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Research Article

Effects of Induced Ageing on Metabolic Enzymes in Castor Seed Accessions

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Abstract
In Nigeria, the castor seeds available to farmers are imported overtime. Changes during storage are vital tool for determining seed stability as well as viability. This study was aimed at comparing the metabolic enzymes' activities, changes in accelerated aged seeds of local and imported castor genotypes. A total of six (6) castor accessions (local and exotic) were assessed for accelerated ageing at a temperature of 40^0C and 100% relative humidity for six (6) days. Activities of enzymes (catalase, amylase, protease and lipase) were determined in the aged seeds using standard biochemical procedures. The result showed a progressive decrease in enzyme activities of all enzymes studied. The decrease was only significant ($p< 0.05$) at day six (6) indicating that both local and exotic castor seeds have long shelf-life.

Keywords*:* Castor, Enzymes, Seeds, Ageing, Accessions

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Introduction

Castor is a member of the Euphorbiaceae family that is found across all the tropical and semi tropical regions of the world (Weiss, 2000). Castor bean (*Ricinuscomminus* L.) is cultivated for its seeds which yield viscous, pale and non-volatile yellow oil (Tchuenteu *et al*., 2013). The oil is inedible and has been used almost entirely for pharmaceutical and industrial purposes. Notably it is used in the manufacture of paints, dyes, inks, waxes, varnishes, lubricants and brake fluids (Ogunniyi, 2006). The castor oil obtained by cold pressing of seeds is also used for soap production, purgatives and laxatives in household (Weiss, 2000).

In order to ensure the availability of seeds for farmers as well as for industrial purposes, it is necessary to develop appropriate storage methods that would prolong the viability of seeds. Accelerated ageing is one of the common tests used to predict the storability of seeds, during which seeds are exposed to high temperature and high relative humidity (Delouche and Baskin, 1973). The temperature levels, moisture content as well as storage duration are the most important factors which individually or collectively affect viability of stored seeds. Under unfavourable storage conditions such as the temperature above 30°C and relative air humidity between 80 to 90 percent, the variation in seed germination rate can be significantly high (Sisman, 2005). Seed aging during storage is an inevitable phenomenon, but the degree and speed of decline in seed quality depend strongly on storage conditions, plant species and initial seed quality (Elias and Copeland, 1994; Balesevic *et al*., 2005). The rate at which the seed aging process takes place depends on the ability of seed to resist degradation

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changes. Seed longevity is therefore determined by seed moisture content, ambient or storage temperature and seed quality attributes that are influenced by genetic and environmental interactions during seed maturation, harvesting and storage (Walters *et al*., 2010).

Metabolism of the seeds is greatly affected during their storage. This is mainly due to modulation of various enzymes activities in the seeds. The alterations in the activity of enzymes could be brought about by compositional or configurational changes in their structure such as partial folding or unfolding, degradation to subunits, and condensation to form polymer (Sharma *et al*., 2012). There is also activation of some enzymes especially the hydrolytic enzymes during storage, but, this is highly dependent upon the moisture level of the seeds. If moisture content reaches acceptable level, physiological germination may occur, however if moisture levels for germination are not attained, the seed deteriorates because of energy expenditure or accumulation of broken-down products (Spickett *et al*., 2015). The various hydrolytic enzymes activated by high moisture levels include lipase, phospholipase, protease, DNase, phosphatase and amylase (Fu *et al*., 2015). Annual losses due to deterioration can be as much as 25% of the harvested crop and therefore is one of the basic reasons for low productivity (Shelar *et al*., 2008). Loss of seed quality, viability and vigour during storage is attributed to seed deterioration triggered by metabolic enzymes under adverse environmental factors (Kapoor *et al*., 2010). Therefore, there is need to determine the enzymatic activity of the seed over a storage period. The study was aimed at determining the effects of ageing on metabolic enzymes' activitiesin castor seeds.

Materials and Methods

The castor seeds (exotic and local accessions) were obtained from the National Cereals Research Institute (NCRI), Badeggi, Niger State, Nigeria.The local and exotic accessions were then selected for accelerated ageing in a biochemical incubator at a temperature and relative humidity of 40^0C and 100% respectively for 2, 4 and 6 days. The aged seeds were then subjected to the various biochemical analyses.

Protease activity determination

The extraction of protease enzyme was done as described by Klomklao *et al*. (2011). To 0.1mL of the extracted enzyme, 1mL each of casein and phosphate buffer 50mM (pH 7.0) was added. It was then made up to 3ml by adding 0.9ml of distilled water. The mixture was then incubated at 55^oC for 30min. To 1ml of the incubated mixture, 1mL of ninhydrin solution was added and the test tube transferred into boiling water for three minutes. The mixture was then made up to 5mL by adding 3mL of distilled water and absorbance was read at 540nm. Standard tyrosine (0.05mg/mL) was also measured at 540nm and the protease activity was calculated.

Determination of amylase activity

The enzyme extraction was carried out as reported by De-Morias and Takaki (1998). In cold tris malate buffer (0.005M, pH 7), 2g of seed cotyledon was grinded into powder using mortar and pestle. The homogenate was centrifuged for 20min at 3000g and the supernatant used as crude extract.To 0.1ml of the crude extract, 1ml of 0.1% starch solution was added and allowed to stand for 5min. Then 1ml of DNS was added to the mixture and incubated in boiling water for 5mins, cooled and the absorbance read at 540nm. In a separate tube, standard glucose (1mg/ml) was also mixed with 1ml of DNS, incubated in boiling water for 5mins, cooled and the absorbance read at 540nm.

Determination of lipase activity

The seed coats were removed manually and 20g cotylydonswere homogenized in chilled acetone at 4°C. The suspension was centrifuged at 3000rpm and residue obtained was dissolved in 100mL distilled water followed by centrifugation at 7500 rpm. The supernatant was used as source of crude enzyme and was precipitated by ammonium sulphate (80% saturation) according to Michael *etal*. (2001). The precipitate was obtained by centrifugation at 10,000rpm for 20min. Precipitate was dissolved in 20ml Tris-Cl buffer (10mm, pH 8.5) and dialyzed overnight against the same buffer. The dialyzed enzyme was used as partially purified enzyme and used for enzyme characterization.

Lipase assay: The titrimetric method of Maliks*et*

al. (2000) was used for determination of lipase activity. Olive oil emulsion was prepared in 180 mL distilled water containing 20ml olive oil, 0.4g of sodium benzoate and 1g gum-arabic. Assay mixture contained 5 mL olive oil emulsion, 5ml0.1M Tris buffer (pH 8) and 1ml crude enzyme was incubated at 35°C for 10min. The reaction was stopped by 10 mL of acetone and methanol mixture (1:1). Each sample was titrated against 0.025N NaOH using 1% phenolphthalein as indicator. The volume of NaOH used in the titration was noted and used for enzyme activity calculations. One unit of lipase is defined as the amount of enzyme required to liberate 1µmol of free fatty acid from olive oil per min under the standard assay conditions.

Catalase activity determination

Catalase was estimated as described by Ayşe *et al*. (2012). Two grams (2g) of the seed was homogenized in extraction buffer (pH7.6) containing 10mm EDTA and 10% (w/v) PVPP. Homogenates were centrifuged at 12000g for 15 min. Catalase activity was spectrophotometrically determined. The decomposition of H_2O_2 was monitored at 240nm in a reaction mixture that contained 50Nm potassium phosphate buffer (pH 7.0), the sample and 10 mm H_2O_2. The assay was performed 25°C in a 3ml cuvette. The protein concentration was measured according to Bradford (1976) using bovine serum albumin as standard.

Results and Discussion

The results showed that enzymes' activity decreases with ageing (Tables 1 - 4). The protease activity of local castor accessions decreased from 23.92±2.22mg/mL/min on day zero to 16.54±1.79 mg/mL/min on day sixth while that of exotic accessions decreased from (20.64±3.02 mg/ml/min to 14.39±2.24mg/mL/min). Amylase activity in local accession decreased from day zero to day six (38.83±2.42 mg/ml/min to 32.10±2.06 mg/ml/min),while the amylase activity of the exotic accessions decreased from 46.53±0.88 to 33.65±0.57 mg/ml/minute. The lipase activity of local castor accessions decreased from day zero to day six of ageing with activity values ranging from 33.14±3.37 mg/ml/min to 23.51±3.10mg/ml/min, while the exotic accessions recorded activity between (30.47±2.96 and 24.10±1.46mg/ml/min). Catalase activity decreased from day zero to day six (44.13±3.41mg/ml/min to 37.21±4.56 mg/ml/min) when compared to the exotic accessions with activity between 43.37±3.17mg/ml/min and 36.68±3.32mg/mL/min. However, there were no significant ($p<0.05$) differences in enzymes activities between local and exotic accessions within the period of ageing (Figures 1 - 4);though, local accessions had higher average activity in protease, lipase and catalase than the exotic. The exotics had higher average amylase activities than the locals,which can be attributed to climatic factors. However, the decrease in enzyme activity with time became significant as from day four. The observed decrease in enzymes' activities is in agreement with the findings of Begum *et al.* *(*2013). Several studies have shown that decrease occur in the activity of enzymes present in aged seeds (Goel *et al.,* 2002; Bailly, 2004; McDonald, 2004). Certain anabolic enzymes aid in maintenance of seed viability while some catabolic enzymes decrease seed viability (Begum *et al.,* 2013).

The concentration of some seed enzymes are markers of ageing in stored seeds. A change in enzyme activity due to ageing of seeds was reported by many researchers. Cakmak *et al*. (2009) observed a decrease in the activities of catalase enzyme in both the old dry seeds of legumes during storage for 40 years. Chauhan*et al*. (2011) studied the reduction in catalase activities in wheat during ageing and the rate of decreasing was higher with increase ageing. The decreased protease activity may be due to reduced de novo protein synthesis (Osmond *et a*l., 1975) or as a result of induced deterioration that increase the extent of protein oxidation thus inducing loss of functional properties of proteins and enzymes (Lehner *et al*., 2008). A gradual decline in amylase activity was reported in natural aged seedlot as time of ageing increased (Petruzzeli and Taranto, 1990). Similar result of decrease in amylase activity was also reported by Norastehnia *et al*. (2007). The study has shown that castor seeds loose viability and stored nutrients with ageing but has a longer period of viability and stability when compared with other oil seeds such as soybean Kabinza *et al.* (2011), sunflower

(Yadollhhi and Mashayekhi, 2013) and groundnut (Huang and Moreau, 2007) .

Conclusion

The castor accessions under investigation showed decrease in the activities of all the enzymes studied. This is an indication that castor seed loses its vigor, viability and nutrient quality with time of storage. The study also revealed that castor seeds have long period of viability and nutrient retention during storage.

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Table 1. Effect of Induced Ageing on Amylase Activity (mg/mL/min) of Local and Exotic Castor Seed Accessions

	Days of induced ageing				
Local accessions	**0**	**2**	**4**	**6**	**% Reduction**
NCRICAS 006	12.02±0.55^{b}	12.40±0.26^{b}	9.60±0.33^{a}	8.01±0.64^{a}	33.36
NCRICAS 036	12.37±0.87^{ab}	12.87±0.69^{ab}	11.43±0.75^{a}	10.57±0.44^{a}	14.55
NCRICAS 019	12.03±0.36^{b}	13.13±0.41^{b}	10.97±0.72^{a}	10.27±0.33^{a}	14.63
NCRICAS 012	19.00±0.50^{b}	18.73±0.28^{b}	14.57±0.58^{a}	13.27±0.19^{a}	30.16
NCRICAS 039	23.63±0.76^{b}	24.03±0.69^{b}	17.87±0.60^{a}	15.83±0.51^{a}	33.01
NCRICAS 044	15.37±0.81^{b}	14.27±0.37^{b}	12.10±0.47^{a}	10.13±0.44^{a}	34.09
Exotic accessions					
NCRI-P-38	19.03±0.58^{c}	16.40±0.42^{b}	12.9±0.25^{a}	11.43±0.49^{a}	39.94
NCRI-P-40	15.97±0.77^{b}	16.03±0.50^{b}	12.43±0.41^{a}	12.30±0.55^{a}	22.98
NCRI-P-45	16.20±0.82^{b}	15.4±0.49^{b}	11.30±0.67^{a}	10.26±0.37^{a}	36.67
NCRI-P-007	17.83±0.61^{b}	16.5±0.31^{b}	12.73±0.50^{a}	11.53±0.29^{a}	35.37
NCRI-P-055	15.37±0.56^{b}	15.60±0.40^{b}	12.23±0.61^{a}	11.50±0.71^{a}	25.18
NCRI-P-017	19.57±0.39^{b}	18.97±0.83^{b}	16.10±0.36^{a}	14.90±0.45^{a}	23.86

Values are mean ± standard error of mean of three determinations. Values with different superscript along the row are significantly ($p < 0.05$) different

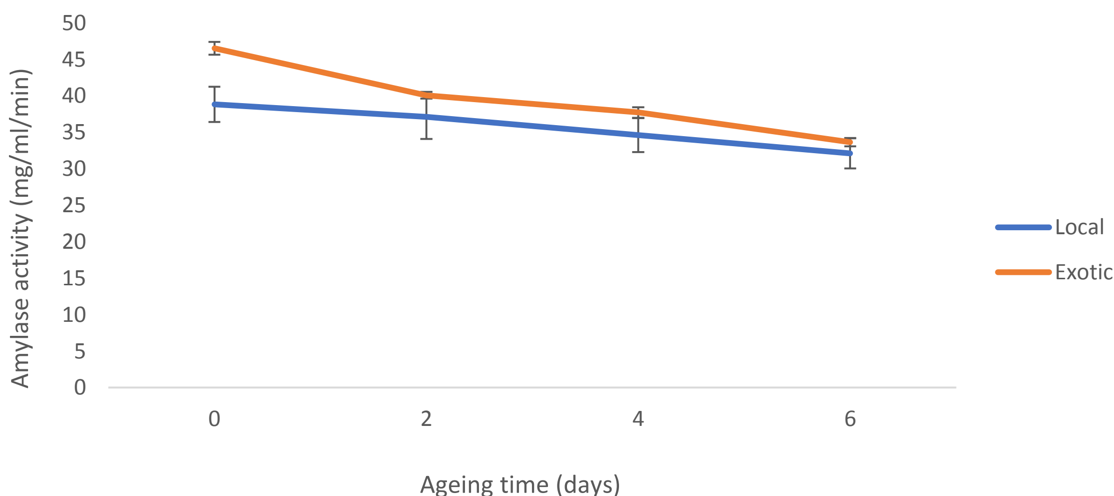

Figure 1. Comparison of Amylase Activity of Aged Castor Seed Accessions

Table 2. Effect of Induced Ageing on Catalase Activity (mg/mL/min) of Local and Exotic Castor Seed Accessions

	Days of induced ageing				
Local Accessions	**0**	**2**	**4**	**6**	**% Reduction**
NCRICAS 006	54.93±0.18^{b}	56.15±0.42^{bc}	53.48±0.33^{b}	46.36±0.27 ^{a}	15.60
NCRICAS 036	52.28±0.22^{b}	52.98±0.54^{b}	49.62±0.81^{b}	43.19±0.46 ^{a}	17.39
NCRICAS 019	38.86±0.35^{b}	39.61±0.28^{bc}	36.49±0.43^{b}	31.68±0.30^{a}	18.48
NCRICAS 012	41.48±1.03^{ab}	41.67±0.47^{a}	40.24±0.61^{a}	37.23±0.24^{a}	10.26
	35.83±0.76^{b}	36.14±0.55^{b}	34.23±0.47^{a}	32.17±0.63^{a}	10.22
NCRICAS 044	37.38±0.44^{b}	38.23±0.26^{b}	36.19±0.51^{a}	32.63±0.43^{a}	12.71
Exotic accession					
NCRI-P-38	54.39±1.02^{b}	55.19±0.85^{bc}	52.18±0.66^{ab}	47.32±0.71^{a}	13.00
NCRI-P-40	49.18±0.55^{b}	51.37±0.62^{c}	50.29±0.69^{bc}	46.13±0.47^{a}	6.20
NCRI-P-45	42.62±0.88^{c}	42.51±0.51^{c}	40.72±0.64^{b}	33.69±0.53^{a}	20.95
NCRI-P-007	40.93±0.39^{b}	41.09±0.11^{b}	40.67±0.45^{b}	34.59±0.32^{a}	15.49
NCRI-P-055	36.75±0.27^{b}	35.69±0.41^{b}	32.69±0.33^{ab}	29.67±0.45^{a}	19.27
NCRI-P-017	34.63±0.52^{b}	34.38±0.61^{b}	31.92±0.36^{a}	28.68±0.70^{a}	21.96

Values are mean ± standard error of mean of six determinations. Values with different superscript along a row are significantly ($p < 0.05$) different

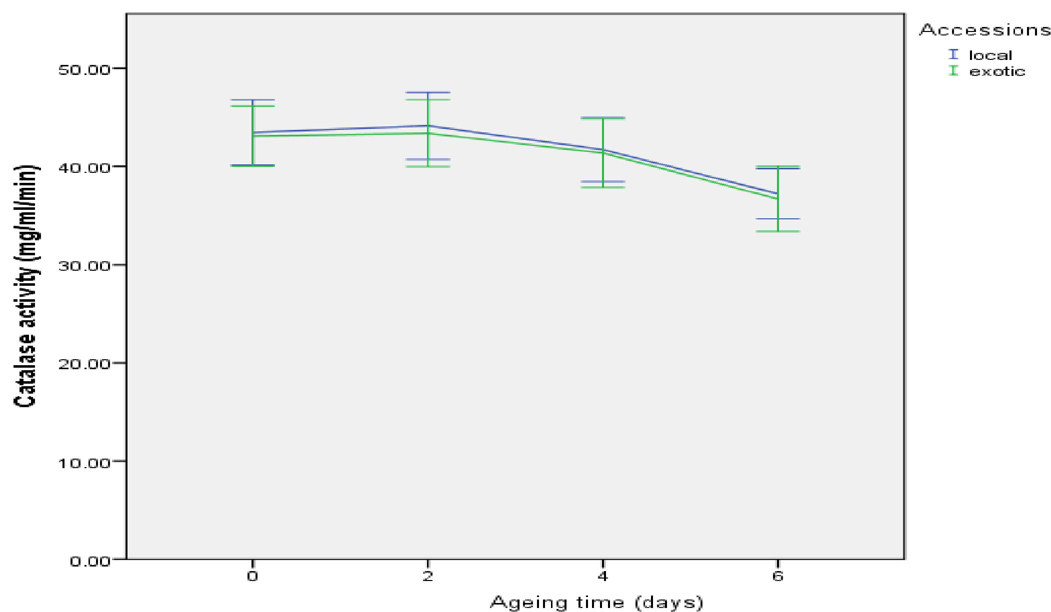

Figure 2. Comparison of Catalase Activity of Aged Castor Seed Accessions

Table 3. Effect of Induced Ageing on Protease Activity (mg/mL/min) of Local and Exotic Castor Seed Accessions

	Days of induced ageing				
Local Accessions	**0**	**2**	**4**	**6**	**% Reduction**
NCRICAS 006	28.89±0.57^{d}	21.85±0.88^{bc}	17.10±0.43^{b}	14.73±0.77^{a}	49.01
NCRICAS 036	19.69±0.43^{b}	19.61±0.55^{b}	14.86±0.47^{a}	12.49±0.51^{a}	36.57
NCRICAS 019	24.19±0.25^{b}	23.84±0.36^{b}	22.29±0.49^{ab}	20.87±0.66^{a}	13.73
NCRICAS 012	29.89±0.33^{d}	22.55±0.48^{c}	18.66±0.50^{ab}	16.72±0.32^{a}	44.06
NCRICAS 039	15.55±0.36^{c}	13.99±0.30^{ab}	12.61±0.15^{a}	11.92±0.26^{a}	23.34
NCRICAS 044	25.31±0.40^{b}	24.36±0.52^{ab}	23.15±0.48^{a}	22.54±0.53^{a}	10.94
Exotic accession					
NCRI-P-38	15.63±0.52^{b}	14.25±0.47^{ab}	12.96±0.11^{a}	10.83±0.32^{a}	30.71
NCRI-P-40	13.73±0.61^{ab}	13.04±0.34^{ab}	12.96±0.55^{a}	10.24±0.23^{a}	25.42
NCRI-P-45	22.29±0.45^{d}	18.57±0.28^{c}	15.20±0.36^{b}	13.68±0.47^{a}	38.63
NCRI-P-007	27.64±0.57^{b}	27.12±0.39^{b}	26.61±0.64^{b}	22.67±0.48^{a}	17.98
NCRI-P-055	13.82±0.24^{b}	13.47±0.58^{b}	12.35±0.71^{b}	9.37±0.27^{a}	32.20
NCRI-P-017	30.75±0.81^{c}	29.20±0.63^{c}	24.88±0.70^{b}	19.52±0.77^{a}	36.52

Values are mean ± standard error of mean of six determinations. Values with different superscript along a row are significantly ($p < 0.05$) different.

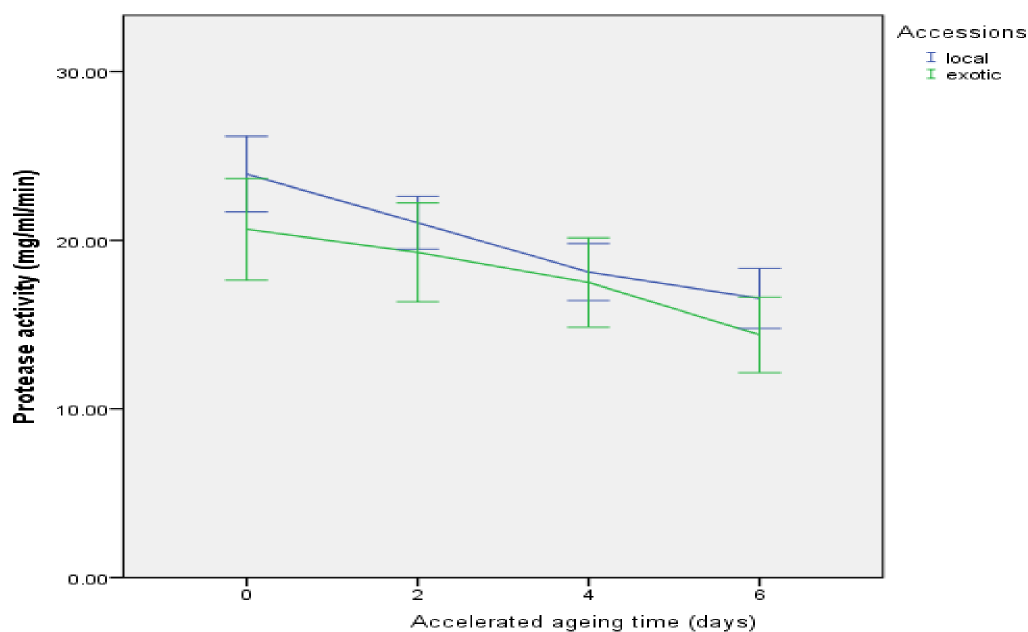

Figure 3. Comparison of Protease Activity of Aged Castor Seed Accessions

Table 4.Effect of Induced Ageing on Lipase Activity (mg/mL/min) of Local and Exotic Castor Seed Accessions

	Days of induced ageing				
Accessions	**0**	**2**	**4**	**6**	**% Reduction**
NCRICAS 006	42.61±0.41^{b}	42.42±0.53^{b}	36.93±0.22^{a}	34.28±0.50^{a}	19.55
NCRICAS 036	38.13±0.18^{a}	36.19±0.29^{b}	31.26±0.54^{ab}	28.14±0.26^{a}	26.20
NCRICAS 019	37.67±1.06^{c}	37.98±0.93^{c}	29.97±0.68^{b}	25.12±0.98^{a}	33.32
NCRICAS 012	32.61±0.23^{c}	33.18±0.71^{c}	27.15±0.66^{b}	22.17±0.45^{a}	32.02
NCRICAS 039	28.15±0.58^{b}	27.49±0.36^{b}	22.63±0.60^{a}	19.19±0.59^{a}	31.83
NCRICAS 044	19.69±0.33^{c}	19.15±0.57^{c}	16.32±0.41^{b}	12.18±0.17^{a}	
Exotic accessions					
NCRI-P-38	39.12±0.77^{c}	38.19±1.04^{c}	33.74±0.87^{b}	29.18±0.65^{a}	25.41
NCRI-P-40	38.48±0.88^{c}	38.68±0.48^{c}	34.47±0.72^{b}	29.68±0.70^{a}	24.13
NCRI-P-45	29.95±0.43^{bc}	26.13±0.58^{b}	21.72±0.62^{a}	19.44±0.44^{a}	35.09
NCRI-P-007	28.72±0.69^{b}	28.15±0.66^{b}	21.28±0.71^{a}	21.92±0.39^{a}	23.68
NCRI-P-055	26.18±0.61^{b}	26.24±0.44^{b}	24.83±0.86^{ab}	21.92±0.48^{a}	16.27
NCRI-P-017	20.38±0.74^{b}	19.79±0.55^{b}	17.48±0.43^{ab}	14.77±0.92^{a}	27.53

Values are mean ± standard error of mean of six determinations. Values with different superscript along a row are significantly ($p < 0.05$) different.

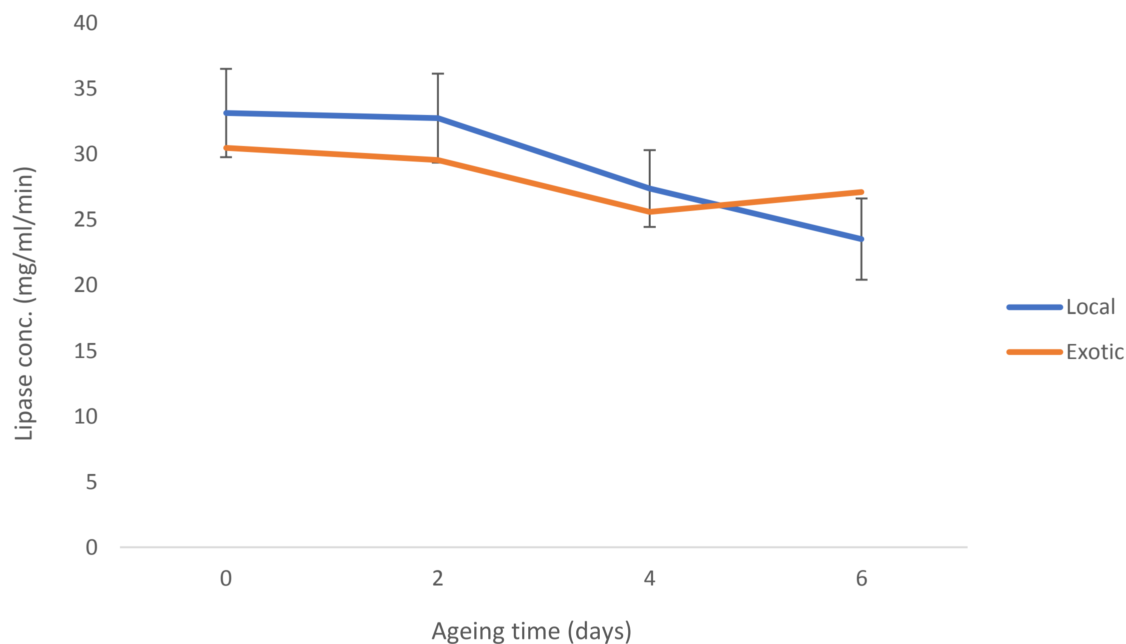

Figure 4. Comparison of Lipase Activity of Aged Castor Seed Accessions