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Effect of Artificial Ageing on Relative Storability of Primed Seed Lots of Fenugreek (*Trigonella foenum-graecum*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OSD guided all the authors during whole period of study and article writing author PS managed the analyses of the study. Authors SK and VSM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The primed seeds lot of two varieties of fenugreek HM-57 and HM-103 having two lots each (L_1 was having standard germination above 70% and L_2 was having standard germination below 70%) were stored under ambient condition and the observations was recorded during 2013-14 to assess the storability of primed seed lots of before artificial ageing (40 ±1°C for 72 h) and after artificial agein g of primed seed lots. It was observed that standard germination (%), root length (cm), shoot length (cm), seedling dry weight (mg) and vigour indices significantly decreased whereas, electrical conductivity (µS/cm/seed) of seed leachates increased with artificial ageing. Maximum germination was retained by HM-57 with, hydration (6 h) and dehydration at room temperature followed by dry dressing with thiram @ 0.25% treatment in good quality seed lot L_1 (standard germination above 70%) after artificial ageing.

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1. INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the sub-family papilionaceae of Leguminosae is an important multiuse seed spice crop cultivated in India, Argentina, Egypt, Morocco, Southern France, Spain, Turkey, China, Algeria and Ethiopia. India has a unique place in world which produces 90 metric tonnes of fenugreek seeds from 66 thousand ha area. (Anonymous, 2013-2014 [1]). Seed is an important component and availability of viable and vigorous seed at the planting time is important for achieving increase agricultural productivity because good quality seed acts as a catalyst for realizing the potential of other inputs. AA test and the conductivity test are the only approved vigor tests used to determine seed vigor (ISTA, 2005. Main objective of the seed storage is to preserve the seed quality and to prolong the self life under different sets of storage conditions. Use of quality seeds increased productivity of crop by 15-20% Sidhawani, [2].

The unfavorable environment (especially exposure to rains just for one week) during the post maturation, pre-harvest environment can greatly accelerate the seed ageing phenomenon, resulting in 20 to 30% loss of seed viability. The seed of particular harvest cannot be used immediately for sowing the following crop because of the time required for processing and for seed certification prior to the marketing of seeds. Therefore, the fresh seeds have to be stored for 6-8 months after harvest and under uncontrolled storage conditions. Accelerated ageing techniques have great potential for understanding the mechanism of ageing and associated deterioration processes of seeds The sensitivity of seeds to accelerated ageing is dependent on temperature and on their moisture content. At a constant temperature, loss of seed viability is faster with increasing moisture content, seed moisture and storing temperature plays a key role in seed longevity McDonald, [3]. Meanwhile, the process of deterioration under accelerated ageing conditions are essentially similar to those under normal conditions where we can predict the rate of deterioration of the seeds through the a.a test so that the relative storability of the seeds can be estimated Goel et al. [4]. At the cellular level, seed ageing is associated with various alterations including loss of membrane integrity, reduced energy metabolism, impairment of RNA and protein synthesis, and DNA degradation Kibinza et al. [5]. During storage, a number of physiological and physicochemical changes occur, termed ageing (Sisman, [6]). The rate at which the seed ageing process takes place depends on the ability of seed to resist degradation changes and protection mechanisms, which are specific for each plant species (Mohammadi,et al. [7]). In seed ageing damage at cellular membranes, decrease in mitochondrial dehydrogenises activities, chromosomal aberrations and DNA degradation increases.

To study the physiological and biochemical changes in seeds during ageing, accelerated ageing has been widely used. In accelerated ageing, the seeds are selfaged by subjecting them to high relative humidity (>90%) and temperatures of (≥40ºC). The seeds, so aged, are compared for morphological, physiological, biochemical and genetic changes with controls. The present study has been set up to investigate the physiological and biochemical aspects of seed deterioration in cottonseeds during accelerated ageing (Sveinsdottir et al. [8]).

2. MATERIALS AND METHODS

The present study was conducted during 2013-14 at the laboratories and research work at the department of Seed Science & Technology CCS Haryana Agricultural University, Hisar. Seed material comprised of two varieties viz. HM-57, HM-103 of fenugreek crop was procured from the Department of Vegetable Science, CCS Haryana Agricultural University. Two seed lots of each variety include – L_1= Standard germination above Indian minimum seed certification standards (>70%), L_2 = Standard germination below Indian minimum seed certification standards (<70%) and each seed lot of both varieties was invigorated with following priming treatments prior to lab and field study.

- T_0 (Control)
- T_1 Hydration (6 h) and dehydration at room temperature
- T_2 2% $CaCl_2$ (6 h) at room temperature and surface drying at room temperature
- T_3 Hydration with 50 ppm GA_3 (6 h) and surface drying at room temperature
- T_4 As in T_1 followed by dry dressing with thiram @ 0.25% (6 h) and surface drying at room temperature

T_5 0.5% KNO_3 hydration (6h.) and dehydration at room temperature

After each treatment seeds were dried back to original moisture content under shade and observations on following parameters were recorded. The primed seed lots were stored under ambient condition and following observation were recorded before artificial ageing and after artificial ageing.

2.1 Laboratory Parameters

2.1.1 Standard germination (%)

One hundred seeds of each lot in three replicates were placed in between sufficient moistened rolled towel papers (BP) and kept at 20℃ in seed germinator. The first count was taken on 5^{th} day and final count on 14^{th} day and only normal seedlings were considered for percent germination according to the rules of International Seed Testing Association (ISTA, [9])

2.1.2 Root length (cm)

Five normal seedlings were selected randomly at the time of final count of standard germination and average length of five root lengths was measured in centimeters.

2.1.3 Shoot length (cm)

Five normal seedlings were selected randomly at the time of final count of standard germination and average length of five shoot length was measured in centimeters.

2.1.4 Seedling dry weight (mg)

Seedling dry weight was assessed after the final count in the standard germination test (14 days). Ten seedlings of each lot replicated thrice were taken. Seedlings were dried in a hot air oven for 24 hrs at 80±1℃. The dried seedlings of each replication were weighed and average seedling dry weight of each variety was calculated.

2.1.5 Seedling vigour indices

Seedling vigour indices were calculated according to the method suggested by Baki and Anderson 1973 [10].

I. Vigour index-I(on seedling length basis):
Vigour index–I = Standard Germination (%) × seedling length (cm)

II. Vigour Index–II (on seedling dry weight basis):
Vigour index–II =Standard Germination (%) × seedling dry weight (mg)

2.2 Accelerated Ageing

To observe the optimum time for accelerated ageing test (i.e. period of reduction of initial germination up to 50 %). Seeds of two varieties were placed in a single layer on the wire mesh trays fitted in the plastic boxes. Each plastic box had 40 ml of distilled water. The boxes were placed in the ageing chamber after closing of lids. The seeds were aged at 40 ±1ºC temperature and 100% relative humidity for 72 hours followed by standard germination in three replications of 100 seeds each.

2.3 Electrical Conductivity (µS/cm/seed)

To measure the electrical conductivity, 50 normal and uninjured seeds in three replications were soaked in 75 ml deionized water in 100 ml beakers. Seeds were immersed completely in water and beakers were covered with foil. Thereafter, these samples were kept at 25℃ for 24 h. The electrical conductivity of the seed leachates was measured using a direct reading conductivity meter and expressed in µS/cm/seed.

2.4 Statistical Analysis

Statistical analysis of data collected during the study was done by applying the technique of analysis of variance (ANOVA) as suggested by Gomez 1984 [11] and Panse and Sukhatme 1961 [12]. All the statistical analysis was carried out by using OPSTAT statistical software.

3. RESULTS AND DISCUSSION

3.1 Standard Germination

The values of standard germination ranged from 51.67 to 76.00 (Table 1). Reduction in germination was observed in both the varieties and lots. Minimum reduction (12%) in germination (from 68.60 to 60.36) was recorded in lot L_2 of HM-103 with treatment T_4 in Fig. 1. The results revealed that standard germination percentage decreased significantly as period of Artificial ageing in both of fenugreek showed a considerable variability in response to viability. The germination of both varieties decreased after accelerated ageing at 40±1℃ for 72 hr. Maximum germination (74.73%) was recorded in L_1 of HM-57 show in Fig. 1. Seed germination, seedling emergence and crop establishment are important aspects of canola production, and are the main components of seed/seedling vigor (Devaiah et al. [13]). Aging is manifested as reduction in germination percentage and those seeds that do germinate produce weak seedling (Veselova and Veselovsky, [14]). Oil seeds are deteriorating more rapidly during storage, which reduces the quality of seeds (Afzal et al. [15]). Woltz and Tekrony (2000 [16]) stated that the accelerated aging (AA) test consistently better than standard germination tests for predicting seed vigor. Under AA conditions oil seed absorbed more water than other seeds (Kausar et al. [17]). Hydropriming of brassica aged seed increased total protein content, whereas total starch and total sugar content decreased (Bedi et al. 2005 [18]).On the basis of treatments dry dressing with thiram @ 0.25% and 0.5% KNO_3 hydration (6h) and dehydration at room temperature were found effective for enhancing the relative storability of good quality seed lot of both the varieties as the standard germination after artificial ageing remained above 50 per cent. Overall, the relative storability was much improved by hydration (6h) and dehydration at room temperature followed by dry dressing with thiram @ 0.25%) followed by hydration (6h) and dehydration at room temperature and 0.5% KNO_3 hydration (6h) and dehydration at room temperature.

3.2 Root Length (cm)

The overall mean values of root length ranged from 6.27 to 9.66 (Table 1). Reduction in root length was observed in both the varieties and lots. Minimum reduction (4%) in root length (from 9.98 to 9.48) was recorded in lot L1 of HM-57 with treatment T4 in Fig. 2. The decrease in radical length, germination speed, germination and seed germination and increased time to complete 50% by accelerated aging may be a result of progressive loss of seed viability and vigor, which was evident in the results of this study by Jain et al. 2006 [19].

Table 1. Mean value of different laboratory parameters of fenugreek before artificial ageing

Variety	Treatments	S.G.%	RL (cm)	SL (cm)	DW (mg)	VI-I	VI-II	EC
HM-57 (L_1)	**T_0**	73.00(58.67)	8.60	15.54	7.15	1761.73	521.88	274.80
	T_1	75.00(59.99)	8.86	15.91	7.36	1857.96	551.88	254.73
	T_2	71.33(57.61)	8.63	15.79	7.61	1742.17	542.68	265.35
	T_3	74.00(59.32)	8.98	15.72	7.31	1827.40	541.17	267.23
	T_4	76.00(60.65)	9.66	16.05	7.91	1953.92	601.25	241.29
	T_5	70.00(56.77)	8.95	15.84	7.64	1735.07	534.65	253.39
HM-103(L_1)	**T_0**	72.67(58.48)	8.50	14.62	7.39	1679.62	537.12	288.99
	T_1	74.33(59.55)	8.85	14.86	7.76	1762.16	576.42	283.24
	T_2	70.33(56.99)	8.73	14.78	7.46	1653.98	523.92	278.36
	T_3	67.33(55.15)	8.84	14.86	7.30	1596.27	491.86	279.81
	T_4	76.00(60.65)	8.99	15.23	7.96	1840.70	605.06	272.26
	T_5	70.00(56.79)	8.57	15.08	7.54	1655.36	527.47	271.79
Mean value of different laboratory parameters of fenugreek after artificial ageing								
HM-57 (L_2)	**T_0**	55.33(48.04)	6.27	12.74	6.34	1051.91	350.68	390.15
	T_1	62.00(51.92)	6.77	12.92	6.56	1220.82	406.55	379.32
	T_2	58.00(49.59)	6.64	12.78	6.44	1126.58	373.19	370.44
	T_3	56.00(48.43)	6.63	12.75	6.45	1085.22	361.54	374.77
	T_4	63.33(52.72)	6.89	13.18	6.68	1270.74	423.44	361.86
	T_5	53.67(47.09)	6.43	12.92	6.52	1038.57	350.27	376.11
HM-103 (L_2)	**T_0**	55.67(48.24)	6.98	13.21	6.36	1124.24	354.05	379.52
	T_1	60.33(50.95)	7.22	13.55	6.60	1253.03	398.54	375.54
	T_2	59.67(50.56)	7.10	13.55	6.55	1231.95	391.41	372.68
	T_3	51.67(45.94)	7.27	13.56	6.71	1076.05	346.54	377.54
	T_4	62.00(51.93)	7.55	13.86	7.01	1327.48	434.34	363.05
	T_5	55.00 (47.85)	7.35	13.63	6.62	1153.79	364.52	370.60
CD(P=0.05) AXBXC		**N.S**	**0.18**	**0.29**	**0.14**	**0.10**	**N.S.**	**0.05**

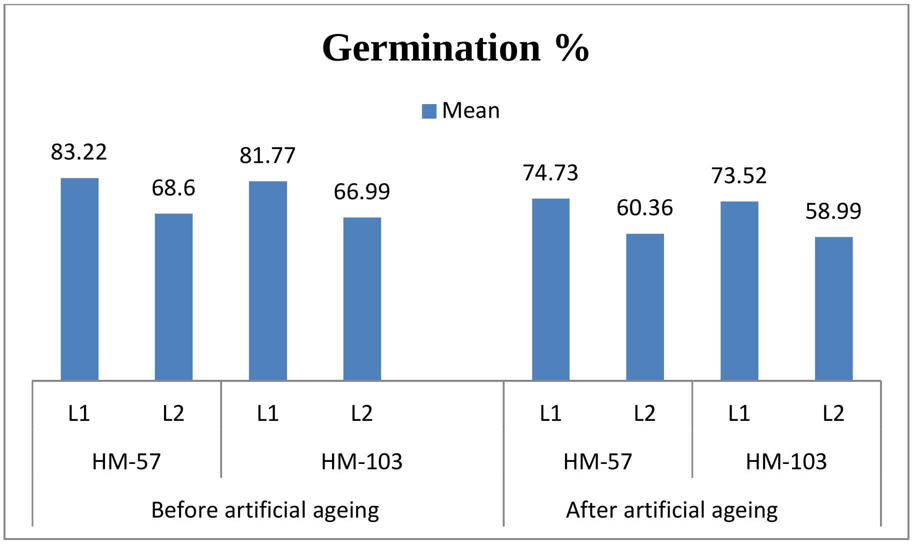

Fig. 1. Effect of artificial ageing on standard germination

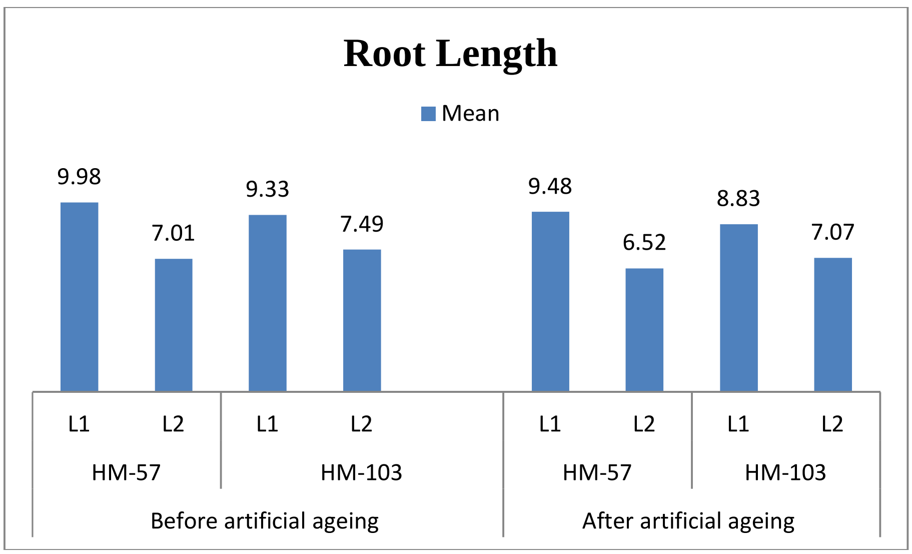

Fig. 2. Effect of artificial ageing on root length (cm)

3.3 Shoot Length (cm)

The overall mean values of shoot length ranged from 12.74 to 16.05 (Table 1). Reduction in shoot length was observed in both the varieties and lots. Minimum reduction (4.49%) in shoot length (from 10.68 to 10.20) was recorded in lot L_2 of HM-103 with treatment T_4 (Fig. 3).

3.4 Seedling Dry Weight (mg)

The overall mean values of seedling dry weight ranged from 6.34 to 7.91 (Table 1). Reduction in seedling dry weight was observed in both the varieties and lots. Minimum reduction (1.00%) in seedling dry weight (from 6.98 to 6.91) was recorded in lot L_2 of HM-103 with treatment T_4 (Fig. 4). The seed deterioration had led to reduced seedling growth as a consequence of both lower respiration and reduced mitochondria in cells (McDonald, 1999 [3]) and accelerated aging test is characterized by the loss of germination and poor seedling development (Mosavi et al. [20]).

3.5 Vigour Index-I

The overall mean values of vigour index-I ranged from 1051.9 to 1953.9 (Table 1). Reduction in vigour index-I was observed in both the varieties and lots. Minimum reduction (16.33%) in vigour index-I (from 1218.25 to 1019) was recorded in lot L_1 of HM-57 with treatment T_4 (Fig. 5). Yari et al. [21] also reported that maximum SVI obtained from wheat seed primed with KH_2PO_4 0.5%.

3.6 Vigour Index-II

The overall mean values of vigour index-II ranged from 605.06 to 346.54 (Table 1). Reduction in vigour index-II was observed in both the varieties and lots. Minimum reduction (14.15%) in vigour index-II (445.97 to 382.93) was recorded in lot L_2 of HM-103 with treatment T_4 (Fig. 6). Accelerated ageing of seed, i.e. exposure of seeds to high temperature and high relative humidity leads to the loss of vigour and eventually viability, and it is an excellent method to determine the vigour changes during seed storage (Tian et al. [22]).

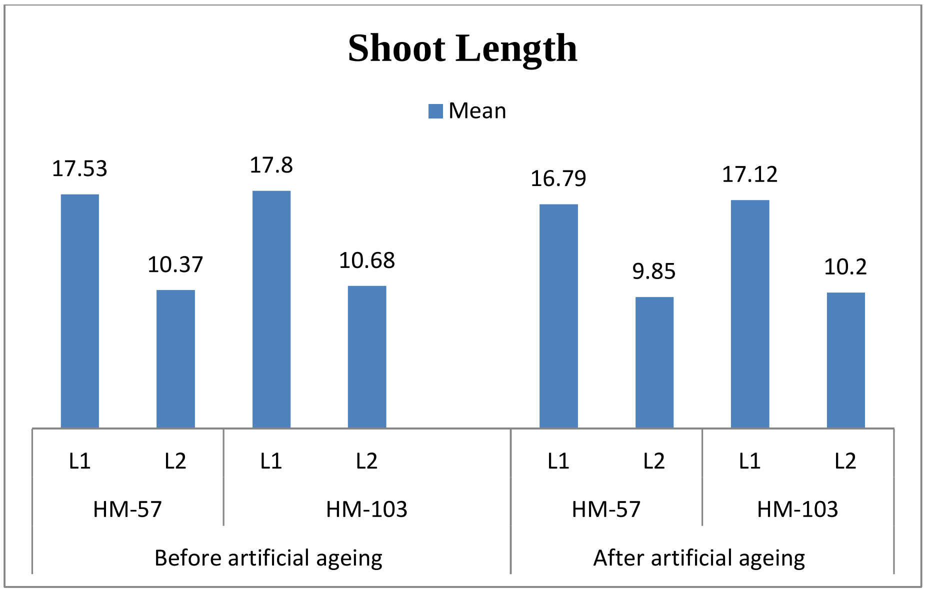

Fig. 3. Effect of artificial ageing on shoot length (cm)

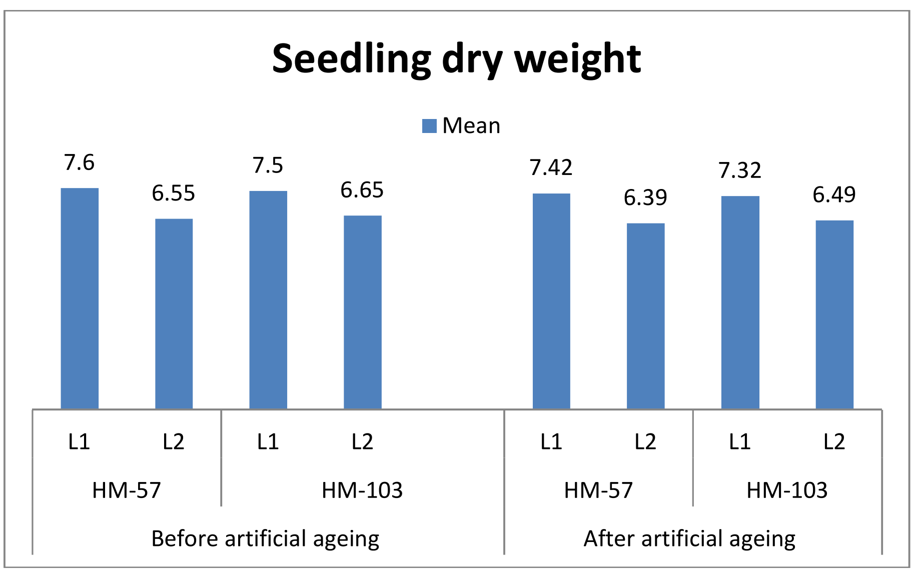

Fig. 4. Effect of artificial ageing on seedling dry weight (mg)

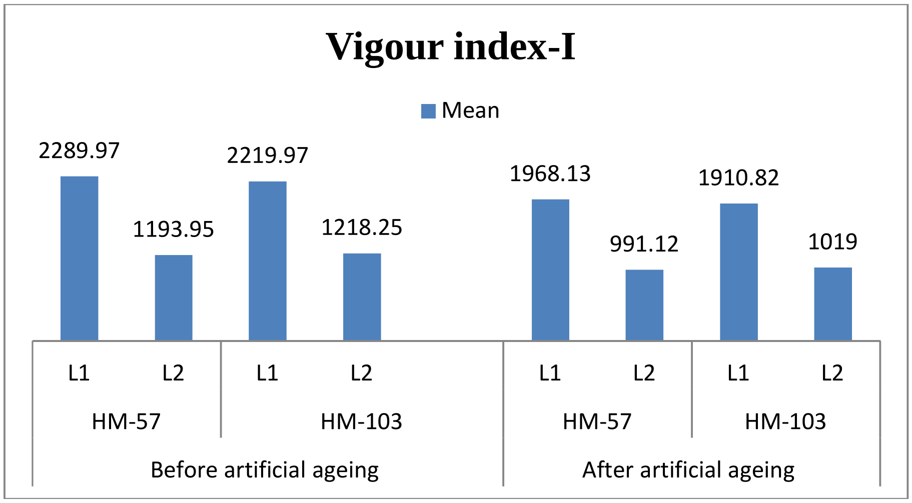

Fig. 5. Effect of artificial ageing on Vigour index-I

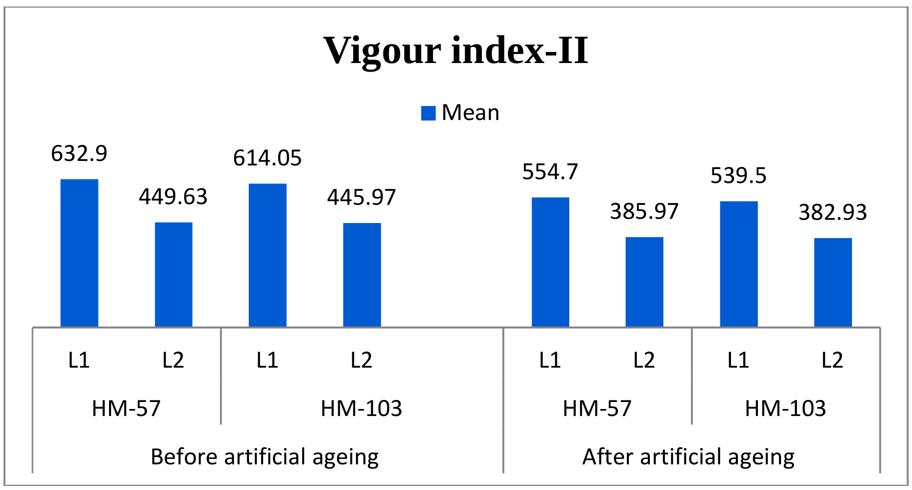

Fig. 6. Effect of artificial ageing on vigour index-II

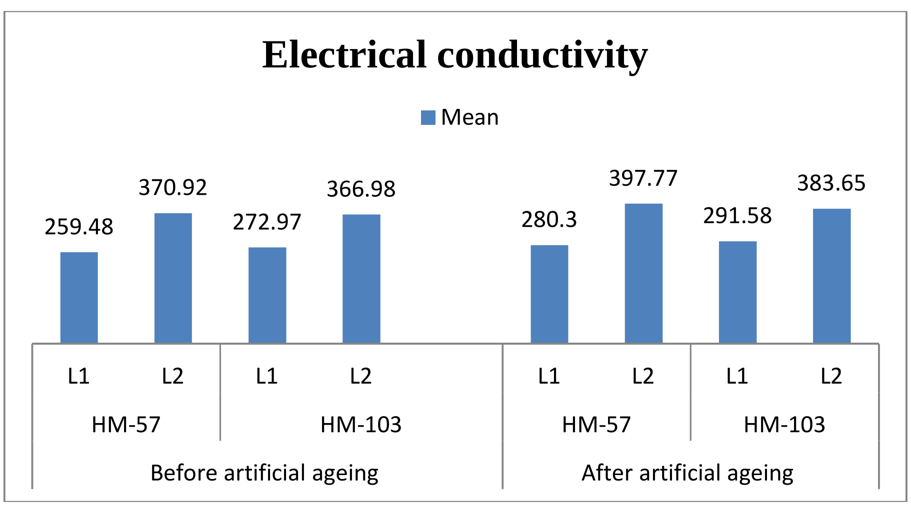

Fig. 7. Effect of artificial ageing on electrical conductivity (µS/cm/seed)

3.7 Electrical Conductivity (µS/cm/seed)

The overall mean values of electrical conductivity ranged from241.6 to 390.15 (Table 1). Reduction in electrical conductivity was observed in both the varieties and lots. Minimum increase (4.64%) in electrical conductivity (from 366.98 to 383.65.) was recorded in lot L_2 of HM-103 with treatment T_4 in Fig. 7. These findings support the earlier work on improve EC by hydropriming and solid matrix priming after 12 and 24 hours in rapeseed by Bijanzadeh et al. [23].

4. CONCLUSION

On the basis of present investigation it is concluded that artificial ageing had significant reduction in seed quality parameters i.e. standard germination, root length, shoot length, seedling dry weight, and vigour indices significantly decreased whereas, electrical conductivity of seed leachates increased with artificial ageing. Maximum germination was observed in HM-57 with hydration (6 h) and dehydration at room temperature followed by dry dressing with thiram @ 0.25% treatment in good quality seed lot (L_1) after artificial ageing. HM-57 seeds had more viability and more germination as compared to HM-103, So HM-57 was found best for seed quality parameters after artificial ageing. Thiram treatment was found best in all the parameters before and after artificial ageing test during storability. It is also concluded that primed seed lots (L_1) showed more reduction in both the varieties (HM-57 and HM-103) in all the parameters after artificial ageing as compared to other seed lot (L_2).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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