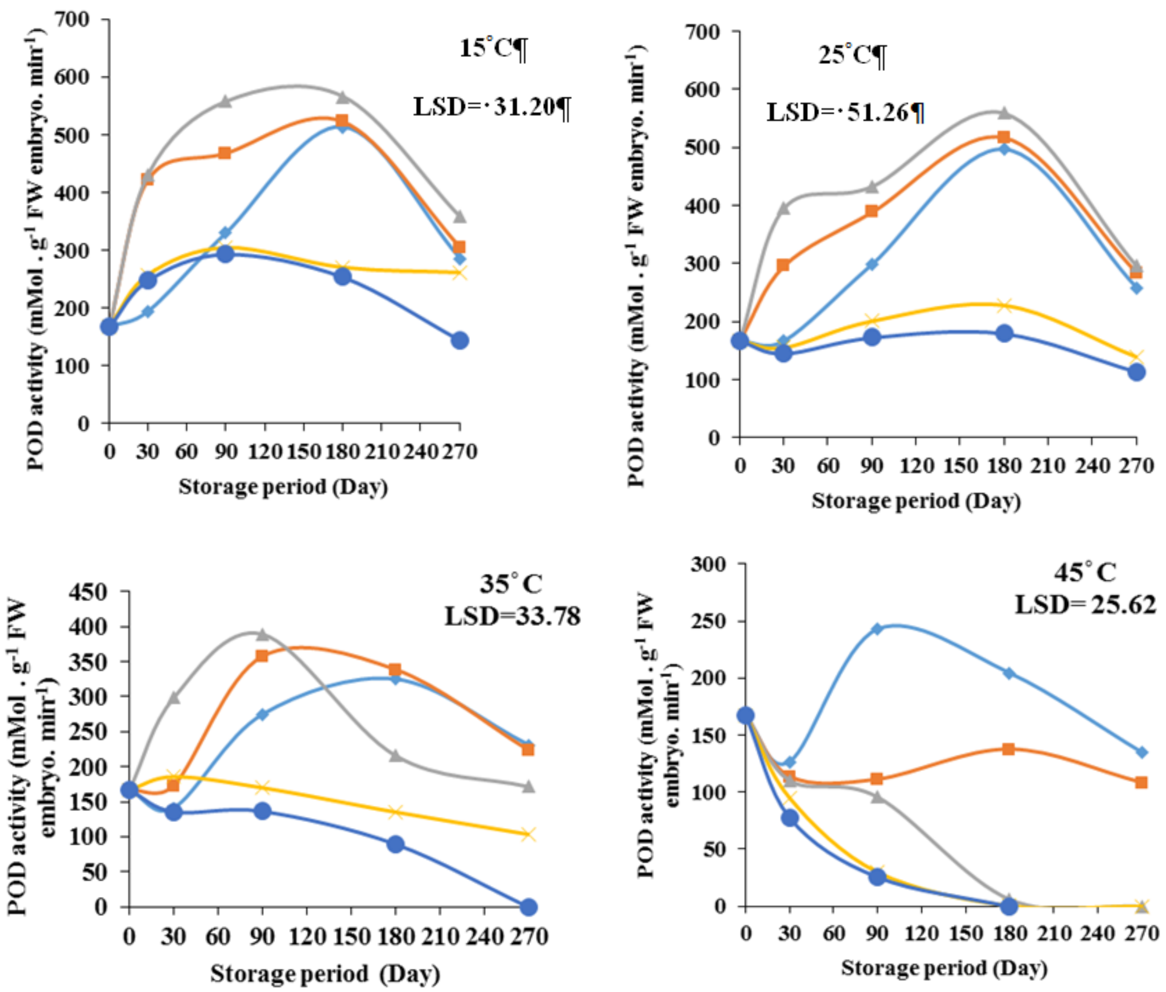

Fig 8. Peroxidase (POD) activity of Faba bean seed, stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ● at different temperatures.

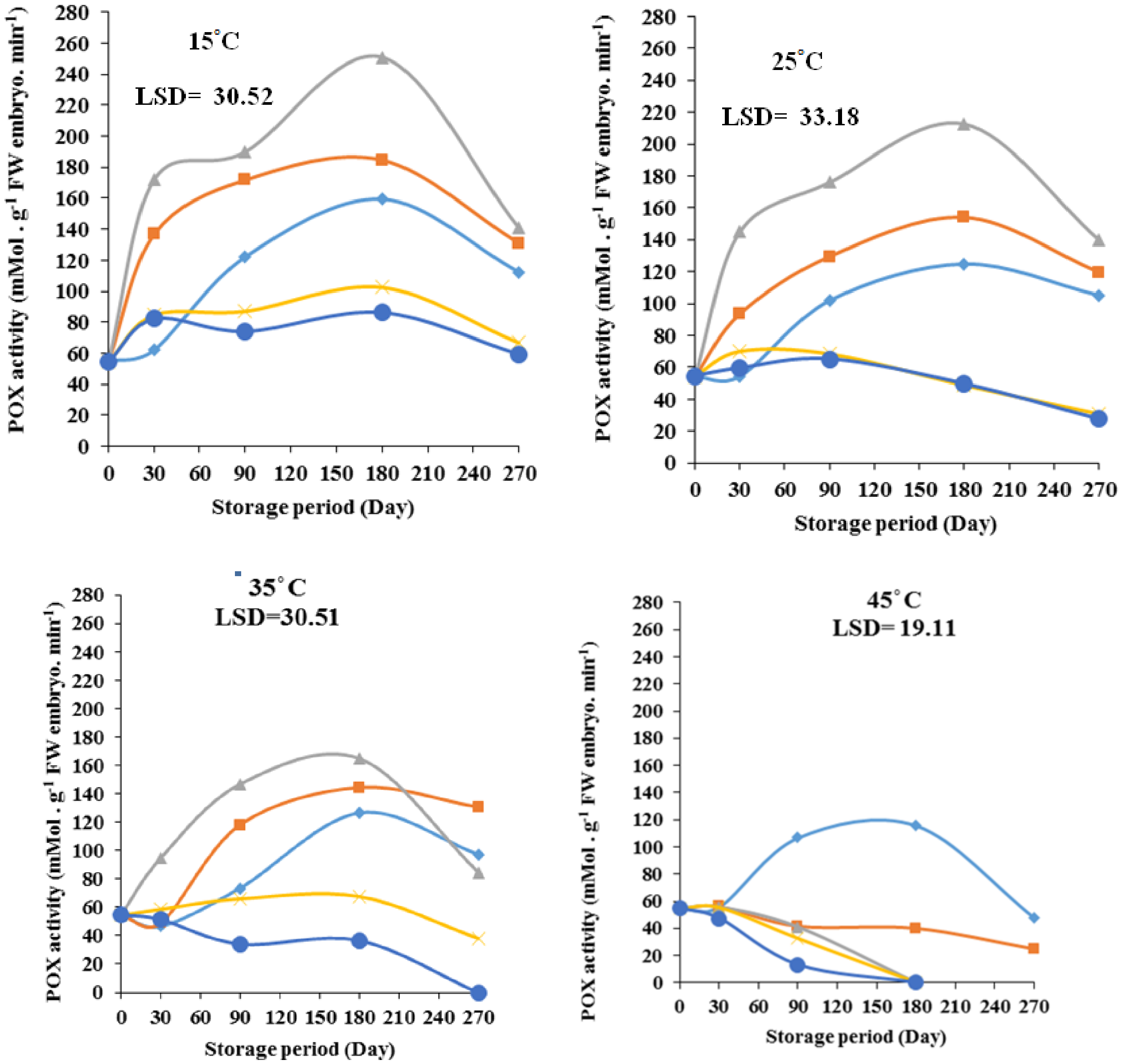

Fig 9. Polyphenol oxidase (POX) activity of Faba bean seed, stored with seed moisture content of 6%: ♦, 10%: ■, 14%: ▲, 18 %: × and 22 %: ● at different temperatures

activity of POD enzyme in the seeds stored at 35 °C was high in lower SMC levels, with the difference that the highest activity (388 mMol min^{-1} per gram fresh weight of the embryo) was observed in the seeds stored at SMC 14% after 90 days of storage. With an increase in temperature from 35 °C to 45 °C, the activity of this enzyme in the SMC 6 remained high (243 mMol min^{-1} per gram fresh weight of the embryo) up to 90 days after storage, while in other SMCs, the enzyme activity reduced from the beginning of storage and in the SMCs of 18% and 22%, dropped to zero from 180 days of storage.

In a study by Goel *et al*., (2003) on cotton seeds under accelerated aging condition, it was found that germination ability was reduced, which correlated with the increase in hydrogen peroxide accumulation and decreased activity of peroxidase enzyme. Bernal *et al*., (2000) have also reported a decrease in the activity of peroxidase as a result of seed deterioration.

The pattern of activity of polyphenol oxidase (POX) in response to temperature and SMC was similar to that of peroxidase enzyme activity. The results showed that the activity of POX enzyme in the seeds stored at 15, 25 and 35°C with 6, 10 and 14% SMCs, increased up to 180 days of storage and then began to decrease (Figure 9). The increase was higher at the temperatures of 15 and 25°C and moisture contents of 6, 10 and 14 %. At 45 ˚C and 6% SMC the activity of this enzyme increased by 54% after 180 days of storage and then decreased. However, in other moisture contents, this trend was reducing from the beginning of storage.

Seiadat *et al*., (2012) and Demirkaya *et al*., (2010) reported that antioxidant enzyme activities decreased by increasing seed deterioration. During the deterioration of the seeds, the active species of oxygen increases and this leads to degradation in the activity of antioxidant enzymes and, as a result, the activity of antioxidant enzymes decreases.

Regarding Figures 8 and 9, the trend of increase and decrease in POD and POX activities during storage can be evidence of increased resistance to ROSs during storage and the subsequent destruction of the enzyme itself, so that, before to mid-warehousing, the activity of these enzymes increased to counteract with ROSs. However, at the end of storage, with the increase of ROS, the structure of the enzymes are degraded and, subsequently, their the activity decreases; thus it can be stated that the decrease of activity of antioxidant enzymes is probably one of the reasons for decrease in the germination indices during storage.

Conclusion

The results obtained in this study showed that temperature and seed moisture content influence seed survival. Therefore, by increasing the storage temperature, the seed moisture content and storage duration, germination and seedling growth characteristics decreased significantly (by 2 to 90%) and this decrease was more intense in higher seed moisture contents (18 and 22%) and storage temperatures (35 and 45 ˚C). The lowest deterioration rate was observed after 270 days of storage at 15 °C and seed moisture content of 6% (with 81 % germination) and the highest deterioration rate was related to the seeds stored at 45 °C, seed moisture content of 22% which dropped to zero after 30 days of storage. Contrary to the observed results for other traits, by increasing the storage periods, the leakage was increased and reached maximum after 270 days of storage. POD and POX activities were increased till 180 days of storage and then decreased. In conclusion, the present results demonstrate that ageing of faba bean seeds is associated with changes in antioxidant enzyme activity and electrolyte leakage. At the end of the storage period, temperatures of 25 and 35 °C, and moisture contents of 18 and 22 %, had the lowest levels of antioxidant enzymes and highest electrolyte leakage, which coincided with the decline of the germination at these temperatures and moisture.

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