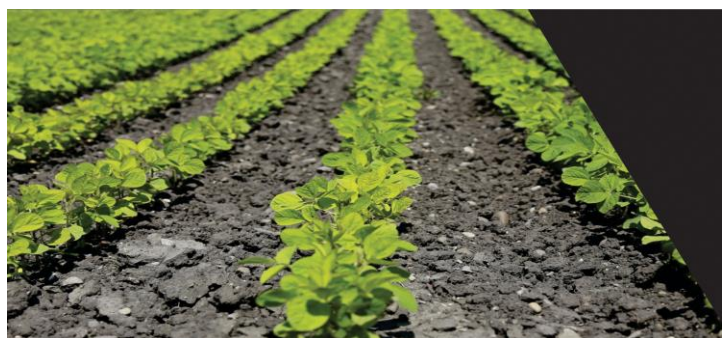




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Influence of accelerated ageing on germination and seedling vigour in different varieties of linseed (*Linum usitatissimum* L.)

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Abstract

Seed deterioration during storage poses a significant constraint to crop establishment and productivity in linseed (*Linum usitatissimum* L.). However, limited information is available on the differential response of linseed varieties to ageing-induced deterioration. The present study was conducted to evaluate the influence of accelerated ageing on germination and seedling vigour parameters in four linseed varieties and to identify genotypes with superior tolerance to seed deterioration. Four linseed varieties, viz. Kiran, Deepika, RLC 92 and Kartika were subjected to accelerated ageing at 45 °C and approximately 95% relative humidity for 0, 2, 4 and 6 days using a factorial completely randomized design with three replications. Data were recorded on germination percentage, germination speed index, seedling length, seedling dry weight, vigour index-I and vigour index-II. Accelerated ageing caused a progressive decline in all germination and vigour parameters across all varieties. Among the varieties, RLC-92 consistently maintained superior performance under prolonged ageing conditions, recording 54.00% germination at 4 days and 16.00% at 6 days of ageing, along with higher vigour index-I (407.03) and vigour index-II (1.14) at 4 days. Conversely, Kiran and Kartika exhibited marked susceptibility to ageing stress. The superior tolerance of RLC 92 to ageing-induced deterioration suggests its better storability and greater seed longevity, with practical implications for seed storage management and germplasm conservation in linseed.

Keywords: Seed deterioration, accelerated ageing, humidity

Introduction

Linseed or flaxseed (*Linum usitatissimum* L.) is one of the oldest cultivated oilseed crops belonging to the family Linaceae. It is grown worldwide for its seed oil, fibre and occupies a significant position in agriculture, industry and human nutrition (Freeman *et al.*, 1995) [8]. The crop is valued for its high-quality oil rich in alpha-linolenic acid (ALA), an essential omega-3 fatty acid that contributes to various health benefits, including the prevention of cardiovascular diseases, certain cancers and other metabolic disorders (Tripathi *et al.*, 2013) [21]. In addition to oil, linseed seeds contain considerable amounts of protein, dietary fibre, lignans, vitamins and antioxidants making them an important functional food source. The fibre obtained from flax stems is also extensively utilized in the textile and paper industries.

Linseed is cultivated in several countries, including Canada, China, India, Russia and Kazakhstan (Rubilar *et al.*, 2010) [19]. In India, it is a major rabi oilseed crop and contributes significantly to the oilseed economy. Despite its economic importance and diverse industrial applications, linseed productivity remains relatively low compared with its genetic potential. Among the various constraints, deterioration of seed quality during storage is one of the primary factors limiting successful crop establishment and productivity.

Seed ageing is a natural and inevitable process that results in the gradual decline of seed quality and viability. During storage, seeds undergo various physiological and biochemical changes, including membrane disruption, enzyme inactivation, degradation of proteins and nucleic acids and impairment of energy metabolism (Priestley, 1986) [18]. These changes adversely affect seed germination, seedling growth and overall vigour. Accelerated ageing, which involves exposing seeds to high temperature and relative humidity, is widely employed as a reliable method for

evaluating seed storability and predicting seed longevity.

In oilseed crops such as linseed, seed deterioration is often more pronounced due to their high lipid content. During ageing, reactive oxygen species (ROS) accumulate within seed tissues and initiate oxidative damage to cellular components. Polyunsaturated fatty acids present in seed membranes are particularly susceptible to lipid peroxidation, resulting in loss of membrane integrity, leakage of cellular contents and reduced metabolic activity (Berger *et al.*, 2001) [5]. Consequently, aged seeds exhibit poor germination, delayed seedling emergence and reduced vigour.

Although accelerated ageing has been extensively studied in several crop species, systematic information on the comparative response of different linseed varieties to ageing stress remains limited. Identification of varieties with superior tolerance to seed deterioration is essential for improving seed storage management and maintaining seed quality during storage. Therefore, the present investigation was undertaken to evaluate the influence of accelerated ageing on germination and seedling vigour parameters of different linseed varieties and to identify varieties possessing better tolerance to ageing-induced deterioration.

Materials and Methodology

In experiment, four different varieties of linseed *i.e.* Kiran, Deepika, RLC 92 and Kartika were tested against four ageing duration for 0, 2, 4 and 6 days. Experiment was conducted using factorial completely randomized design (FCRD) and repeated for 3 times. Data was analysed using agri analyse software. Treatment means were compared using the Critical Difference (CD) test at 5% significance level. The five-gram seeds of four varieties in three replications were tied in a fine muslin cloth bag. The tied seeds were placed in desiccators on a wire mesh. The lower part of the desiccators was filled with water, so that there was not any direct contact between water and the seed. The desiccators were covered with the lid and sealed with paraffin wax to make it airtight. The desiccators were then placed in the hot air oven maintaining at 45 °C temperature and approximately 95% relative humidity for 0 (control) 2, 4 and 6 days. The desiccators were removed after this period and the seeds were cooled at room temperature. The seeds were then tested for normal germination test.

Observations Recorded

Germination (%)

The standard germination test was conducted on 100 seeds with three replications by using top of paper method at 20-30 °C/20 °C temperature and 95 ± 1% relative humidity, Final count was taken on 7th day as per ISTA (ISTA, 2015) [12].

Germination speed index (GSI)

The Association of Official Seed Analyst's (AOSA) methodology for calculating the germination speed index (GSI) was followed.

$$GSI = \frac{\text{No. of Germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of Germinated seeds}}{\text{Days of final count}}$$

Seedling root length (cm)

Seedling root length was observed on 7th day and 10 normal seedlings were selected randomly and their root length was measured. The root length was measured from tip of primary root to base of the hypocotyls and the mean root length was expressed in centimetre.

Seedling shoot length (cm)

Seedling shoot length was observed on 7th day and 10 normal

seedlings were selected for measurement of shoot length. The shoot length was measured from the base of primary leaf to the base of hypocotyl and the mean shoot length was expressed in centimetre.

Seedling length (cm)

The seedling length was computed by using the following formula.

$$\text{Seedling length (cm)} = \text{Seedling root length (cm)} + \text{Seedling shoot length (cm)}$$

Seedling fresh weight (mg)

To record seedling fresh weight, ten seedlings were counted, cut free from their cotyledon and weighed while still moist.

Seedling dry weight (mg)

Ten normal seedlings selected for measurement of seedling fresh weight was used for the seedling dry weight measurement. These seedlings were kept in hot air oven for 24 hours at the temperature of 80 °C. The weight of the dried seedlings was recorded and expressed in milligrams (mg).

Seedling vigour index-I

The seedling vigour index-I was calculated using the procedure suggested by Abdul-Baki and Anderson (1973) [1] and expressed in whole number.

$$\text{Seedling vigour index-I} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

Seedling vigour index-II

The seedling vigour index-II was calculated using the procedure suggested by Abdul-Baki and Anderson (1973) [1] and expressed in whole number.

$$\text{Vigour index-II} = \text{Germination (\%)} \times \text{Seedling dry weight (g)}$$

Results and Discussion

Germination (%) and Germination speed index (GSI)

The interaction effect between varieties and ageing durations was showed significant influence on germination percent and GSI, indicating differential response of linseed varieties to ageing stress. Among all treatment combinations, the highest seed germination percentage (93.00%) was recorded in varieties RLC 92 and Deepika in control condition (0-day ageing) and it was found statistically at par with variety Kiran in control condition (89%). In contrast, the lowest seed germination percentage (8.00%) was observed in variety Kiran and Deepika at the longest ageing duration of 6 days. Among all treatment combinations, the highest germination speed index (20.69) was recorded in unaged seeds of variety Deepika and RLC 92 (18.53). In contrast, the lowest germination speed index (1.81) was observed in Kiran and Deepika varieties at the longest ageing duration of 6 days. All varieties exhibited a reduction in seed germination percentage and germination speed index with increasing ageing duration. The interaction effect between varieties and ageing durations indicated differential responses of linseed varieties to ageing stress. Variety RLC 92 maintained comparatively higher germination under prolonged ageing conditions, recording 54.00% at 4 days and 16.00% at 6 days ageing duration. Similarly, it sustained comparatively higher germination speed index values under prolonged ageing (10.00 at 4 days and 3.31 at 6 days), demonstrating superior seed vigour, greater tolerance to seed

deterioration and better storage potential. This performance highlights the greater capacity of RLC 92 to maintain rapid and successful germination even under ageing stress conditions. Conversely, Kiran and Kartika varieties displayed a marked decline in both germination percentage and germination speed index with increasing ageing duration, indicating their susceptibility to ageing stress (Fig. A and B).

The reduction in seed germination and seed vigour in aged seeds may be attributed to decreased activities of antioxidant enzymes such as catalase and ascorbate peroxidase with advancement of ageing period. Lower antioxidant enzyme activity leads to accumulation of reactive oxygen species, resulting in oxidative damage to cellular membranes and metabolic impairment. Furthermore, the decline in seed germination percentage and germination speed index in aged seeds could also be due to reduced activity of α -amylase and other hydrolytic enzymes along with depletion of carbohydrate reserves required for seed germination and seedling growth (Bailey, 2004) [4]. Seed ageing generally reduces metabolic efficiency and delays seed germination due to membrane deterioration and oxidative damage, resulting in delayed and non-uniform germination and consequently lower germination speed index. Similar findings were reported by Kapilan and Thiagarajah (2015) [13] in sunflower, Diederichsen and Jones-Flory (2005) [7] in linseed, Hussein *et al.* (2011) [10] in sunflower, Hussein (2012) [11] in sunflower. Who stated that seed ageing adversely affects enzymatic activity and metabolic processes, thereby reducing seed germination and vigour.

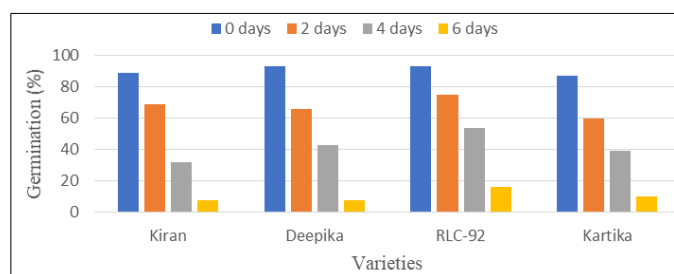


Fig A: Effect of varieties and accelerated ageing duration on germination (%) in Linseed

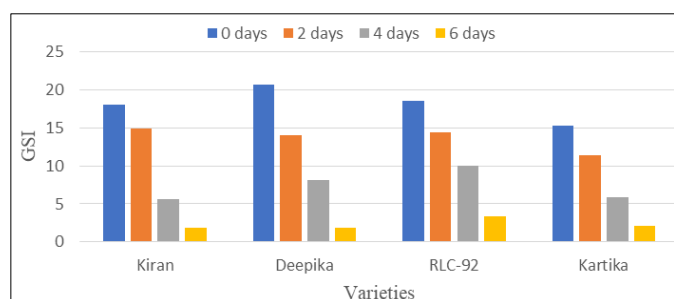


Fig B: Effect of varieties and accelerated ageing duration on germination speed index in linseed

Seedling root length (cm)

Among all treatment combinations, the highest root length (3.60 cm) was recorded in unaged seeds of variety RLC 92, it was found statistically at par with unaged seeds of Deepika (3.55 cm) and Kartika (3.52 cm). In contrast, the lowest root length (0.83 cm) was observed in Deepika and Kartika varieties at the longest ageing duration of 6 days. Root length decreased progressively with increasing ageing duration across all varieties. However, variety RLC 92 maintained comparatively higher root growth under prolonged ageing conditions, demonstrating enhanced

tolerance to seed deterioration. Conversely, Kartika exhibited a pronounced reduction in root length at higher ageing durations, suggesting its susceptibility to ageing stress (Fig. C). The decline in root length might be attributed to DNA degradation with ageing which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of seed germination (Kapoor, 2010) [14]. The results confirmed the report of Hussein *et al.* (2011) [10] and Hussein (2012) [11] in sunflower and Mali *et al.* (2014) [15] in soybean.

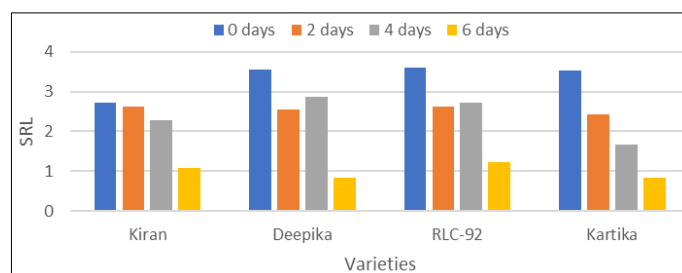


Fig C: Effect of varieties and accelerated ageing duration on seedling root length (cm) in linseed.

Seedling shoot length (cm) and Seedling length (cm)

The interaction effect between varieties and ageing durations was found significant for both seedlings shoot length and seedling length (Fig. D, E); indicating that linseed varieties responded differently to accelerated ageing conditions. Among all treatment combinations, the maximum shoot length was recorded in Kiran (V_1) under D_1 (0 days ageing) with a value of 7.72 cm, followed closely by Deepika (V_2) under D_1 (7.60 cm). Similarly, the highest seedling length was also observed in Kiran (V_1) under D_1 (11.24 cm), closely followed by Deepika (V_2) at D_1 (11.15 cm). The minimum shoot length (1.89 cm) and seedling length (2.72 cm) were both recorded in Kiran (V_1) under D_4 (6 days ageing).

A progressive reduction in both shoot length and seedling length was observed with increasing ageing duration across all varieties. In Kiran (V_1), shoot length decreased from 7.72 cm at D_1 to 3.73 cm, 4.50 cm and 1.89 cm at D_2 , D_3 and D_4 , respectively, while seedling length declined from 11.24 cm at D_1 to 6.92 cm, 5.42 cm and 2.72 cm at D_2 , D_3 and D_4 , respectively. Deepika (V_2) recorded shoot lengths of 7.60 cm, 4.67 cm, 4.37 cm and 2.34 cm under D_1 , D_2 , D_3 and D_4 , respectively and seedling lengths of 11.15 cm, 6.92 cm, 7.54 cm and 3.17 cm under the corresponding durations. Variety RLC-92 (V_3) maintained comparatively better shoot growth under prolonged ageing conditions, recording 5.52 cm at D_2 , 4.40 cm at D_3 and 2.63 cm at D_4 , and seedling lengths of 10.15 cm, 7.02 cm, 8.25 cm and 3.85 cm at D_1 , D_2 , D_3 and D_4 , respectively. Kartika (V_4) exhibited shoot lengths of 6.42 cm, 4.25 cm, 4.62 cm and 2.22 cm under D_1 , D_2 , D_3 and D_4 , respectively, and seedling lengths of 9.13 cm, 7.24 cm, 6.53 cm and 3.31 cm under the corresponding durations.

The relatively higher shoot length and seedling length maintained by RLC-92 under advanced ageing conditions suggests its better tolerance to seed deterioration and superior storability compared with the other varieties. The progressive reduction in both parameters across all varieties with increasing ageing duration may be attributed to ageing-induced DNA degradation, which hampers transcription and results in defective or insufficient synthesis of enzymes required during the early stages of seed germination and seedling growth (Kapoor, 2010) [14]. Similar observations were reported in sunflower by Hussein *et al.* (2011) [10] and Hussein (2012) [11] and in soybean by Mali *et al.* (2014) [15].

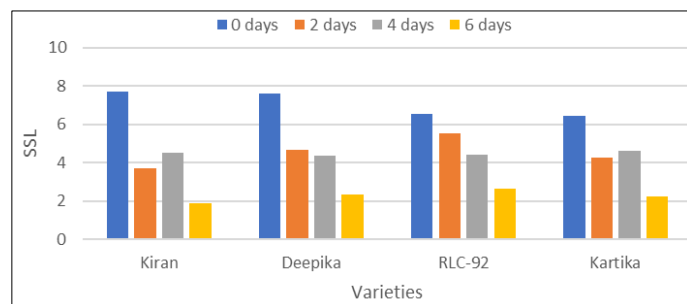


Fig D: Effect of varieties and accelerated ageing duration on seedling shoot length (cm) of linseed

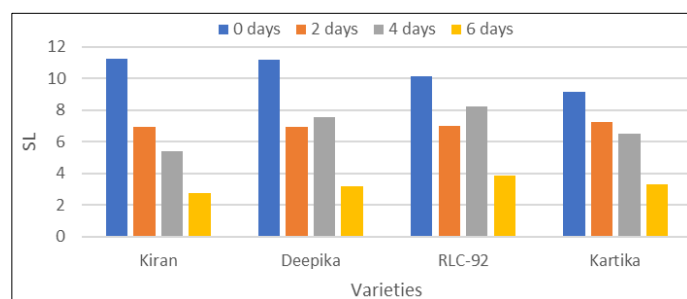


Fig E: Effect of varieties and accelerated ageing duration on seedling length (cm) of linseed

Seedling Fresh Weight, Seedling Dry Weight and Seedling Vigour Indices

The interaction effect between varieties and ageing durations was found significant for seedling fresh weight, seedling dry weight, vigour index-I and vigour index-II (Fig. F, G, H, I); indicating that linseed varieties responded differently to accelerated ageing stress. Among all treatment combinations, the highest seedling fresh weight (486.89 mg), seedling dry weight (46.66 mg), vigour index-I (1037.03) and vigour index-II (4.33) were consistently recorded in RLC-92 (V_3) under D_1 (0 days ageing). Kiran (V_1) under D_1 recorded the next highest values for fresh weight (464.61 mg), dry weight (45.34 mg), vigour index-I (1000.22) and vigour index-II (4.03). Conversely, the lowest values for all four parameters were observed in Kartika (V_4) under D_4 (6 days ageing), recording 108.03 mg fresh weight, 11.04 mg dry weight, 33.11 vigour index-I and 0.11 vigour index-II.

A progressive decline in seedling fresh weight, dry weight and vigour indices was observed with increasing ageing duration across all varieties. In Kiran (V_1), fresh weight decreased from 464.61 mg at D_1 to 163.01 mg, 216.84 mg and 127.90 mg at D_2 , D_3 and D_4 , respectively; dry weight declined from 45.34 mg to 15.95 mg, 20.91 mg and 12.29 mg; vigour index-I declined from 1000.22 to 477.33, 173.38 and 21.77; and vigour index-II declined from 4.03 to 1.10, 0.67 and 0.10 under the corresponding durations. Deepika (V_2) recorded fresh weights of 409.53 mg, 252.75 mg, 217.44 mg and 137.93 mg; dry weights of 39.96 mg, 24.77 mg, 20.97 mg and 13.26 mg; vigour index-I values of 944.08, 463.17, 354.78 and 30.81; and vigour index-II values of 3.71, 1.63, 0.90 and 0.11 under D_1 , D_2 , D_3 and D_4 , respectively. Variety RLC-92 (V_3) maintained comparatively higher values under prolonged ageing conditions, recording fresh weights of 256.75 mg, 217.83 mg and 154.62 mg; dry weights of 25.17 mg, 21.00 mg and 14.88 mg; vigour index-I values of 519.18, 407.03 and 50.73; and vigour index-II values of 1.88, 1.14 and 0.24 at D_2 , D_3 and D_4 , respectively. Kartika (V_4) exhibited fresh weights of 417.42 mg, 198.89 mg, 200.56 mg and 108.03 mg; dry weights of

40.82 mg, 19.43 mg, 19.33 mg and 11.04 mg; vigour index-I values of 794.39, 434.23, 254.84 and 33.11; and vigour index-II values of 3.55, 1.16, 0.75 and 0.11 under D_1 , D_2 , D_3 and D_4 , respectively.

The comparatively higher fresh weight, dry weight and vigour indices maintained by RLC-92 under advanced ageing conditions suggest better tolerance to seed deterioration and superior storability. Conversely, Kiran (V_1) showed a sharp decline at higher ageing durations, particularly at D_4 (fresh weight: 127.90 mg; dry weight: 12.29 mg; vigour index-I: 21.77; vigour index-II: 0.10), suggesting greater susceptibility to ageing stress.

The reduction in seedling fresh and dry weight may be attributed to decreased protein content in aged seeds. Ageing causes degradation of stored proteins and reduction in protein biosynthesis, which adversely affects metabolic activities and enzyme synthesis required for seedling growth. Since proteins serve as important reserve materials during seed germination, their deterioration limits mobilization of nutrients and biomass accumulation in developing seedlings. Similar decline in protein content with seed ageing was reported by Cherry and Skadsen (1986) [6] and Hallam *et al.* (1973) [9], who associated protein deterioration with loss of seed viability.

The progressive reduction in vigour index-I and vigour index-II across all varieties with increasing ageing duration can be attributed to the multiplicative nature of these indices. Vigour index-I is calculated by multiplying seed germination (%) and seedling length (cm), while vigour index-II is derived from seed germination (%) and seedling dry weight (mg). As both germination percentage and seedling growth parameters declined with increasing ageing duration (as shown in Fig. A, E and G), the vigour indices exhibited a corresponding progressive decrease. These results are in close agreement with the findings of Hussein *et al.* (2011) [10] in sunflower, Mali *et al.* (2014) [15] in soybean, Ahamed *et al.* (2014) [2] in chilli and Nigam *et al.* (2019) [16] in tomato.

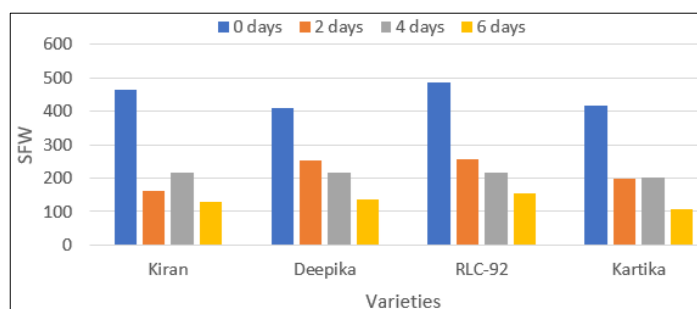


Fig F: Effect of varieties and accelerated ageing duration on seedling fresh weight (mg) of linseed

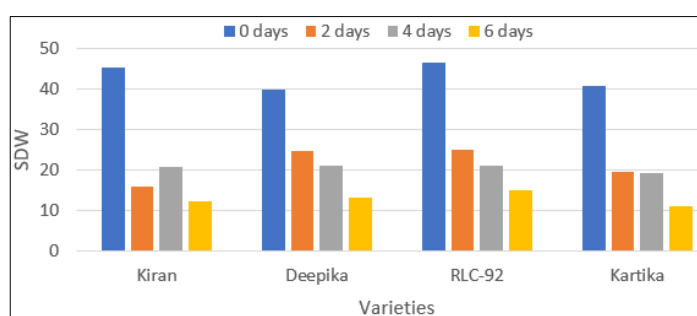


Fig G: Effect of varieties and accelerated ageing duration on seedling dry weight (mg) of linseed

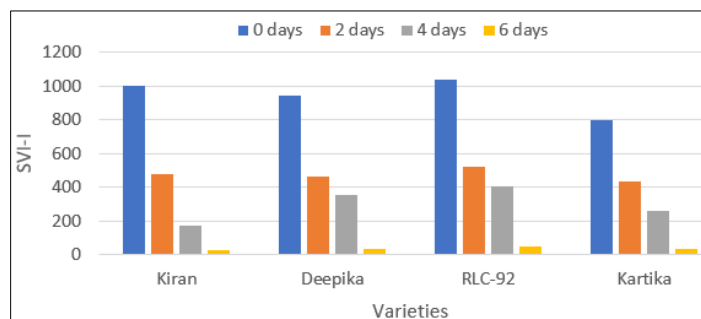


Fig H: Effect of varieties and accelerated ageing duration on seedling vigour index - I in linseed

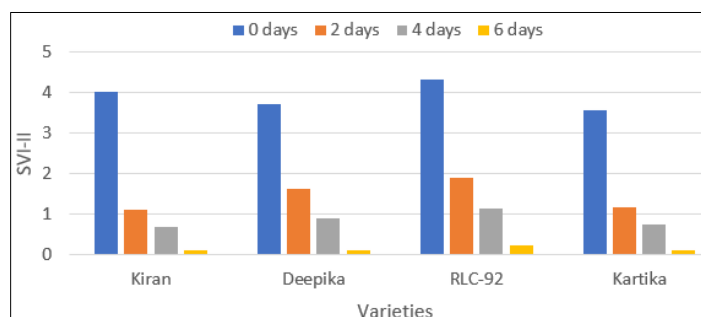


Fig I: Effect of varieties and accelerated ageing duration on seedling vigour index - II in linseed

Conclusion

Accelerated ageing significantly affected seed quality and vigour parameters in all four linseed varieties, causing a progressive decline in germination percentage, germination speed index, seedling growth, seedling biomass, and vigour indices with increasing ageing duration from 0 to 6 days. The adverse effects of ageing became more pronounced at higher ageing durations, particularly after 4 days of exposure. Among the tested varieties, RLC-92 exhibited the highest tolerance to ageing stress by maintaining comparatively greater germination (54.00%), Vigour Index-I (407.03), Vigour Index-II (1.14), seedling growth, and biomass accumulation under prolonged ageing conditions, indicating superior seed longevity and storability. In contrast, Kiran and Kartika showed a rapid deterioration in seed quality traits with increasing ageing duration, reflecting their greater susceptibility to seed ageing. The superior performance of RLC-92 suggests its potential suitability for long-term seed storage and conservation programmes.

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