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Evalution of biochemical changes associated with seed quality under naturally stored conditions in fennel

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Abstract
An experiment was conducted on fifteen varieties/genotypes of fennel having three seed lots *viz*., freshly harvested seed (Lot-1), one year old seed (Lot-2) and two year old seed (Lot-3). All the seed lots were analyzed for different seed quality parameters like Tetrazolium test (viability %), pH exudate test (%), Dehydrogenase activity test (OD g^{-1} ml^{-1}) and Electrical conductivity test (µS cm^{-1} $seed^{-1}$) to study the effect of natural storage on seed deterioration. Results revealed that value of all the seed quality parameters decreases significantly as the age of the seed increases except electrical conductivity where value increases significantly as the age of the seed increases due to loss of membrane integrity. Among all the varieties/genotypes, maximum viable seed was found in genotype HF 33 (82.91%) followed by HF 102 (81.33%) and maximum loss of viability was observed in genotype HF 115 (70.25%).

Keywords: Ageing, ambient storage, electrical conductivity, fennel, seed lots, seed quality

Introduction
Fennel *(Foeniculum vulgare* Mill.) is an important seed spice belongs to family Apiaceae and widely grown in the states like Gujarat, Rajasthan and Uttar Pradesh in India. Fennel is used in various traditional systems of medicine like in the Ayurveda, Unani, Siddha, in the Indian, and Iranian traditional systems of alternative and balancing medicine (Rahimi and Ardekani, 2013) [14]. The seeds contain about 9.5% protein, 10.0% fat, 42.3% carbohydrates, 18.5% fibre and 13.4% minerals (Bhunia *et al*., 2005) [2].

Agriculture and civilization have progressed simultaneously along with seed husbandry as well as the history of the development of new crops and their varieties. Seed has been an important entity since the crops were domesticated first. Quality seed is the basic unit for releasing higher yield per unit area. Ageing of seed starts right from physiological maturity. It is one of the most intriguing and challenging scientific problem of universal concern (Moment, 1978) [10]. Rapid deterioration of stored seed is a serious problem in the tropical and subtropical countries like India where high temperature and high relative humidity greatly accelerate the phenomenon of seed ageing.

Fennel being one of the most important spice crop at national and international level, information on the physiological or biochemical basis of seed viability is fragmentary and incomplete, which still has contradictions, errors and difference of opinion but very little research work has been undertaken to study the quality parameters of fennel seeds stored under ambient conditions. Therefore, the present study was aimed to study the seed quality deterioration of different varieties of fennel stored under ambient conditions.

Material and Methods
The present investigation was carried out on three seed lots of fifteen fennel genotypes *viz*., HF 33, HF 101, HF 102, HF 103, HF 104, HF 105, HF 106, HF 107, HF 108, HF 109, HF 114, HF 115, HF 118, HF 122 and HF 124 stored for different periods under ambient condition in the Department of Vegetable Science, CCS HAU, Hisar during 2014-15.

Storage period of seed
L1 : Freshly harvested seed
L2 : One year old seed
L3 : Two year old seed

All the seed lots stored under ambient condition and subjected to Tetrazolium test (viability %), pH exudate test (%), Dehydogenase activity test (OD g^{-1} ml^{-1}) and Electrical conductivity test (µS cm^{-1} $seed^{-1}$) in seed testing laboratory, Department of Seed Science and Technology, CCS Haryana Agricultural University.

Tetrazolium test (%)

In tetrazolium viability test (Moore, 1973) [11], 50 seeds of each seed lot replicated four times were soaked for 24 h in distilled water at room temperature. The pericarp of each seed was removed with the help of needle and forceps. Seeds were stained in 0.7 per cent tetrazolium chloride solution at 30ºC for four hours and the evaluation was done based on staining of seeds. After that, the solution was poured off and seeds were rinsed briefly in water and examined under magnifications. The number of seeds stained entirely red was considered as viable seeds and expressed in percentage.

pH exudates test (%)

Four replications of 100 seeds of each seed lot were placed in a hundred cells of plastic trays, 100 seeds per tray and one seed per cell and 2ml of distilled water was added to each cell. The seeds were then allowed to imbibe for 30 minutes at 25ºC. At the end of imbibition period 25µl of phenolphthalein solution and 50µl of Na_2CO_3 solution were added to soaking water in each cell. The colour change was noticed and rosy colour indicated viable seeds whereas no colour change indicated the seeds to be dead and expressed as percentage.

Electrical conductivity test (µS cm-1 seed-1)

To measure the electrical conductivity, 50 normal and uninjured seeds in four replications were soaked in a beaker contained 50 ml of distilled water for 24 h at 25ºC temperature. The electrical conductivity of seed leachates was measured by conductivity bridge meter and the conductivity was expressed in µ S cm-1 seed-1.

Dehydrogenase activity test (OD g-1 ml-1)

Sample of 2 g of each genotype was grounded to pass through a 20-mesh screen to obtain 200 mg flour. The flour was soaked in 5 ml of 0.5 % tetrazolium solution at 35ºC and was centrifuged after 2 h at 10,000 rpm for 3 minutes and supernatant was poured off. Formazan was extracted with 10 ml acetone for 16 h followed by centrifugation at room temperature and then absorbance reading of the solution was taken in spectrophotometer at 520 nm.

Result and Discussion

The change in the seed viability under ambient storage conditions is a function of a complex interaction of genetic constitution and environmental conditions. Naturally aged seeds resulted in the loss of germinability and viability in all the varieties/genotypes of fennel. The data on effect of ambient storage on seed viability based on tetrazolium chloride test is presented in Table 1. In freshly harvested seed lot, the maximum viability was found in HF 33 (96.25 %) followed by HF 102 (94.00 %) which were statistically at par and minimum viability was found in HF 115 (82.75%). Thereafter viability decreased gradually with the advancement of storage period among all the genotypes. Among different seed lots, the viability based on tetrazolium test was found maximum (63.75%) in HF 33, HF 102 and HF 107 whereas minimum (54.00%) was observed in HF 115 for two year storage under ambient condition. The maximum mean value (82.91%) was observed in HF 33 followed by HF 102 (81.33%) and minimum in HF 115 (70.25%). The present results are also in corroborate with the findings of Kumar *et al.,* 2015 [7] where loss of seed viability and vigour increased with increase in period of storage in coriander. Above results are substantiate with the findings of various workers in different crops such as okra (Narwal, 1995) [12], fenugreek (Kumari *et al*., 2014) [8], carrot (Maskri *et al*., 2003) [9] and turnip (Khan *et al*., 2005) [4] under natural ageing.

Table 1: Effect of natural ageing on viability (%) of fennel

Genotypes	Seed lots			Mean
	L1	L2	L3	
HF 33	96.25 (79.32)	88.75 (70.59)	63.75 (52.97)	82.91 (67.62)
HF 101	91.75 (73.52)	84.25 (66.71)	61.75 (51.79)	79.25 (64.01)
HF 102	94.00 (76.04)	86.25 (68.29)	63.75 (52.98)	81.33 (65.77)
HF 103	91.75 (73.42)	81.25 (64.35)	62.00 (51.93)	78.33 (63.23)
HF 104	90.25 (71.90)	78.75 (62.56)	59.50 (50.45)	76.16 (61.64)
HF 105	86.25 (68.25)	77.75 (61.86)	62.00 (51.93)	75.33 (60.68)
HF 106	86.00 (68.16)	83.25 (65.82)	60.75 (51.19)	76.66 (61.72)
HF 107	90.75 (72.46)	82.75 (65.45)	63.75 (52.97)	79.08 (63.63)
HF 108	91.75 (73.42)	82.00 (64.91)	59.00 (50.17)	77.58 (62.83)
HF 109	92.25 (74.04)	81.50 (64.56)	62.00 (51.93)	78.58 (63.51)
HF 114	88.75 (70.51)	81.00 (64.20)	61.75 (51.78)	77.16 (62.17)
HF 115	82.75 (65.50)	74.00 (59.36)	54.00 (47.27)	70.25 (57.38)
HF 118	86.50 (68.51)	82.00 (64.91)	57.75 (49.44)	75.41 (60.95)
HF 122	91.00 (72.57)	82.75 (65.49)	62.00 (51.93)	78.58 (63.33)
HF 124	84.25 (66.61)	75.75 (60.49)	55.00 (47.85)	71.66 (58.32)
Mean	89.61 (71.62)	81.46 (64.64)	60.58 (51.10)	

C.D. (p=0.05) for genotypes=1.906, lots =0.852, Genotypes x lots=3.302

Figures in parenthesis are arcsine transformed values

The pH exudates test is a calorimetric method that predicts the germinability of individual seed based on colour change in the seed exudates from colourless to rosy colour. Data presented in the Table 2 shows that the genotype HF 33 recorded highest mean value (81.33%) followed by HF 102 (79.16%) and pH was lowest in HF 115 (65.00%). However, in two year old seed lot, highest pH of exudates recorded in HF 33 (63.75%) followed by HF 102 (62.25%), which was statistically at par. The results further indicated that pH

exudates declined significantly in all the genotypes with the passage of storage time, which was 78.25% to 94.75% in freshly harvested seed lot, 71.75% to 85.50% in one year seed lot, 41.75%to 70.00% in two year seed lot. The present study is confirmed by the Kumari *et al*. (2014) [8] in fenugreek and by Kumar *et al*. (2017) [6] in coriander where viability of seed decreased with ageing of seed.

Table 2: Effect of natural ageing on pH exudate of fennel

Genotypes	Seed lots			Mean
	L1	L2	L3	
HF 33	94.75 (77.77)	85.50 (67.76)	63.75 (52.97)	81.33 (66.17)
HF 101	90.75 (72.32)	82.25 (65.29)	58.25 (49.75)	77.08 (62.45)
HF 102	92.50 (74.30)	82.75 (65.47)	62.25 (52.08)	79.16 (63.95)
HF 103	90.25 (71.86)	79.75 (63.25)	58.25 (49.77)	76.08 (61.62)
HF 104	81.25 (64.41)	74.75 (59.84)	58.75 (50.05)	71.58 (58.10)
HF 105	86.25 (68.36)	76.25 (60.91)	55.25 (48.01)	72.58 (59.10)
HF 106	87.50 (69.72)	80.25 (63.68)	61.75 (51.79)	76.50 (61.73)
HF 107	81.75 (64.82)	75.25 (60.18)	59.50 (50.50)	72.16 (58.50)
HF 108	85.50 (67.70)	75.50 (60.36)	53.50 (47.00)	71.50 (58.35)
HF 109	84.75 (67.15)	76.50 (61.10)	57.75 (49.45)	73.00 (59.23)
HF 114	85.75 (67.87)	71.75 (57.90)	60.50 (51.06)	72.66 (58.94)
HF 115	78.25 (62.36)	75.00 (60.02)	41.75 (40.20)	65.00 (54.19)
HF 118	86.25 (68.48)	77.75 (61.97)	70.00 (56.81)	78.00 (62.42)
HF 122	88.25 (70.32)	75.50 (60.35)	58.25 (49.78)	74.00 (60.15)
HF 124	79.25 (62.95)	72.75 (58.57)	48.50 (44.11)	66.83 (55.21)
Mean	86.20 (68.69)	77.43 (61.78)	57.86 (49.56)	

C.D. (p = 0.05) f or genotypes =2.72, lots =1.22, Genotypes x lots = 4.71

Figures in parenthesis are arcsine values

Leaching of electrolytes, nitrogen, and amino acids due to lose membrane integrity of aged seed causes increase in electrical conductivity of seed which cause less vigour and viability of seed. The seed leachates increased with increased in storage period of sunflower (Pallavi *et al.,* 2003) [13]. Sadik (2012) [15] also observed increase in leaching of electrolytes for ajwain seed with increased period of storage. All the varieties/genotypes lost their membrane integrity significantly and gradually with advancement of storage period (Figure 1). The range of electrical conductivity of seeds varied from 0.12 (HF 102) to 0.20 (HF 115, HF 124) in freshly harvested seed lot whereas it ranged from 0.37 (HF 102) to 0.56 (HF 115) in one year old seed lot. The data on electrical conductivity of leachates indicated that the highest membrane integrity was present in the HF 102 (0.12) in freshly harvested seed lot. In comparison with other seed quality parameters, altogether a reverse trend was observed for electrical conductivity test. The genotype HF 33, HF 102 showed minimum mean value of electrical conductivity (0.40) and thus they were rated as good storer. Electrical conductivity test have been used by many researchers to indicate seed vigour. The electrical conductivity is related to the deterioration processes of seeds as degradation of cell membranes and leackage out of the cell. In aged seeds or partially deteriorated seed, the electrical conductivity will be higher, owing to decrease in membrane integrity on account of detrimental changes in biomembranes occurring in stored seeds Higher leachates which leach out with increase in storage period due to loss of membrane integrity and due to peroxidation of unsaturated fatty acids. The results obtained by Kaewnaree *et al.* (2011) [3] in sweet pepper, Singh *et al.* (2015) [16] in fenugreek and by Basra *et al*. (2003) [1] in cotton double check the results of present finding.

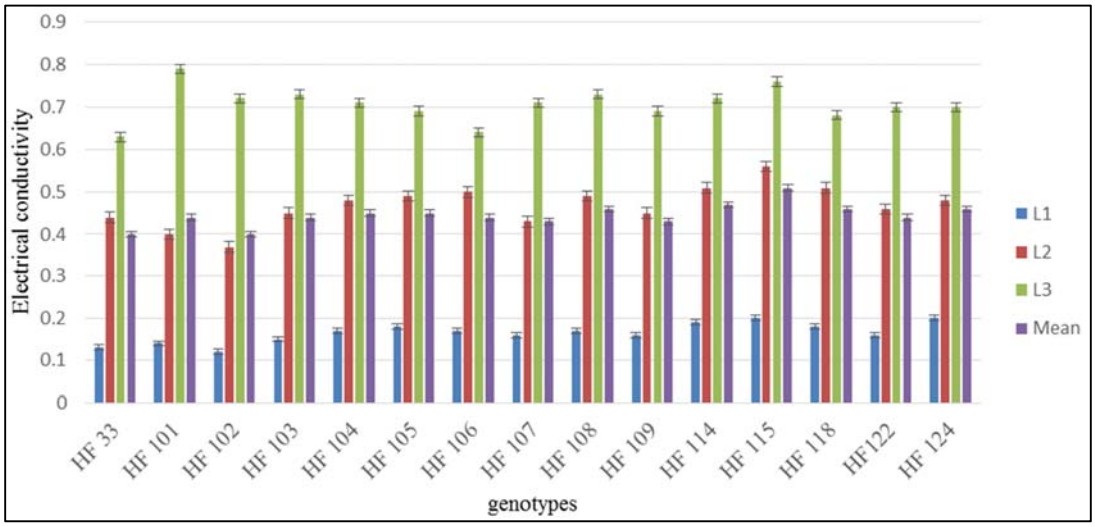

Fig 1: Effect of natural ageing on electrical conductivity (µS cm-1 seed-1) of fennel

Spectrophotometer was used to to examine the the range of optical density of formazan. The results as periodic profile of absorbance of formazan have been presented in Figure 2. The results also revealed that dehydrogenase activity decreased significantly as the age of the seeds increased. Mean dehydrogenase activity decreased as the ageing period increased which was found 0.14, 0.08 and 0.05 in freshly harvested seed lot, one year old seed lot and two year old seed lot, respectively. The range of optical density of the formazan in different varieties/genotypes varied from 0.02 to 0.18 from freshly harvested seed lot to two year old seed lot depending upon the genotype and period of ambient storage. The genotypes HF 33 (0.18) showed highest value of dehydrogenase activity in freshly harvested seed lot and statistically at par with HF 102 (0.16) and also in one year old and two year old seed lot HF 33 showed the maximum value of dehydrogenase activity which was 0.11 and 0.06 respectively. In *Brassica* spp. (Verma *et al*., 2003) [17] dehydrogenase activity was also reduced as the ageing progressed and was found lowest after four year of storage. These results are substantiate with the findings of Narwal (1995) [12] in okra seeds and by Kumar and Verma (2008) [5] in fenugreek.

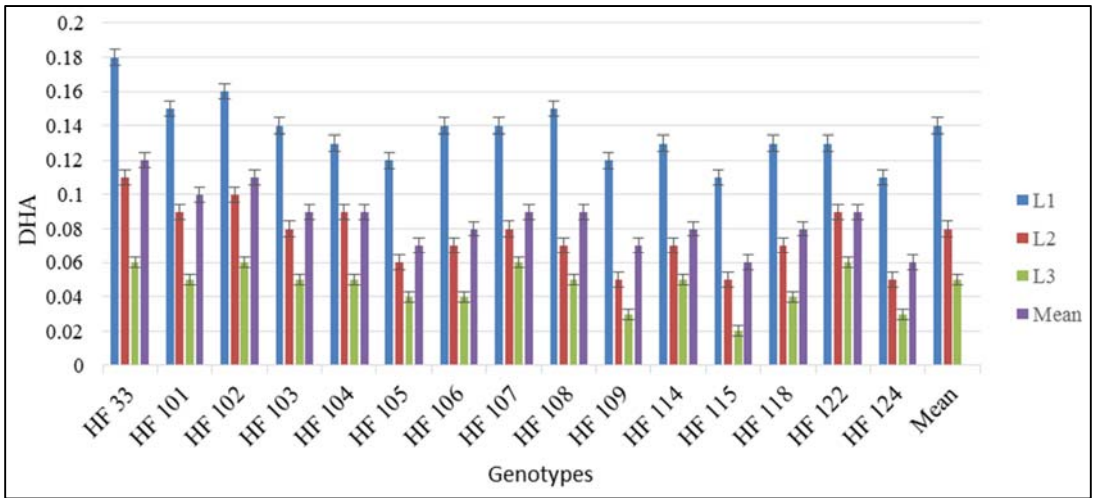

Fig 2: Effect of natural ageing on dehydrogenase activity (OD g-1 ml-1) of fennel

Conclusion

From the present study, it can be concluded that the quality of fennel seeds deteriorate rapidly under ambient storage condition as the age of the seeds increased and two year old seeds should not be used for sowing purpose by the farmers as the viability and vigour of the seeds of all the varieties/genotypes declined with fast rate after two year of storage. Among the varieties/genotypes, the genotype HF 33 and HF 102 were found most promising in terms of storability and these genotype may be used for further breeding programme.

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