Seed quality assessment in natural and artificially aged seed of fenugreek (*Trigonella foenum-graecum* L.)

BY

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CERTIFICATE – I

This is to certify that the thesis entitled, **"Seed quality assessment in natural and artificially aged seed of fenugreek (*Trigonella foenum-graecum* L.)"** submitted for the degree of **Doctor of Philosophy** in the subject of **Seed Science & Technology** from Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. Sunil Kumar** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been duly acknowledged.

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CERTIFICATE – II

This is to certify that this thesis entitled, **"Seed quality assessment in natural and artificially aged seed of fenugreek (*Trigonella foenum-graecum* L.)"** submitted by **Mr. Sunil Kumar** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in the subject of **Seed Science &Technology** has been approved by the Student's Advisory Committee after an oral examination on the same.

MAJOR ADVISOR **EXTERNAL EXAMINER**

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LIST OF ABBREVIATION

cm	:	Centimetre
kg	:	kilogram
mg	:	Milligram
MT	:	Metric tone
%	:	per cent
ha	:	hectare
SG	:	Standard germination
SDW	:	Seedling dry weight
SL	:	Seedling length
EC	:	Electrical conductivity
DHA	:	Dehydrogenase activity test
et al.	:	*et alia* (and others)
SEI	:	Seedling emergence index
SE	:	Seedling establishment
VI-I	:	Vigour index-I
VI-II	:	Vigour index-II
PGRs	:	Plant growth regulators
GA_3	:	Gibberellic acid
KNO_3	:	Potassium nitrate
$CaCl_2$	:	Calcium chloride
SOD	:	Superoxide dismutase
POX	:	Peroxidase activity

CHAPTER-I

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.*)* belongs to fabaceae family and is a major seed spice crop. The major fenugreek producing countries are Afghanistan, Pakistan, India, Iran, Nepal, Bangladesh, Argentina, Egypt, France, Spain, Turkey and Morocco. In India, it is mainly grown in Rajasthan, Gujarat, Uttarakhand, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and Punjab. Being a leguminous crop, the root nodules of fenugreek improves the soil fertility status by fixing of atmospheric nitrogen in the soil. In India, during 2016-17, fenugreek was grown in an area of 210 thousand hectares with the production of 297 thousand MT and productivity of 1.4 MT/ha. In Haryana, fenugreek was grown in an area of 3.53 thousand hectares with the production of 9.35 thousand MT and productivity of 2.65 MT/ha during 2016-17 (Anonymous, 2017).

Seed is an important component of agriculture and quality seed plays an important role in agricultural production as well as in national economy. So, good quality seed is prerequisite to enhance the production and productivity. It has been realized that the use of quality seeds increased productivity of crop by 15-20 per cent (Sidhwani, 1991). Fenugreek is a small seeded crop and therefore seed quality is very important for its good germination and early plant stand. Seed possess maximum vigour and viability at the time of physiological maturity (Meena *et al.*, 1994). Deterioration of seed quality and vigour starts immediately after attaining the physiological maturity on the plant itself (Abdul-Baki and Anderson, 1973a or 1973b). Physiological maturity attainment is a genotypic character which is influenced by several environmental factors (Mahesha *et al*., 2001).

Seed ageing cause loss of seed quality with time. It is a natural process which involves cytological, physiological, biochemical and physical changes in seeds. These changes reduce the viability, vigour and ultimately cause death of the seed. The rate of deterioration of seed quality varies among species and varieties. It also depends on environmental conditions and storage time (Walters *et al*., 2010). The rate of seed ageing is enhanced, when seed exposure to external challenges increases and decreases the ability of the seed to survive which is an undesirable attribute of agriculture. Annual losses due to seed deterioration can be as much as 25 per cent of the harvested crop. This may be one of the basic reasons for low productivity (Shelar *et al*., 2008). The process of seed ageing has been described as cumulative, irreversible, degenerative and inexorable process (Kapoor *et al*., 2011).

Deterioration of seed is a more serious problem in tropical and subtropical countries like India, where high temperature and humidity accelerates the seed ageing phenomenon.

Therefore after knowing the quality of the seed lot, the other important aspect is to define the storage/shelf life of seed. Jyoti and Malik (2013) indicated that seeds are highly susceptible to damage and mechanical injury during post-harvest handling. Several environmental factors contribute to seed deterioration and these conditions make it very difficult to maintain viability during storage. The seed quality depends upon the initial seed quality, temperature, moisture content and microflora. As seed aged, it comes to germinate more slowly than fresh seeds, respire slower and become more susceptible to disease and chromosomal abnormalities. Deterioration leads to increased proportion of morphologically abnormal seedling and reduction in seed quality performance and stand establishment (Christiansen and Rowland, 1981).

The relative storability of different seed lots/species can be assessed by using artificial ageing test performed under controlled conditions. This test provides valuable prediction on seed storability and seedling field emergence potentials. Seeds are hydrated to a specific level when exposed to relatively high temperature (40 to 45º C) and relative humidity (around 100 per cent). It increases catabolic changes at the cellular levels beyond the threshold of tolerance leading to reduction in seed quality parameters. Seeds are subjected to a germination test and higher vigour seed lots tolerate this ageing condition better than lower vigour seed lots and produce a higher percentage of normal seedlings (Baalbaki, 2009). The development of the artificial ageing test came from observations of Crocker and Groves (1915) which suggested that seed deterioration during storage was caused by protein coagulation and this process was accelerated by increase in seed mass temperature.

Lipid auto-oxidation is another cause of seed ageing which involves the production of free radicals. The protective mechanisms involve several free radicals and scavenging enzymes such as catalase, peroxidase and superoxide dismutase activity has evolved within the seed, keeping these deleterious compounds to a minimum (Halmer and Bewely, 1984).

Seed invigoration implies an improvement in seed vigour by any post harvest treatment resulting in improved germinability, greater storability and better field performance than the corresponding untreated (control) seed. Improving the seed quality is an approach, which is likely to produce significant benefits in almost all circumstances without any significant increase in risk. It will be of immense use to seed industry and farming community that how best the seeds can be stored by treating the seeds with chemicals and inert matter at relatively low cost under ambient storage and refrigerated conditions, with minimum quantitative and qualitative losses.

Seed priming is an active technology to enhance rapid and uniform germination and to achieve high vigour seed, leading to better field crop establishment and yield. It is a simple and low cost hydration technique in which seeds are partially hydrated to a point where pre-germination metabolic activities start without actual germination, and then again dried until close to the original seed dry weight of this crop. Priming with GA_3 @ 50 ppm for 2 hrs

enhanced field establishment, standard germination and seedling emergence index in soybean seed as compared to unprimed seed lots (Bassi *et al.*, 2011).

In the light of the above facts, the present study has been undertaken with the following objectives:

1. **To assess seed quality during natural ageing**
2. **To study the physiological and biochemical changes associated with seed deterioration after accelerated ageing**
3. **To study the effect of priming on seed quality of marginal seed lots after natural and accelerated ageing**

CHAPTER-II

REVIEW OF LITERATURE

Seed is a living entity which is bound to lose its life due to extrinsic and intrinsic factors such as genetic, pre-harvest climatic conditions, length of storage, initial seed quality, seed type, seed structure, seed health, storage temperature, relative humidity of the atmosphere, seed moisture content and seed treatments (Roberts, 1961). During storage, a number of physiological and physio-chemical changes occur, termed ageing (Silva *et al*., 2005).

Seed ageing is recognized by some parameters like delay in germination and emergence, slow growth and increasing susceptibility to environmental stresses (Walters, 2010). Seed quality (viability and vigor) decreases under long storage conditions due to ageing. It is the reason of declining in germination characteristics in wheat (Soltani *et al*., 2008). The aim of seed vigor tests is to distinct low and high vigor seed mass with each other, also these tests provide ways to examine the ability of field performance of different seeds in laboratory conditions (Ghasemi- golezani *et al*., 2010).

Increasing deterioration severity caused decreasing germination characteristics and seed reserve utilization (Mohammadi *et al*., 2011; Ansari and Sharif Zadeh, 2013). Low vigor seeds emergence lower than high vigor seeds in field conditions and produce less plantlets. Seeds deteriorate and lose their germinability during periods of prolonged aging (Ansari and Sharif Zadeh, 2013; Seiadat *et al*., 2012). The two most important environmental factors influencing the rate of deteriorative processes in seed ageing are the relative humidity of the air, which controls seed moisture content, and the temperature (McDonald, 1999).

In seed ageing damage to cellular membranes, decrease in mitochondrial dehydrogenase activity, chromosomal aberration and DNA degradation increases (Parrish and Leopold, 1978). Genetic damage may accumulate until the embryos are unable to germinate and grow. Besides being the main cause of seed damage, lipid peroxidation causes initial biochemical changes in seed that can be observed during storage. Structural changes associated with seed deterioration are reduced membrane fluidity, altered folding of DNA, lost elasticity of proteins and increased brittleness of the cellular matrix. Molecules oxidation leads to either smaller molecules with reactive carbonyl or nitrogen groups that easily diffuse through cells or adducts between carbohydrates, proteins and nucleic acids that cause intermolecular cross-linking and further degrade into advanced glycogen end-products (Walters *et al*., 2010).

Therefore, it becomes necessary to characterize the changes in seed characteristics which may get affected by natural and artificial ageing. This review of literature here covers

those all aspects, which are correlated to seed quality of fenugreek. The literature on fenugreek was scanty therefore, related work from other crops was undertaken into consideration.

A BRIEF RESUME OF WORK DONE IN INDIA AND ABROAD

The information related to the present study entitled "**Seed quality assessment in natural and artificially aged seed of fenugreek (*Trigonella foenum-graecum* L.)"** has been briefly reviewed here as under:

Effect of natural ageing on physiological parameters of seed quality

Seed ageing is a process which results in delayed germination, reduction in germination rate, and sometimes even a total loss of seed yield (Priestley, 1986). Seeds of different plant species under the identical natural storage conditions lose its viability to a greater or a lesser degree. Seed storage has marked effects on seed quality. Resolution of this problem must begin in the field during seed production, and it should be continued till the harvesting period. Viable seed is capable of producing new healthy plant under both favorable and unfavorable climatic conditions. The rate at which the seed ageing process takes place depends upon the ability of seed to resist degradation, protection mechanisms and plant genetic behavior (Gupta and Aneja, 2004). Mohammadi *et al*. (2011) observed in soybean seed that deterioration results in the decreased percentage of germination and percentage of normal seedlings. Ghasemnezhad and Honermeier (2007) observed that quality parameters of seed such as oil content, fatty acid composition and protein content were significantly influenced by storage conditions in most cases.

Successful germination of seeds is a prerequisite for plants to commence their life cycle and distribute their progeny is largely determined by the seed vigour (Rajjou *et al.*, 2012). The standard germination test is an excellent measure of seed viability. It is a universally acceptable viability and vigour test which predicted the maximum plant producing potential of seed lots and found to be correlated with field emergence under favorable conditions (ISTA, 2003).

Kharb and Dahiya (2000) revealed that marginal reduction in germination (6-10 %) was recorded in the 18 months old seed, which further reduced to 50 per cent after 30 months of storage in pigeon pea. Freitas *et al.* (2006) recorded the vigour and viability of cotton seeds decreased with the increase in artificial or natural ageing.

Singh *et al.* (2015) observed that test weight (g), seed density (g/cc), standard germination (%), seedling length (cm), dry weight per seedling (mg), vigour index-I & II, viability (%) by tetrazolium test, speed of emergence, seedling establishment (%) decreased in fenugreek seed whereas, mean emergence time (days) and electrical conductivity (µS/cm/seed) of seed leachates increased with the ageing period. Tirakannanavar *et al.* (2006)

revealed that there was decrease in germination percentage after 12 month natural ageing in bitter gourd.

Vijay *et al*. (2015) suggested that sunflower seeds can be stored at extreme temperature and relative humidity for about four months without losing the minimum seed certification standard for germination. Zaheer *et al*. (2016) observed in wheat seed that rate of deterioration is also useful in searching sensitive seed ageing signals for developing tools to monitor seed viability under storage.

Kavitha *et al*. (2017) reported that reduction of germination per cent in sesame was correlated with the reduction of seed quality parameters. After three days of artificial ageing treatment, it was equivalent to ten months of natural ageing in storage and maintained the germination above Indian Minimum Seed Certification Standards (IMSCS). Perez-Camacho *et al.* (2008) studied the effect of deterioration caused by natural ageing on husk tomato seed and reported that, the viability decreased from 84.5 to 50.8 percent after five years of storage.

Priya and Rao (2008) observed that freshly harvested seed had the highest germination, seedling vigour and it gradually decreased in all vegetables (carrot, cucumber, onion and tomato) seeds when the storage duration was extended. Juan *et al.* (2014) found that germination, vigour and respiratory activity decreased significantly from first to seventh year generation of ageing in husk tomato.

The length of seedling after a specified period is the product of time taken to germination *i.e*. initiation of growth and the subsequent rate of growth. Measuring plumule growth as a vigour test for cereals and sugar beet was first suggested by Germ (1960).

The measurement of seedling dry weight has been suggested as a parameter of vigour by AOSA vigour testing committee and Woodstock (1976). Nagarajan *et al.* (2004) observed that as the ageing period increased, the dry weight of seedling decreased in okra. The seedling dry weight and seed reserve depletion decreased significantly as seed ageing progressed in soybean seed (Mohammadi *et al.*, 2011).

Seed vigour is important quality parameter which needs to be assessed to supplement germination and viability test to gain insight the performance of seed lot on the field or in storage. Therefore, the availability of genetically pure and vigorous seeds at planting time is important for achieving target of agriculture production. Various seed variability and vigour test have been used extensively in a number of crops to predict the planting value of seed lots in the field. The vigour index offers the possibility of categorizing seed lots in to various classes of seed quality.

Singh *et al.* (2003) observed that the seed vigour index decreased with the increase in ageing duration in urd and mung bean. Rajkumar *et al.* (2004) also observed that the rate of germination percentage and vigour index decreased with progressive ageing period in pea.

Sudharani and Padmasri (2014) observed that final count of germination suitable for assessing seed vigour and accelerated ageing for 48 hours for estimating of storage potential of cotton seed lots. Deepika *et al.* (2014) revealed that there was significantly higher germination, plant height, number of leaves and vigour index with seeds sown at zero days of extraction, followed by 10 days of extraction in karonda seeds.

Basu *et al.* (2004) observed that the vigour indices decreased as the duration of the storage period increased in maize. Dahiya *et al.* (2006) reported that the physiological parameters like germination percentage, seedling length and vigour indices decreased with natural ageing in three seed lots of onion. Suthar *et al.* (2006) observed that onion seed germination decreased with the increase in storage period. An initial germination per cent was reduced from 86 to 62 per cent and thereafter reduced sharply. Seed viability and seedling vigour parameters were considerably reduced in all accessions at high relative humidity irrespective of the species of mustard (Suma *et al.* 2013).

The tetrazolium test is one of the most valuable techniques for analyzing the seed quality and viability potential of seed. It was developed by Lakon (1942). The action of a tetrazolium molecule to react with hydrogen atoms released dehydrogenase enzyme activity in living tissue. The tetrazolium test has a wide acceptance not only as a rapid technique for estimating quality, but also as a powerful tool for assessing seed vigour recommended. Tetrazolium test as an important indirect method for testing seed viability of some selected species where, the germination test takes more days ISTA (1999). Santos *et al*. (2007) indicated that reducing the pre-conditioning period is feasible and represents an important advance permitting faster decisions in seed quality programmes. The tetrazolium test permits the development of clear and clean colouration of seed and adequate for the identification of critical areas related to germination and also provided consistent data for the assessment of viability and vigour of tomato seeds. Demirkaya *et al*. (2010) investigated that high level of correlation was found between the loss of seed viability and decreased activities of catalase and superoxide in onion seeds.

Effect of artificial ageing on physiological parameters of seed quality

The Accelerated ageing test was first developed to measure the relative storability of seeds (AOSA 1983) and later on evaluated as indicator of seed vigour in a wide range of species (Hampton and Tekrony, 1995). Delouche and Baskin (2016) observed that the germination percentage after accelerated ageing was correlated with vigour of the lot and hence to the lots capacity to perform well under field conditions.

Hussein *et al.* (2012) suggested that accelerated ageing of sunflower seeds up to three days showed significant effect on germination percentage. Germinability was lost completely after seven days of accelerated ageing.

Tian *et al.* (2008) suggested the relationships between seed viability, cell death production and scavenging of reactive oxygen species during accelerated ageing. Water content of soybean seeds gradually increased while final germination percentage, germination rate and fresh weight of seedling decreased correspondingly with the increase in accelerated ageing time. Basra *et al.* (2003) found that vigour level, mean emergence time and quality parameters could be predicted by accelerated ageing test in cotton seed.

Silva *et al*. (2006) showed that accelerated ageing test is most commonly used to determine the physiological potential of different seed lots of beetroot and provide good relationship with field emergence. Komba *et al.*, (2006) observed that the artificial ageing test provides information about seed vigour, storability and other quality parameters of Kale (*Brassica oleracea*) seed.

The degree of seed damage and the ability of seed to resist the negative consequences of ageing were influenced, by duration of ageing period, type of storage and characteristics of sunflower seed (Balesevic *et al.*, 2005).

Maryam *et al.* (2014) indicated that germination percentage, germination index, normal seedling percentage, seedling dry weight and weight of utilizing (mobilized) seed reserve decreased significantly as accelerated ageing progressed in two wheat cultivars. But, mean time of germination, electrical conductivity and malondialdehyde content increased significantly as seed ageing progressed. Kapilan and Thiagarajah (2015) concluded that highest germination characteristics and enzyme activity were attained in the initial and the lowest at the final stage of the accelerated ageing in sunflower seed.

The accelerated and natural aged seeds showed the first signs of physiological deterioration by the slower germination of the radical emergence and delayed radical emergence or a high mean germination time (Matthews *et al.*, 2012).

Somasundaram and Bhaskaran (2017) considered that accelerated ageing test has good correlation to field emergence and storage potential of the seed. In this regard, an attempt was made to standardize accelerated ageing duration for screening rice genotypes for seed longevity. Hence, 20 days of accelerated ageing is considered as optimum duration of ageing for screening the genotypes for seed longevity in rice.

Accelerated aged seeds of rice have physiological mechanisms responsible for the slow root growth of the seedlings and led to the deterioration of both germinability and seed viability (Kapoor *et al.*, 2011). Farhadi *et al.* (2012) indicated the germination percentage, germination speed, root and shoot length decreased with artificial ageing in basil.

Soltani *et al.* (2009) reported in wheat crop that maximum rate of emergence reduced significantly with the increase in the duration of accelerated ageing. Seed ageing significantly reduced leaf area and seedling dry weight at first harvest, but reduction in these characteristics

was not significant at the second harvest. Gupta *et al.* (2005) observed that the pearl millet seed showed a gradual reduction in seedling vigour as artificial ageing duration increased.

Kapoor *et al.* (2010) explained that all the physiological parameters *viz*., germination percentage, seed viability, seedling length and consequently the seed vigour index in all varieties of chickpea were significantly decreased with accelerated ageing treatment. An increase in moisture content was observed during accelerated ageing.

Chauhan *et al.* (2011) reported that seed vigour decreased due to artificial ageing of six wheat varieties based on seedling length and seedling dry weight. Tabatabaei (2015) evaluated the effects of accelerated ageing on germination indices and enzyme activity of barley seed. He found that increase in accelerated ageing duration resulted in higher reduction in germination percentage, vigour index, mean time of germination, normal seedling percentage, catalase and peroxidase activity.

Da Silva *et al.* (2014) evaluated that using accelerated ageing saturated salt solution for 48 or 72 hours at 41˚C is an alternative and efficient way to evaluate the physiological quality of tomato seeds.

Biochemical changes associated with seed ageing (Natural & Artificial)

Seed ageing leads to various cellular and metabolic alterations, loss of membrane integrity, degradation of DNA and reduced primary metabolism (Kibinza *et al*. 2011). Reactive oxygen species (ROS) resulted in the peroxidation and degradation of lipids, which eventually damaged the integrity of cellular membranes. Generally, ROS regarded as the main factor that leads to seed ageing during storage (Priestley, 1986). Mahjabin *et al.* (2015) observed that free fatty acid can damage, lipid bilayer particularly of mitochondria leading to reduced energy production and free radicals have potential to damage the membrane, DHA, enzymes, protein and ultimately cellular repair mechanism.

Special attention is paid to enzyme activities due to their possible usage as significant indicators of vigour or seed longevity. Superoxide dismutase (SOD) is enzyme belongs to the first group of the protective mechanisms of plant cells against oxidative damage. Due to superoxide dismutase activity low concentration of superoxide in cells is maintained and thus preventing formation of harmful oxidative products in plant cells (Alscher *et al.*, 2002). Superoxide dismutase activity serves as a protective role in respiring cells through its elimination of the reactive superoxide radical. Level of superoxide dismutase activity in stored seed can be significant factor that determined the level of seed protection against oxidative stress (Blokhina *et al.* 2003).

Enzymatic activity test (CAT, POX, DHA, SOD, E.C.)

Dehydrogenase enzymatic activities are also known as tetrazolium reduction ability test. Kittock and Law (1968) gave an indirect method, *i.e.* by colorimetric estimation of formazan (product of reaction tetrazolium solution with dehydrogenase enzyme). The estimation can differentiate between the vigour levels of different seed lots. The activity of dehydrogenase

enzyme is directly correlated with the vigour of seed. Puntralo and Boveris (1990) noticed that gradual reduction of peroxidase activity during natural and artificial seed ageing. It was evident based on the research that many enzymes could be seriously degraded as a consequence of seed ageing and others failed to activate at normal levels during germination.

Dahiya *et al.* (1999) observed that the standard germination had significantly positive correlation with dehydrogenase activity in cotton. Rajjou *et al.* (2008) studied the comparison between natural and accelerated ageing on the basis of deterioration rate in Arabidopsis seeds. Radha *et al.* (2014) recorded that the dehydrogenase and peroxidase activities were less in the naturally aged seed lot as compared to fresh seed lot and the loss in seed viability and decrease in enzyme activity are highly correlated with each other.

Goel and Sheoran (2003) suggested that the activities of peroxidase, catalase and superoxide dismutase decreased with accelerated ageing period increased in cotton seed. Kumar (2004) observed that enzyme activities of catalase, superoxide dismutase and peroxidase decreased but the electrical conductivity of onion seed increased with advancement of ageing period. Sahu *et al.* (2017) observed that reactive oxygen species showed reduction in lipid peroxidation, protein oxidation and dehydrogenase enzymes in Karanj seed during storage.

Bhanuprakash *et al.* (2010) also recorded that enzyme activity change due to accelerated ageing period increased in bell pepper. Cakmak *et al.* (2010) evaluated the effect of long-term natural ageing on legume seed with a decrease in enzymatic activity and antioxidant in alfalfa seed. Seeds stored for 42 years showed decreased germination with low H_2O_2 content and decreased activities of catalase, peroxidase and superoxide dismutase enzymes.

Chauhan *et al.* (2011) studied that in naturally aged seed lots, the catalase and peroxidase activity decreased as the ageing period increased in all the six varieties of wheat, the rate of decreasing was higher in enzyme activity after 18 months of storage period. Singhal *et al*. (2017) revealed that the value of all seed quality parameters decreased significantly in fennel as ageing periods increased except electrical conductivity where value increased significantly due to loss of membrane integrity. Kumari *et al.* (2014) evaluated eleven genotypes of fenugreek kept under ambient condition to change in biochemical and viability of seed during storage. Yin *et al.* (2014) observed that artificially aged seeds of rice have decreased catalase activity and resulted in decreased percentage of germination from 99 to 2 per cent.

Yadollhhi and Mashayekhi (2013) evaluated in soybean seeds that with the increase in ageing duration resulted in higher reduction in germination characteristics associated with catalase activity and ascorbate peroxidase activity decreased significantly. Lakshmi *et al.* (2014) observed that total soluble proteins, sugars and starch content decreased during the accelerated ageing process. There was a gradual decrease in the activity of peroxidase, while the activity of amylase increased in the edible bamboo seeds. This may be due to the changes

in the biochemical content and the activity of enzymes involved in the degradation of seed reserves.

Seed deterioration is always correlated with an increased electrical conductivity of seed leachates and this is being used as one of the methods for evaluating seed ageing. The electrical conductivity test measures the amount of electrolytes that leach out from the cotton seeds as they deteriorate. The test has been also used to measure seed viability (Presely, 1958). Cyrus Mansouri-Far *et al.* (2015) indicated that there was damaged cell membrane and antioxidant enzyme activity in the maize hybrids under ageing. In general, loss of viability and increase in electrolyte leakage reflected the seed deterioration during storage under accelerated ageing conditions.

Khan *et al.* (2005) observed that electrical conductivity value increased due to loss of membrane integrity and seed leachates when the ageing duration increased, which played a key role in loss of seed quality in turnip. Mirdad *et al.* (2006) suggested that increased solute leakage from seed had been linked to poor quality seed and decreased germination percentage in mustard seeds. Kaewnaree *et al.* (2011) demonstrated that after accelerated ageing, cell membrane damage decreases the ability of carrier proteins and causes for increasing electrolyte leakage in sweet pepper. The biochemical parameters like electrical conductivity of seed leachates and lipid peroxidation under different treatments showed significantly increased value with seed deterioration in karanj (Kumar *et al.*, 2011).

Seed priming

Seed priming is a pre-sowing treatment that involves the exposure of seeds to low external water potential that limits hydration. Seed priming is a controlled hydration treatment in which seeds are allowed to imbibed before radical protrusion and improve germination rate, uniformity of germination and some time greater germination percentage (Bradford, 1986). This hydration is sufficient to permit pre-germinative metabolic events but allow radical protrusion through seed coat (Heydecker *et al*., 1975). Several reports had been proved that seed priming was effective in promoting seed germination, enhancing seedling growth of rice (*Oryza sativa* L.) under chilling (Zheng *et al*., 2016). In seed priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing in enough water for radical protrusion, thus suspending the seeds in the lag phase of seed germination but seeds are prohibited to enter into the next phase. During the second phase of seed germination most of metabolic pathways and recovering processes starts so that the embryo is assured that everything is prepared for successful germination (Varierf *et al.*, 2010). Kanwar *et al.* (2014) observed in bitter gourd seeds that priming improved germination and vigour indices of seeds through initiation of cell cycle, metabolic repair and reduction in the deleterious effects of ageing.

Osmo-priming is a low cost technique for improving seed germination and vigour of the seed. It involves controlled imbibitions of seeds and starts the initial germination followed by seed drying up to its original weight or moisture content. Osmo-priming has many advantages including rapid and uniform emergence of healthy seedling and better field establishment under any climatic and soil conditions (Chiu *et al*., 2002).

Seeds have a low water potential, determined by their osmotic and matrix potential, which remain low during initial swelling (McDonald, 1999). Generally, any type of priming treatment would cause an effective seed invigoration of the dry seed, which is the inception of metabolic processes that normally occur during imbibitions and subsequently fixed by drying the seed (Heydecker, 1978).

Wang *et al*. (2018) concluded that primed rice seeds were recommended to store at low relative humidity or low temperature condition to ensure good crop establishment. Nawaz *et al.* (2017) observed that priming with MLE30 was a natural and low cost effective priming tool for maximizing wheat yield due to its stress ameliorating potential. Ajirloo *et al.* (2013) also observed that interactive effect of varieties and priming technique were not significant for mean emergence time and coefficient of uniformity of emergence in forage maize. Sadeghi *et al.* (2011) recorded in soybean seeds that 12 hrs. osmo-priming duration by using PEG-6000 had most effective than other duration or control.

Tavili *et al.* (2012) categorized osmo- priming with PEG 6000 and NaCl into two different categories. Drought condition had stimulated by using PEG 6000 and for salinity NaCl and priming was a good seed enhancement technique for improving seed germination at faster rate of Bromus seeds. Ahmadvand *et al.* (2012) evaluated that seed priming with KNO_3 significantly increased germination and emergence percentage, radical, plumule length, seedling dry weight, plant height and plant leaf area of soybean. Farahani and Maroufi (2011) observed that the effect of hydro-priming and NaCl was significant on germination, seedling dry weight were significant in fenugreek. Pandey *et al.* (2017) recorded in cucumber crop that enhancement in seed viability and vigour in primed seeds was due to low membrane injury coupled with high enzyme activities (dehydrogenase and amylase).

Mahmoodi *et al.* (2011) recorded in maize that all hydro-priming durations significantly improved mean germination rate, seed vigour index as well as root shoot ratio, seedling dry weights and reduced electrical conductivity of seed leachates, compared with unprimed seeds. Maiti *et al.* 2011 studied the effect of seed priming on seedling vigour and yield of tomato and chilli.

Kalsa *et al.* (2011) studied that effect of storage duration and hydro-priming on seed germination and vigour of common vetch. Sori (2014) observed that chickpea hydro-priming decreased electrical conductivity of seeds by 20 per cent as compared to osmo-priming. Therefore,

it can be concluded that hydro priming can step-up economic benefit of chickpea growing farmers.

Kumar *et al.* (2017) observed that hydration (6 h) and dehydration at room temperature followed by dry dressing with thiram (0.25 %) recorded significantly maximum germination over the treatments throughout the storage period of fenugreek.

Huarte *et al.* (2007) reported that incubation with 1.44 mm GA_3 solution increased final germination of *Tripascum dactyloides*. Hammouda *et al.* (2017) observed that hydro-priming improved the morphology of germinated seeds in watermelon. Priming with GA_3 at low dose had no effect on seed germination.

Abdnadani and Ramezani, (2012) suggested that polyethylene glycol and KNO_3 solutions increased the fresh and dry weight of roots in maize by 2 and 5 per cent concentration primed for 12 and 18 h, respectively. In addition, they also increased the vigour index. Selvarani *et al.* (2011) found that priming treatments increased the speed of germination, germination percentage, seedling length, protein content and enzyme activity but lowered the electrical conductivity of seeds when compared to control in onion seed. Hamidi *et. al.* (2013) reported that increasing effect of urea and KNO_3 seed priming on seedling growth was more than PEG due to seed nutrition in sunflower crop. Bobak *et al.* (2015) observed that application of phytohormone and KNO_3 on germination characteristics in aged seed.

Vimala and Pratap (2014) observed in China aster that among the various priming treatments, priming of seed with KNO_3 with the dose of 0.5 per cent was found best with regard to all the physiological and biochemical parameters followed by hydro priming. However, storage of the six months old KNO_3 treated seed was found to be good compared to one year old seed regarding all the quality parameters. Krishnakumary *et al.* (2010) reported that among the different concentrations and duration of KNO_3, best result was obtained when the seeds of bitter gourd were treated with 15 mm concentration for 32 hrs duration followed by 30 mm concentration for 16 hrs duration. Tiryaki (2009) revealed that priming of seeds with 3 per cent KNO_3 did not enhance germination of Amaranthus species, while treating seeds with KNO_3 in the presence of methyl jamonates gave better results.

Kumar and Misra (2015) observed that all the priming methods showed significant differences with the control and the highest per cent germination, seedling length, weight and vigour index were observed with PEG 6000 priming for 20 hrs in cluster bean. Pandita and Nagarajan (2004) reported that osmo-priming (PEG 6000 and mannitol) and hydration 48 hrs prior to sowing improved seedling emergence and vigour in bitter gourd.

Khan *et al.* (2015) concluded that a combination of ridge sowing and osmo-priming with $CaCl_2$ can play a vital role in mitigating the adverse effects of drought stress, increasing the production of maize and net returns under normal and deficit water conditions. Chinanat

et al. (2015) found that seed priming with KH_2PO_4 and PEG 6000 improved the quality of hybrid cucumber seeds and biochemical parameters at a significant level. Seed priming significantly reduced amount of malondialdehyde and total peroxide. The seed priming affects various results of biochemical changes and expressed with increasing the level of antioxidant activity.

CHAPTER-III

MATERIALS AND METHODS

The present study entitled "Seed quality assessment in natural and artificially aged seed of fenugreek (*Trigonella foenum-graecum* L.)" was conducted during *rabi* 2015-16 and *rabi* 2016-17 at Research Farm and laboratory of the Department of Seed Science & Technology, CCS Haryana Agricultural University, Hisar. There were three set of experiments and the details of materials and methods used in the present investigation are as follows:

3.1 EXPERIMENTAL SITE

3.1.1 Location

Department of Seed Science & Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar is situated in semi-arid subtropics located at 29º 10' N latitude and 75º 46' E longitudes and at an altitude of about 215.2 m above the mean sea level in Haryana state of India.

3.1.2 Climate

The climate of Hisar (Haryana) region is semi-arid with hot and dry desiccating winds accompanied by frequent dust storms of high velocity in summer, severe cold during winter and warm humid conditions during rainy season. The mean monthly maximum and minimum temperatures showed a wide range of fluctuations during both the years. The mean monthly maximum temperature of 43-45°C was common during the summer months of May to June while minimum temperature during the winter months of December and January sometimes went as low as 0 ºC.

3.1.3 Seed procurement

Seed material comprised of six genotypes of fenugreek *viz.* **HM-57** (Hisar Sonali), **HM-103** (Hisar Suvarna), **HM-273, RMT-361**, **FGK-49** (UM-355), **FGK-80** (JFG-266) having germination (>70%) above Indian Minimum Seed Certification Standard (IMSCS) were collected from the Department of Vegetable Science, Choudhary Charan Singh Haryana Agricultural University, Hisar.

3.1.4 Experimental layout

Seeds of all six genotypes of fenugreek were stored under ambient conditions in the laboratory of Seed Science & Technology. All three experiments were laid out in factorial Complete Randomized Design (CRD) for laboratory studies and factorial Randomized Block Design (RBD) for field studies with three replications during 2015-16 and 2016-17. In case of natural ageing, fenugreek seed was stored in cotton bags under ambient conditions up to 18 months and all physiological, biochemical and field parameters were observed at three months intervals and enzyme activity was estimated at first and last observation.

Average weather data of Hisar during the experimentation season (2016-17)

MONTHS	TEMPERATURE (°C)		RELATIVE HUMIDITY (%)		RAIN	
	MAXIMUM	MINIMUM	MORNING	EVENING	RAINFALL (mm)	RAINYDAYS
Jan.2016	19.6	7.1	94.9	64.5	0.0	Nil
Feb. 2016	24	7.4	92.0	49.0	5.3	1
Mar. 2016	29.9	13.7	88.0	46.0	25.2	4
Apr. 2016	37.9	18.4	61.0	25.0	0.0	Nil
May. 2016	41	24.8	63.0	35.0	44.3	2
Jun. 2016	39.6	27.6	71.0	44.0	91.1	4
Jul. 2016	35.1	26.1	90.0	72.0	244.8	11
Aug. 2016	34	25.6	90.0	69.0	80.4	4
Sep. 2016	35.2	24.2	86.0	54.0	2.8	1
Oct. 2016	34.6	18.4	84.0	42.0	12.0	1
Nov. 2016	29.3	10.6	91.0	47.0	0.0	Nil
Dec. 2016	24.2	7.5	98.0	57.0	0.0	Nil
Jan. 2017	18.6	6.9	99.0	71.0	41.2	4
Feb. 2017	24.3	7.8	92.0	46.0	0.0	Nil
Mar. 2017	29	11.3	90.0	38.0	7.5	1
Apr. 2017	38.6	18.9	57.0	22.0	1.0	0
May. 2017	40	24.4	57.0	25.0	10.9	1
Jun. 2017	37.2	25.6	77.0	50.0	283.8	10
Jul.2017	35.1	27	88.0	67.0	83.0	5
Aug.2017	34.7	26.3	90.0	69.0	95.5	6
Sep. 2017	34.9	23.5	87.0	50.0	56.6	2
Oct. 2017	35	17.2	85.0	28.0	0.0	0
Nov. 2017	27.2	10.8	90.0	40.0	0.0	0
Dec. 2017	21.7	6.1	90.7	42.9	3.8	1

Source:- Agro Metrology Department CCS HAU, Hisar

For defining the artificial ageing, fresh seed of all six genotypes of fenugreek were artificially aged (41±1ºC /72 hrs.) and observations were recorded. After that, different priming treatments were given to both (natural & artificial) aged seed to check the best priming treatments and better improvement in natural or artificial aged seed.

3.1.5 Natural ageing test:

Seeds were stored under ambient condition in a cotton bag and observations were taken at three months interval up to 18 months. Enzyme activities were estimated at 1st and last observation.

3.1.6 Artificial ageing test:

Sufficient number of seeds from seed lots were taken and put on in a single layer on a wire mesh tray fitted in plastic boxes having 40 ml of distilled water in the bottom. The boxes

were placed in the accelerated ageing chamber after closing their lids. The seeds were aged at 40±1°C temperature and about 100 per cent relative humidity for 72 hrs and then tested for germination according to ISTA (2003). The number of normal seedlings were counted and expressed in percentage.

The natural aged seeds of all the six genotypes were evaluated for the following parameters:

3.2 EXPERIMENT:-1 To assess seed quality during natural ageing.

3.2.1 Observations recorded

3.2.1.1 Physiological parameters

1. Standard germination (%)
2. Seedling length (cm)
3. Seedling dry weight (mg)
4. Seed vigour indices
5. Tetrazolium test (%)
6. Electrical conductivity test (dS/cm/seed)

3.2.1.2 Biochemical parameters

1. Catalase activity test (CAT, mg protein^{-1} min^{-1}) (Aebi, 1983)
2. Peroxidase activity test (POD, mg protein^{-1} min^{-1}) (Shannon *et al*., 1966)
3. Dehydrogenase activity test (OD g^{-1} ml^{-1}) (Kittock & Law,1968)
4. Superoxide dismutase activity test (SOD, mg protein^{-1}min^{-1}) (Giannopolitis & Ries, 1977)

3.2.1.3 Field parameters

1. Seedling emergence index (Maguire, 1962)
2. Mean emergence time (Ellis and Roberts,1977)
3. Seedling establishment (%)

3.3 EXPERIMENT-2:-To study the physiological and biochemical changes associated with seed deterioration after accelerated ageing

Accelerated ageing (40±1ºC for 72 hrs & 100 % RH) was given to the freshly harvested seed of all six genotypes of fenugreek.

3.3.1 Observations recorded

3.3.1.1 Physiological parameters

1. Standard germination (%)
2. Seedling length (cm)
3. Seedling dry weight (mg)
4. Seed vigour indices
5. Tetrazolium test (%)
6. Electrical conductivity test (dS/cm/seed)

3.3.1.2 Biochemical parameters:-

1. Catalase activity test (CAT, mg protein^{-1} min^{-1}) (Aebi, 1983)
2. Peroxidase activity test (POD, mg protein^{-1} min^{-1}) (Shannon *et al*., 1966)
3. Dehydrogenase activity test (OD g^{-1} ml^{-1}) (Kittock & Law,1968)
4. Superoxide dismutase activity test (SOD, mg protein^{-1}min^{-1}) (Giannopolitis & Ries, 1977)

3.4 EXPERIMENT 3:- Effect of priming treatment on seed quality of marginal seed lots after natural and artificial ageing

Priming treatment details:-

T_0 -Control.

T_1 - Hydration (16-18 hrs.) followed by dry dressing with Thiram @ 0.25 per cent and drying at room temperature below 25ºC.

T_2 - Hydration with GA_3 (50 ppm for 16-18 hrs.) and drying at room temperature.

T_3 - Hydration with PEG (6000) (16-18 hrs.) and drying at room temperature.

T_4 - 0.5 per cent KNO_3 hydration (16-18 hrs.) and drying at room temperature.

T_5 - 2 per cent $CaCl_2$ hydration (16-18 hrs.) and drying at room temperature.

After each treatment, seeds were dried back to original moisture content. Then the standard germination test was conducted on the treated seeds to find out the viability percentage of the each genotype.

Chemical used in priming

1. Thiram
2. Gibberlic acid (GA_3)
3. Polyethylene glycol (PEG)
4. Potassium nitrate (KNO_3)
5. Calcium chloride ($CaCl_2$)

Preparation of solutions

1. Thiram (0.25 %): 2.5 g thiram was dissolved in one liter of distilled water.
2. Gibberlic acid (50 ppm) : 50 mg of GA_3 was dissolved in one liter of distilled water to make a one liter solution of GA_3 of 50 ppm concentration. One to three drops of acetone was also added as GA_3 does not dissolve in water.
3. Polyethylene glycol (PEG-6000): 27.3 g of PEG-6000 was dissolved in one liter of distilled water, which providing an osmotic potential of -1 Mpa (Michel and Kaufnann, 1973).
4. Potassium nitrate (0.5 %): 0.5 gm of KNO_3 was dissolved in 100 ml of water.
5. Calcium chloride (2 %): 2 gm of $CaCl_2$ was dissolved in 100 ml of water.

3.4.1 Observations recorded:

3.4.1.1 Physiological parameters

1. Standard germination test (%)
2. Seedling length (cm)
3. Seedling dry weight (mg)
4. Vigour index-I
5. Vigour index-II
6. Tetrazolium test (%)
7. Electrical conductivity (ds/cm/seed)

3.4.1.2 Field Parameters:-

1. Seedling emergence index (Maguire, 1962)
2. Mean emergence time (Ellis and Roberts,1977)
3. Seedling establishment (%)

Detail of observations recorded in Experiment-1, 2 and 3 are given below

Standard germination (%)

One hundred seeds of each genotype in three replicates placed in between sufficiently moistened rolled towel papers (BP) and kept at 20º C in seed germinator. The first count was taken on 5^{th} day and final count on the 14^{th} day and only normal seedlings were considered for per cent germination according to the rules of International Seed Testing Association (ISTA, 2003).

Seedling length (cm)

Ten normal seedlings at the time of final count were randomly selected from each replication of all the seed lots and their length was measured in centimeter. The average length of these seedlings was calculated.

Seedling dry weight (mg)

Ten normal seedlings which were used for the measurement of seedling length were also used for seedling dry weight measurement. These were dried in hot air oven at 80^0C temperature for 48 hrs. Then seedlings were removed from the oven and allowed to cool in desiccators for 30 minutes before weighing on an electronic balance. The average weight of dried seedlings from each replication was calculated and expressed in milligram.

Seed vigour indices

Seedling vigour indices were calculated by using the formula suggested by Abdul-Baki and Anderson (1973a or b) as follows:-

Vigour Index-I

Vigour Index-I = Standard germination (%) × Average seedling length (cm)

Vigour Index-II

Vigour Index-II = Standard germination (%) × Average seedling dry weight (mg)

Tetrazolium test (%)

The tetrazolium viability test (Moore, 1973) was executed on three replications of 50 seeds. These seeds were soaked in 50 ml water for 24 hrs at 25º C to activate dehydrogenase enzymes. After removal of seed coat, the seeds were stained in 0.5 per cent tetrazolium chloride solution (2, 3, 5- triphenyl tetrazolium chloride) (pH 7.0) for 4 hrs at 38º C in petri plates. After that, 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, which were expressed in percentage.

Electrical conductivity test (dS/cm/seed)

To measure the electrical conductivity of seeds 50 normal and undamaged seeds of each seed lot were imbibed in 75 ml deionized water in 100 ml beakers replicated thrice. Seeds were imbibed completely in water and beakers were covered with aluminum foil. Thereafter the samples were kept at 25°C for 24 hrs. The electrical conductivity of the seed leachates was measured using a direct reading conductivity meter. The conductivity was expressed in dS/cm/seed.

Biochemical parameters

Catalase activity test (mg protein^{-1}min^{-1})

For the extraction of catalase enzyme, seeds of each genotype were dipped in water at 30º C in the seed germinator for 24 hrs. Two hundred milligram of imbibed seed was ground in a chilled pestle mortar by adding 10 ml phosphate buffer (pH 7.8) and a pinch of corning sand. The 10 ml homogenate was centrifuged at 12000 rpm for 20 minutes at 4ºC. The supernatant was obtained, then re-centrifuged at 15000 rpm for 10 minutes. The clear supernatant was obtained and that is used for estimating the activity of catalase. The catalase activity was expressed by the method as described by Aebi (1983) based on the reduction of potassium dichromate to chromic acetate by hydrogen peroxide.

Reagents: 0.3 M hydrogen peroxide (H_2O_2)

0.1 M phosphate buffer (pH 7.0)

Dichromate acetic acid reagent (5% potassium dichromate + glacial acetic acid in the ratio of 1:3), 0.5 ml of H_2O_2 and 1.0 ml of buffer phosphate (pH 7.0) was added in 0.5 ml of enzyme extract in a side mouthed test tube. This was mixed rapidly and then incubated at 37º C for 5 minutes. The test tubes were then taken out and 4 ml of dichromate acetic acid reagent was added. These were then heated for 10 minutes in a boiling water bath. The colour that is changed into greenish due to the formation of chromic acetate after cooling was measured by Systronic Spectrophotometer 169 at 570 nm. The activity of catalase has been expressed as the amount of enzyme required to bring about a change in absorbance by 0.01 per minute.

Peroxidase activity test (mg protein^{-1} min^{-1})

For the extraction of peroxidase enzyme, seeds of each genotype were imbibed in water at 30º C in the seed germinator for 24 hrs. Two hundred milligram of imbibed seed samples was ground in a chilled pestle mortar by adding 10 ml phosphate buffer (pH 7.8) and a pinch of corning sand. The 10 ml homogenate was centrifuged at 12000 rpm for 20 minutes at 4º C. The supernatant obtained was then again centrifuged at 15000 rpm for 10 minutes. The clear supernatant, was obtained and used for estimating the activity of peroxidase. Peroxidase activity was determined by the method of Shannon *et al.* (1966) following the oxidation of O-dianisidine in the presence of hydrogen peroxide (H_2O_2).

Reagents: 0.1 M sodium buffer acetate (pH 4.5)

0.2 M hydrogen peroxide (H_20_2)

10 mg O-dianisidine dissolved per 2 ml of methanol.

2 ml of acetate buffer, (pH 4.5) and 0.1 ml of the O - dianisidine solution was added to 0.05 ml of enzyme extract. Then 0.1 ml of 0.2 M hydrogen peroxide also added to start the reaction. The reading was taken at 470 nm wavelength after every 15 second for 1 minute and enzyme unit was expressed as mg protein^{-1} min^{-1}.

The amount of enzyme required to bring about a change in absorbance of 0.01 per minute.

Dehydrogenase activity test (OD g^{-1} m^{-1})

Dehydrogenase activity test was performed using the method given by Kittock and Law (1968). One gram seed of each seed lot replicated thrice was ground to pass through 20 mesh screen. The 200 mg flour was soaked in 5 ml of 0.5% tetrazolium solution at 38°C for 3-4 hrs. Then it was centrifuged at10000 rpm (round per minute) for 3 minute and the supernatant was poured off and the formazan was extracted with 10 ml acetone for 16 hours, followed by centrifugation and absorbance of the solution was determined by Systronic spectrophotometer 169 at 480 nm. The observations were expressed as change in OD g^{-1} ml-$^{1.}$.

Superoxide dismutase activity test (mg protein^{-1} min^{-1})

The enzyme activity was assayed as per method given by Giannopolitis and Ries (1977).

Reagents: 1.3 µM riboflavin (1 ml)

13 mM methionine (1 ml)

63 µM nitroblue tetrazolium (NBT)

0.1 M phosphate buffer (pH 7.0)

Procedure:- In 3.0 ml of 0.1 M phosphate buffer (pH 7.0) containing 1.3 µM riboflavin13 mM methionine and 63 µM nitroblue tetrazolium, 0.1ml of enzyme extract was added. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. Glass tubes containing the mixture were exposed to light (two 15 Watt fluorescent lamps). Identical tubes, which were not illuminated, served as blanks. After illumination for 10 minutes, they were covered with black cloth and absorbance

was measured at 560 nm. Long A-560 was plotted as a function of volume of enzyme extract used in the reaction mixture. From the resultant graph, volume of enzyme extract corresponding to 50 per cent inhibition of the photochemical reaction was obtained and considered as one enzyme unit (Beauchamp and Fridovich, 1971).

Units: One unit of SOD was defined as the enzyme activity which inhibited the photo reduction of NBT to blue formazan by 50 per cent and expressed as units SOD mg protein^{-1} min^{-1}.

Field parameters

One hundred seeds of six genotypes of fenugreek stored under ambient conditions were sown in a factorial Randomized Block Design, during *Rabi*, 2015-16 and 2016-17 in the Research farm of Department of Seed Science & Technology, Choudhary Charan Singh Haryana Agricultural University, Hisar. The following observations were recorded in the field.

Seedling Emergence Index (SEI)

Line sowing of 100 seed of each variety in three replications was done in the well prepared field. The number of seedling emerged were counted daily up to the final seedling establishment. The seedling emergence index was calculated using formulae as suggested by Maguire (1962).

$$\text{Seedling Emergence Index (SEI)} = \frac{\text{No. of seedlings emerged}}{\text{Day of first count}} + \text{- - - - - - - - -} + \frac{\text{No. of seedlings emerged}}{\text{Day of final count}}$$

Mean Emergence Time (MET)

The mean emergence time was calculated for each treatment combination using the formula cited by Ellis and Roberts (1977).

$$MET = \Sigma nt / \Sigma n$$

Where,

n- Number of seeds newly germinated at time‘t’

t - Days from sowing

Σn - Final emergence of seedlings

Seedling establishment (%)

In field conditions, daily newly emerged seedlings were counted till seedling emergence became constant and no further seedling was emerged. The final day count of the total number of seedling emerged was used for calculation of average number of seedling establishment.

3.4 Statistical analysis

The statistical analysis was carried out for each observed character under the study using OP Stat (Sheoran *et al*., 2006). The mean values of data were subjected to analysis of variance as described by Factorial Randomized Block Design (RBD) and Factorial Completely Randomized Block Design (CRD) through the online statistical tool.

CHAPTER-IV

RESULTS

The results obtained during the course of the investigation are presented in this chapter. The results for effect of natural and artificially ageing and seed priming on a marginal lot of both (Natural and artificial) aged seed of all the six genotypes of fenugreek are presented under the following heads.

4.1 To assess the seed quality during natural ageing

4.2 To study the physiological and biochemical changes associated with seed deterioration after accelerated ageing.

4.3 To study the effect of priming on seed quality of marginal seed lots after natural and accelerated ageing.

4.1 To assess the seed quality during natural ageing

Fresh seeds comprised of six genotypes of fenugreek which had germination (>70%) above Indian Minimum Seed Certification Standard (IMSCS) were collected at the time of sowing of crop. In case of natural ageing, observations were recorded quarterly on the stored fenugreek seed in cotton bags at ambient condition up to 18 months and enzymes activation was estimated at 1st and last observations.

4.1.1 Standard germination

Standard germination decreased as period of natural ageing increased in all the genotypes of fenugreek (Table 4.1). There was a gradual fall in standard germination at every 3 month interval, but standard germination remained above (70%) after 18 months of storage in all the genotypes. So it is predicted that all genotypes of fenugreek had good storability. At the end of storage period, maximum standard germination was retained in genotype HM-103 (90.67%) whereas minimum was retained in FGK-80 (86.00%).

4.1.2 Seedling length

Seedling length in all the six genotypes decreased with the advancement of storage period (Table 4.2). Reduction rate in all the six genotypes was very slow. At the end of storage period reduction rate was faster as compared to initial stage of storage. Among the six genotypes, HM-57 found better in seedling length (25.47 cm) whereas FGK-80 had minimum (22.17 cm) after 18 months of storage period.

Table 4.1: Effect of natural ageing on standard germination (%) of fenugreek

Genotypes	Ageing period (months)							Mean
	0	3	6	9	12	15	18	
HM-57	96.33 (79.11)	96.00 (78.95)	96.00 (78.49)	95.33 (77.51)	94.00 (75.82)	92.67 (74.29)	89.67 (72.53)	**94.52 (76.67)**
HM-103	95.67 (77.97)	95.33 (77.07)	95.00 (77.09)	94.33 (76.28)	94.00 (75.82)	92.33 (73.95)	90.67 (72.22)	**93.95 (75.92)**
HM-273	95.33 (77.51)	95.00 (76.55)	94.00 (75.82)	93.33 (75.02)	92.67 (74.31)	90.33 (70.61)	88.33 (68.87)	**92.76 (73.56)**
RMT-361	95.00 (77.22)	94.33 (76.21)	93.67 (75.46)	92.67 (74.27)	91.00 (72.53)	88.67 (70.35)	87.00 (68.87)	**91.76 (73.56)**
FGK-49	94.67 (76.67)	94.00 (75.82)	93.00 (74.65)	92.67 (74.27)	91.67 (73.24)	90.33 (70.61)	87.33 (69.13)	**91.95 (73.48)**
FGK-80	93.33 (75.02)	92.67 (74.29)	92.67 (74.31)	90.67 (72.20)	90.67 (72.19)	88.67 (70.33)	86.00 (68.01)	**90.67 (72.34)**
Mean	**95.06 (77.25)**	**94.72 (76.82)**	**94.06 (75.97)**	**93.17 (74.92)**	**92.33 (73.99)**	**90.06 (71.69)**	**87.67 (69.94)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	0.794	0.398	0.282					
Genotypes	0.735	0.369	0.261					
M X G	NS	0.976	0.690					

*Figures in the parenthesis are arcsine value

Table 4.2: Effect of natural ageing on seedling length (cm) of fenugreek

Genotypes	Ageing period (months)							Mean
	0	3	6	9	12	15	18	
HM-57	28.57	27.82	27.57	26.80	26.37	25.80	25.47	**26.91**
HM-103	27.70	27.30	26.47	26.27	25.60	25.20	24.43	**26.14**
HM-273	27.50	26.73	26.33	25.70	24.72	23.90	23.47	**25.48**
RMT-361	27.48	26.58	26.12	24.90	24.53	23.83	23.30	**25.25**
FGK-49	26.50	26.15	25.32	24.77	23.77	23.08	22.65	**24.60**
FGK-80	26.27	25.17	25.20	24.00	23.53	23.17	22.17	**24.21**
Mean	**27.34**	**26.63**	**26.17**	**25.41**	**24.75**	**24.16**	**23.65**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	0.449	0.226	0.159					
Genotypes	0.415	0.209	0.147					
M X G	NS	0.552	0.390					

4.1.3 Seedling dry weight

Seedling dry weight decreased gradually as advancement of storage period up to 18 months (Table 4.3). At the end of storage periods, maximum seedling dry weight was recorded in genotype HM-57 (7.601 mg) and minimum was in FGK-80 (6.736 mg).

Table 4.3: Effect of natural ageing on seedling dry weight (mg) of fenugreek

Genotypes	Ageing period (months)							Mean
	0	3	6	9	12	15	18	
HM-57	8.062	7.975	7.957	7.827	7.783	7.664	7.601	**7.838**
HM-103	7.735	7.650	7.565	7.444	7.372	7.258	7.153	**7.454**
HM-273	7.540	7.470	7.402	7.326	7.222	7.120	7.050	**7.304**
RMT-361	7.441	7.411	7.370	7.294	7.177	7.082	6.973	**7.250**
FGK-49	7.263	7.233	7.200	7.097	6.986	6.897	6.796	**7.067**
FGK-80	7.238	7.212	7.142	7.064	6.970	6.855	6.736	**7.031**
Mean	**7.546**	**7.492**	**7.439**	**7.342**	**7.251**	**7.146**	**7.051**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	0.035	0.018	0.013					
Genotypes	0.033	0.016	0.012					
M X G	NS	0.044	0.031					

4.1.4 Seed vigour index-I

Seed vigour index-I decreased throughout of storage period in all the six genotypes of fenugreek (Table 4.4). After one year of storage, seed vigour declined at very faster rate in all the six genotype of fenugreek. At the end of the storage period, maximum seed vigour index-I was recorded in genotype HM-57 (2317.5) and minimum was in FGK-80 (1978.1).

4.1.5 Seed vigour index-II

Significant reduction was observed in all the six genotypes of fenugreek with the advancement of storage period (Table 4.5). After 18 months of storage periods, maximum seed vigour index-II was recorded in HM-57 (691.7) and minimum was in FKG-80 (570.4).

Table 4.4: Effect of natural ageing on vigour index-1 of fenugreek

Genotypes	Ageing period (months)							Mean
	0	3	6	9	12	15	18	
HM-57	2,752.8	2,680.0	2,646.1	2,561.3	2,478.5	2,391.1	2,317.5	**2,546.8**
HM-103	2,650.0	2,611.7	2,514.4	2,500.8	2,406.3	2,326.5	2,174.7	**2,454.9**
HM-273	2,622.0	2,547.9	2,476.1	2,400.4	2,290.7	2,127.0	2,041.8	**2,358.0**
RMT-361	2,610.2	2,507.8	2,445.7	2,316.7	2,232.6	2,113.2	2,027.2	**2,321.9**
FGK-49	2,508.2	2,458.2	2,354.8	2,333.6	2,178.5	2,054.3	2,013.0	**2,271.5**
FGK-80	2,451.7	2,331.8	2,335.5	2,177.6	2,133.7	2,054.3	1,978.1	**2,194.5**
Mean	**2,599.2**	**2,522.9**	**2,462.1**	**2,381.7**	**2,286.7**	**2,177.7**	**2,075.2**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	46.8	23.5	16.6					
Genotypes	43.3	21.8	15.4					
M X G	NS	57.6	40.7					

Table 4.5: Effect of natural ageing on vigour index-II of fenugreek

Genotypes	Ageing period (months)							Mean
	0	3	6	9	12	15	18	
HM-57	776.6	768.3	763.8	746.2	731.6	710.2	691.7	**741.2**
HM-103	740.0	731.9	718.7	702.2	692.9	670.1	636.6	**698.9**
HM-273	718.8	712.1	695.8	683.8	669.2	633.7	613.4	**675.3**
RMT-361	706.9	699.1	690.3	675.9	653.1	628.0	606.6	**665.7**
FGK-49	687.5	679.9	669.6	657.6	640.3	613.9	593.5	**648.9**
FGK-80	675.5	668.4	661.8	640.5	631.9	607.8	570.4	**636.6**
Mean	**717.5**	**709.9**	**700.0**	**684.4**	**669.8**	**643.9**	**618.7**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	6.971	3.499	2.474					
Genotypes	6.454	3.24	2.291					
M X G	NS	8.571	6.061					

4.1.6 Tetrazolium test

Seed viability decreased with the passage of time in all the genotypes of fenugreek (Table 4.6). After 18 months of storage, maximum seed viability was recorded in genotype HM-57 (91.33 %) where as minimum was recorded in FGK-80 (85.33 %).

Table 4.6: Effect of natural ageing on tetrazolium test (%) of fenugreek

Genotypes	Ageing period (months)							Mean
	0	3	6	9	12	15	18	
HM-57	97.67 (81.22)	97.00 (80.09)	96.67 (79.63)	95.67 (78.03)	94.67 (76.63)	93.00 (74.65)	91.33 (72.89)	**95.14 (77.59)**
HM-103	97.00 (80.09)	96.67 (79.63)	95.67 (78.07)	95.00 (77.09)	94.67 (76.67)	92.33 (73.92)	90.00 (71.55)	**94.48 (76.72)**
HM-273	96.67 (79.47)	96.00 (78.49)	94.67 (76.70)	93.67 (75.44)	93.67 (75.46)	89.67 (71.25)	88.00 (69.72)	**93.19 (75.22)**
RMT-361	95.33 (77.61)	95.00 (77.09)	94.33 (76.21)	93.33 (75.07)	91.33 (72.89)	89.67 (71.23)	88.00 (69.74)	**92.43 (74.26)**
FGK-49	95.00 (77.16)	94.67 (76.63)	94.00 (75.82)	93.00 (74.65)	92.33 (73.92)	89.33 (70.93)	88.00 (69.72)	**92.33 (74.12)**
FGK-80	94.33 (76.28)	93.67 (75.44)	93.00 (74.65)	91.33 (72.89)	89.67 (71.24)	88.00 (69.74)	85.33 (67.46)	**90.76 (72.53)**
Mean	**96.00 (78.64)**	**95.50 (77.89)**	**94.72 (76.85)**	**93.67 (75.53)**	**92.72 (74.47)**	**90.33 (71.95)**	**88.44 (70.18)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	0.98	0.492	0.348					
Genotypes	0.907	0.455	0.322					
M X G	NS	1.205	0.852					

*Figures in the parenthesis are arc sine value

4.1.7 Catalase & Peroxidase activity test

Catalase and peroxidase activities decreased significantly as storage period increased in all six genotypes of fenugreek (Table 4.7). Maximum catalase activities were found in HM-57 (0.205 mg protein^{-1}min^{-1}) and minimum were found in FGK-80 (0.154 mg protein^{-1}min^{-1}). Peroxidase activities were found maximum in HM-57 (0.397 mg protein^{-1}min^{-1}) and minimum was in FGK-80 (0.306 mg protein^{-1}min^{-1}) at the end of storage period.

Table 4.7: Effect of natural ageing on catalase and peroxidase activity test of fenugreek

Catalase activity test(mg protein^{-1}min^{-1})				**Peroxidase activity test (mg protein^{-1}min^{-1})**		
Ageing periods (months)				**Ageing periods (months)**		
Genotypes	**0**	**18**	**Mean**	**0**	**18**	**Mean**
HM-57	0.280	0.205	**0.242**	0.583	0.397	**0.490**
HM-103	0.269	0.185	**0.227**	0.574	0.370	**0.472**
HM-273	0.262	0.179	**0.221**	0.568	0.348	**0.458**
RMT-361	0.266	0.169	**0.218**	0.558	0.360	**0.459**
FGK-49	0.249	0.168	**0.209**	0.556	0.352	**0.454**
FGK-80	0.241	0.154	**0.197**	0.546	0.306	**0.426**
Mean	**0.261**	**0.177**		**0.564**	**0.356**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	**C.D.**	**±SE(d)**	**±SE(m)**
Months	0.004	0.002	0.001	0.007	0.003	0.002
Genotypes	0.007	0.003	0.002	0.0012	0.006	0.004
M X G	0.001	0.005	0.003	0.018	0.008	0.006

4.1.8 Dehydrogenase and superoxide dismutase activity test

Dehydrogenase activity and superoxide dismutase activities decreased significantly as increased in all six genotypes of fenugreek (Table 4.8). More formazan were found in HM-57 (0.528 OD g^{-1} m^{-1}) whereas minimum in FGK-80 (0.490 OD g^{-1} m^{-1}). Superoxide dismutase activities were observed better in HM-57 (0.784 mg protein^{-1}min^{-1}) and less activity in FGK-80 (0.720 mg protein^{-1}min^{-1}).

4.1.9 Electrical conductivity

Electrical conductivity of seed leachates increased significantly in all the six genotypes of fenugreek as the storage period progressed (Table 4.9). At the end of the storage period, HM-57 (351.2 dS/cm/seed) showed minimum leachates whereas maximum leachates were recorded in genotype FGK-80 (376.6 dS/cm/seed).

Table 4.8 Effect of natural ageing on Dehydrogenase and Superoxide dismutase activity test of fenugreek

Dehydrogenase enzyme activity ($OD\ g^{-1}\ m^{-1}$)				Superoxide dismutase activity ($mg\ protein^{-1} min^{-1}$)			
Ageing periods (months)				Ageing periods (months)			
Genotypes	**0**	**18**	**Mean**	**Genotypes**	**0**	**18**	**Mean**
HM-57	0.609	0.528	**0.569**	**HM-57**	0.983	0.784	**0.883**
HM-103	0.600	0.515	**0.557**	**HM-103**	0.977	0.764	**0.87**
HM-273	0.592	0.504	**0.548**	**HM-273**	0.974	0.751	**0.863**
RMT-361	0.591	0.504	**0.547**	**RMT-361**	0.965	0.744	**0.854**
FGK-49	0.580	0.492	**0.536**	**FGK-49**	0.958	0.741	**0.849**
FGK-80	0.576	0.490	**0.533**	**FGK-80**	0.951	0.720	**0.836**
Mean	**0.591**	**0.505**		**Mean**	**0.968**	**0.751**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	**Factors**	**C.D.**	**±SE(d)**	**±SE(m)**
Months	0.004	0.002	0.001	**Months**	0.005	0.002	0.002
Genotypes	0.008	0.004	0.003	**Genotypes**	0.008	0.004	0.003
M X G	NS	0.005	0.004	**M X G**	0.0012	0.006	0.004

Table 4.9: Effect of natural ageing on electrical conductivity test (dS/cm/seed) of fenugreek

Genotypes	Ageing periods (months)		Mean
	0	**18**	
HM-57	295.9	351.2	**323.5**
HM-103	305.5	370.7	**338.1**
HM-273	308.8	374.3	**341.6**
RMT-361	307.6	373.6	**340.6**
FGK-49	313.4	378.1	**345.7**
FGK-80	319.1	376.6	**347.8**
Mean	**308.4**	**370.7**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**
Months	7.161	3.449	2.439
Genotypes	4.135	1.991	1.408
M X G	10.128	4.878	3.439

4.1.10 Seedling emergence index

Effect of natural ageing on seedling emergence index had adverse effect in all six genotypes of fenugreek (Table 4.10). After 18 month of the storage period, HM-57 showed maximum seedling emergence index (7.829) whereas minimum was in FGK-80 (7.465).

Table 4.10: Effect of natural ageing on seedling emergence index of fenugreek

Genotypes	Ageing periods (months)							Mean
	0	3	6	9	12	15	18	
HM-57	8.686	8.592	8.499	8.393	8.292	8.071	7.829	**8.337**
HM-103	8.613	8.541	8.441	8.359	8.258	8.047	7.639	**8.271**
HM-273	8.589	8.470	8.385	8.275	8.169	7.968	7.588	**8.206**
RMT-361	8.588	8.483	8.384	8.286	8.192	7.985	7.584	**8.215**
FGK-49	8.501	8.408	8.303	8.207	8.108	7.909	7.513	**8.136**
FGK-80	8.488	8.398	8.275	8.181	8.076	7.893	7.465	**8.111**
Mean	**8.578**	**8.482**	**8.381**	**8.284**	**8.182**	**7.979**	**7.603**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	0.01	0.005	0.004					
Genotypes	0.01	0.005	0.003					
M X G	0.026	0.013	0.009					

4.1.11 Mean emergence time

Effect of natural ageing on mean emergence time affected adversely in all the six genotypes of fenugreek (Table 4.11). After 18 months of the storage period, HM-57 showed minimum mean emergence time (5.695) whereas maximum in FGK-80 (7.075).

Table 4.11: Effect of natural ageing on mean emergence time of fenugreek

Genotypes	Ageing period (months)							Mean
	0	3	6	9	12	15	18	
HM-57	4.194	4.362	4.477	4.742	4.961	5.395	5.695	**4.832**
HM-103	4.255	4.519	4.78	5.037	5.375	5.542	5.842	**5.05**
HM-273	4.413	4.722	5.222	5.402	5.759	6.193	6.593	**5.472**
RMT-361	4.623	4.892	5.457	5.466	5.768	6.334	6.868	**5.63**
FGK-49	4.667	4.888	5.194	5.556	5.891	6.291	6.657	**5.592**
FGK-80	4.841	5.352	5.57	5.808	6.191	6.558	7.075	**5.914**
Mean	**4.499**	**4.789**	**5.117**	**5.335**	**5.658**	**6.052**	**6.455**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	092	0.046	0.033					
Genotypes	0.85	0.043	0.03					
M X G	0.226	0.113	0.08					

4.12 Seedling establishment

Seedling establishment was found in decreasing order up to 18 months of storage period in all the six genotypes of fenugreek (Table 4.12). After 18 months of ageing period,

maximum seedling establishment was recorded in genotype HM-57 (65.33 %) whereas minimum was in FGK-80 (60.67 %).

Table 4.12: Effect of natural ageing on seedling establishment (%) of fenugreek

Genotypes	Ageing period (months)							Mean
	0	3	6	9	12	15	18	
HM-57	74.67 (59.78)	74.00 (59.32)	73.33 (58.89)	72.33 (58.25)	70.33 (56.98)	67.00 (54.92)	65.33 (53.91)	**71.00 (57.44)**
HM-103	73.00 (58.68)	72.00 (58.03)	71.33 (57.61)	70.33 (56.98)	68.00 (55.53)	65.33 (53.91)	63.67 (52.91)	**69.10 (56.24)**
HM-273	72.67 (58.46)	72.00 (58.03)	71.00 (57.40)	70.00 (56.77)	67.00 (54.92)	63.67 (52.91)	61.67 (51.73)	**68.29 (55.75)**
RMT-361	72.33 (58.25)	72.00 (58.03)	70.67 (57.19)	69.33 (56.36)	67.00 (54.92)	63.67 (52.91)	61.67 (51.73)	**68.10 (55.63)**
FGK-49	71.33 (57.61)	71.00 (57.40)	70.00 (56.77)	68.67 (55.94)	66.33 (54.52)	63.33 (52.52)	61.33 (51.14)	**67.43 (54.95)**
FGK-80	71.67 (57.82)	69.67 (56.56)	69.00 (56.15)	68.67 (55.94)	66.33 (54.52)	63.00 (52.52)	60.67 (51.14)	**67.00 (54.95)**
Mean	**72.61 (58.43)**	**71.78 (57.90)**	**70.89 (57.3)**	**69.89 (56.71)**	**67.50 (55.23)**	**64.33 (53.31)**	**62.39 (52.16)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**					
Months	0.597	0.299	0.212					
Genotypes	0.552	0.277	0.196					
M X G	NS	0.733	0.519					

* Figures in the parenthesis are arc sine value

4.2 To study the physiological and biochemical changes associated with seed deterioration after accelerated ageing.

Accelerated ageing (40±1 ^{0}C for 72 hrs & 100 % RH) treatment was given to the freshly harvested seeds of all six genotypes of fenugreek.

4.2.1 Standard Germination

Germination percentage decreased after natural and accelerated ageing in all the six genotypes of fenugreek (Table 4.13). Effect of ageing was more in artificially aged seed as compared to naturally aged seed. In case of natural ageing, maximum standard germination was observed in genotype HM-103 (90.67 %) and minimum in FGK-80 (86.00 %). In accelerated ageing, maximum germination was recorded in genotype HM-57 (86.33 %) and minimum in FGK-80 (81.67 %).

4.2.2 Seedling length

Seedling length was found lower after artificial ageing in all the six genotypes of fenugreek (Table 4.14). The results indicated that among naturally aged seeds of all six genotypes, HM-57 recorded maximum seedling length (25.47 cm) whereas FGK-80 recorded minimum (22.17 cm). In artificially aged seeds of all six genotypes, HM-57 had maximum seedling length (24.82 cm) and FGK-80 recorded minimum (21.43 cm).

Table 4.13: Effect of natural and artificial ageing on standard germination (%) of fenugreek

Genotypes	Fresh	Natural ageing (18 months)	Artificial ageing	Mean
HM-57	96.33 (79.11)	89.67 (72.53)	86.33 (68.30)	**91.22 (73.31)**
HM-103	95.67 (77.97)	90.67 (72.22)	85.67 (67.74)	**90.67 (72.64)**
HM-273	95.33 (77.51)	88.33 (68.87)	84.00 (65.88)	**88.56 (70.75)**
RMT-361	95.00 (77.22)	87.00 (68.87)	84.67 (66.95)	**88.89 (71.01)**
FGK-49	94.67 (76.67)	87.33 (69.13)	83.00 (65.63)	**88.33 (70.48)**
FGK-80	93.33 (75.02)	86.00 (68.01)	81.67 (65.64)	**87.00 (69.56)**
Mean	**95.06 (77.25)**	**88.17 (69.94)**	**84.33 (66.69)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	
Ageing	0.938	0.461	0.326	
Genotypes	1.326	0.651	0.461	
A X G	NS	1.128	0.798	

*Figures in the parenthesis are arc sine value

Table 4.14: Effect of natural and artificial ageing on seedling length (cm) of fenugreek

Genotypes	Fresh	Natural ageing (18 months)	Artificial Ageing	Mean
HM-57	28.57	25.47	24.82	**25.62**
HM-103	27.70	24.43	23.80	**25.31**
HM-273	27.50	23.47	22.32	**24.43**
RMT-361	27.48	23.30	23.47	**24.75**
FGK-49	26.50	23.65	22.43	**23.99**
FGK-80	26.27	22.17	21.43	**23.51**
Mean	**27.34**	**23.76**	**22.71**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	
Ageing	0.484	0.238	0.168	
Genotypes	0.685	0.336	0.238	
A X G	NS	0.582	0.412	

4.2.3 Seedling dry weight

Seedling dry weight was decreased after artificial ageing in all the six genotypes of fenugreek (Table 4.15). Seedling dry weight was more in natural aged seeds as compared to artificial aged seeds. In natural aged seeds, it was observed that seedling dry weight was higher in HM-57 (7.601 mg) and lower in FGK-80 (6.736 mg). In case of artificially aged seeds, higher seedling dry weight recorded in genotype HM-57 (7.433 mg) and lower in FGK-80 (6.435 mg).

Table 4.15: Effect of natural and artificial ageing on seedling dry weight (mg) of fenugreek

Genotypes	**Fresh**	**Natural ageing (18 months)**	**Artificial Ageing**	**Mean**
HM-57	8.062	7.601	7.433	**7.699**
HM-103	7.735	7.153	6.990	**7.292**
HM-273	7.54	7.050	6.797	**7.129**
RMT-361	7.441	6.973	6.833	**7.082**
FGK-49	7.263	6.796	6.560	**6.873**
FGK-80	7.238	6.736	6.435	**6.803**
Mean	**7.546**	**7.019**	**6.78**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	
Ageing	0.062	0.03	0.021	
Genotypes	0.087	0.043	0.03	
A X G	NS	0.074	0.052	

Table 4.16: Effect of natural and artificial ageing on vigour index-I of fenugreek

Genotypes	**Fresh**	**Natural ageing (18 months)**	**Artificial Ageing**	**Mean**
HM-57	2,752.8	2,317.5	2,142.1	**2,346.6**
HM-103	2,650.0	2,174.7	2,039.0	**2,301.3**
HM-273	2,622.0	2,041.8	1,875.3	**2,174.6**
RMT-361	2,610.2	2,027.2	1,986.2	**2,207.9**
FGK-49	2,508.2	2,013.0	1,862.2	**2,127.8**
FGK-80	2,451.7	1,978.1	1,750.4	**2,064.8**
Mean	**2,599.2**	**2,096.4**	**1,916.0**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	
Ageing	49.284	24.202	17.113	
Genotypes	69.698	34.227	24.202	
A X G	NS	59.283	41.919	

4.2.4 Vigour index-I

Seed vigour index–I was calculated by multiplication of standard germination and seedling length. Vigour index-I was observed more in natural aged seed as compared to artificial aged seed (Table 4.16). The vigour index-I decreased after natural and artificial ageing in all the six genotypes. In case of natural ageing, maximum vigour index-I was found in genotype HM-57 (2317.5) followed by HM-103 (2174.7) and minimum vigour-I index was recorded in FGK-80 (1876.7). After artificial ageing, maximum vigour index-I was recorded in HM-57 (2142.1) followed by HM-103 (2039) and minimum in FGK-80 (1750.4).

4.2.5 Vigour index-II

Seed vigour index–II was calculated by multiplication of standard germination and seedling dry weight. Vigour index-II was observed better in natural aged seed as compared to artificial aged seed (Table 4.17). In case of natural ageing, maximum vigour index-II was found in genotype HM-57 (691.7) followed by HM-103 (636.6). Whereas minimum was in genotype FGK-80 (570.4). In artificial ageing, maximum vigour index-II was recorded in genotype HM-57 (641.7) followed by HM-103 (598.9) and minimum was recorded in FGK-80 (525.6).

Table 4.17: Effect of natural and artificial ageing on vigour index-II of fenugreek

Genotypes	**Fresh**	**Natural ageing (18 months)**	**Artificial Ageing**	**Mean**
HM-57	776.6	691.7	641.7	**686.8**
HM-103	740.0	636.6	598.9	**662.5**
HM-273	718.8	613.4	570.9	**632.9**
RMT-361	706.9	606.6	578.5	**630.7**
FGK-49	687.5	593.5	544.5	**608.5**
FGK-80	675.5	570.4	525.6	**596.4**
Mean	**717.5**	**619.2**	**572.1**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	
Ageing	9.299	4.566	3.229	
Genotypes	13.15	6.458	4.566	
A X G	NS	11.185	7.909	

4.2.6 Tetrazolium test

Tetrazolium test is used as quick seed viability test which is checked on the basis of topographical staining of the living tissues of the seed. The results showed that the viability of the seeds decreased after natural as well as artificial ageing (Table 4.18). But the effect of artificial ageing was more as compared to natural ageing in all six genotypes of fenugreek. In

natural aged seed the highest viability percentage was recorded in genotype HM-57 (91.33 %) and lowest in FGK-80 (85.33 %). In artificial ageing, highest viability was recorded in genotype HM-57 (88.00 %) and lowest was in FGK-80 (83.00 %).

Table 4.18: Effect of natural and artificial ageing on tetrazolium test (%) of fenugreek

Genotypes	Fresh	Natural ageing (18 months stored)	Artificial Ageing	Mean
HM-57	97.67 (81.22)	91.33 (72.89)	88.00 (68.85)	**92.33 (74.32)**
HM-103	97.00 (80.09)	90.00 (72.53)	86.00 (68.01)	**91.00 (73.54)**
HM-273	96.67 (79.47)	88.00 (69.72)	85.67 (66.94)	**90.11 (72.04)**
RMT-361	95.33 (77.61)	88.00 (69.74)	85.33 (67.47)	**89.56 (71.61)**
FGK-49	95.00 (77.16)	88.00 (69.72)	85.00 (67.22)	**89.33 (71.37)**
FGK-80	94.33 (76.28)	85.33 (68.60)	83.00 (66.40)	**87.56 (70.43)**
Mean	**96.00 (78.64)**	**88.83 (70.53)**	**85.33 (67.48)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	
Ageing	0.965	0.474	0.335	
Genotypes	1.365	0.67	0.474	
A X G	NS	1.161	0.821	

*Figures in the parenthesis are arcsine value

4.2.7 Catalase and peroxidase activity test

Catalase and peroxidase activities were decreased after natural and artificial ageing in all six genotypes of fenugreek. Catalase activities were found more in natural aged seed as compared to artificial aged seed (Table 4.20). In catalase activities of natural aged seed, the maximum were found in genotype HM-57 (0.205 mg protein^{-1}min^{-1}) and minimum was recorded in FGK-80 (0.154 mg protein^{-1}min^{-1}). In artificial aged seed, maximum catalase activities were recorded in genotype HM-57 (0.185 mg protein^{-1}min^{-1}) and minimum was recorded in FGK-80 (0.118 mg protein^{-1}min^{-1}). In case of peroxidase activities of natural aged seed, maximum were found in HM-57 (0.397 mg protein^{-1}min^{-1}) and minimum was in FGK-80 (0.306 mg protein^{-1}min^{-1}). Whereas in artificial ageing, maximum peroxidase activities were recorded in genotype HM-57 (0.301 mg protein^{-1}min^{-1}) and minimum was in FGK-80 (0.246 mg protein^{-1}min^{-1}).

Table 4.19: Effect of natural and artificial ageing on catalase and peroxidase activity test of fenugreek

Genotypes	Catalase activity test (mg protein^{-1}min^{-1})			Mean	Peroxidase activity test (mg protein^{-1}min^{-1})			Mean
	Fresh	Natural ageing	Artificial ageing		Fresh	Natural ageing	Artificial Ageing	
HM-57	0.280	0.205	0.185	**0.223**	0.583	0.397	0.301	**0.427**
HM-103	0.269	0.185	0.165	**0.206**	0.574	0.370	0.278	**0.407**
HM-273	0.262	0.179	0.144	**0.195**	0.568	0.348	0.267	**0.394**
RMT-361	0.266	0.169	0.154	**0.196**	0.558	0.360	0.267	**0.395**
FGK-49	0.249	0.168	0.136	**0.184**	0.556	0.352	0.269	**0.392**
FGK-80	0.241	0.154	0.118	**0.171**	0.546	0.306	0.246	**0.366**
Mean	**0.261**	**0.177**	**0.142**		**0.564**	**0.332**	**0.269**	
Factors	**C.D.**	**SE(d)**	**SE(m)**		**C.D.**	**SE(d)**	**SE(m)**	
Ageing	0.004	0.002	0.001		0.004	0.002	0.001	
Genotypes	0.006	0.003	0.002		0.006	0.003	0.002	
A X G	NS	0.005	0.004		0.01	0.005	0.003	

Table 4.20: Effect of natural and artificial ageing on dehydrogenase and superoxide test of fenugreek

Genotypes	Dehydrogenase activity test (OD g^{-1} m^{-1})			Mean	Superoxide activity test (mg protein^{-1}min^{-1})			Mean
	Fresh	Natural ageing	Artificial Ageing		Fresh	Natural ageing	Artificial Ageing	
HM-57	0.609	0.528	0.502	**0.546**	0.983	0.784	0.696	**0.812**
HM-103	0.600	0.515	0.483	**0.533**	0.977	0.764	0.669	**0.799**
HM-273	0.592	0.504	0.476	**0.524**	0.974	0.751	0.661	**0.796**
RMT-361	0.591	0.504	0.477	**0.524**	0.965	0.744	0.670	**0.801**
FGK-49	0.580	0.492	0.465	**0.512**	0.958	0.741	0.654	**0.793**
FGK-80	0.576	0.490	0.450	**0.505**	0.951	0.720	0.628	**0.789**
Mean	**0.591**	**0.505**	**0.476**		**0.968**	**0.763**	**0.664**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**		**C.D.**	**±SE(d)**	**±SE(m)**	
Ageing	0.004	0.002	0.001		0.004	0.002	0.001	
Genotypes	0.006	0.003	0.002		0.005	0.002	0.002	
A X G	NS	0.005	0.003		0.009	0.004	0.003	

4.2.8 Dehydrogenase and superoxide activity test

Intensity of formazan was observed more in natural aged seed as compared to artificial aged seed (Table 4.21). Dehydrogenase activities decreased after natural and artificial ageing in all the six genotypes of fenugreek. In case of dehydrogenase activities of natural aged seed, maximum was recorded in genotype HM-57 (0.528 OD g^{-1} m^{-1}) and minimum was recorded in FGK-80 (0.490 OD g^{-1} m^{-1}) and in artificial aged seed, more dehydrogenase activities were recorded in genotype HM-57 (0.502 OD g^{-1} m^{-1}) and less was recorded in FGK-80 (0.450 OD g^{-1} m^{-1}). In case of superoxide activities during natural ageing, maximum was in genotype HM-57 (0.784 mg protein^{-1}min^{-1}) and minimum was recorded in FGK-80 (0.720 mg protein^{-1}min^{-1}) and in artificial ageing, maximum dehydrogenase activity was recorded in genotype HM-57 (0.696 mg protein^{-1}min^{-1}) and minimum was in FGK-80 (0.628 mg protein^{-1}min^{-1}).

4.2.9 Electrical conductivity

Electrical conductivity of seed leachates increased significantly after natural and artificial ageing in all the six genotypes of fenugreek (Table 4.19). Electrical conductivity is inversely proportion to seed quality. In case of natural aged seed, electrical conductivity of seed leachates was minimum in genotype HM-57 (351.2 dS/cm/seed)) and maximum in FGK-49(378.1 dS/cm/seed). In artificial aged seed, minimum increase in seed leachates was recorded in genotype HM-57 (432.4 dS/cm/seed) and maximum was in FGK-80 (468.1 dS/cm/seed).

Table 4.21: Effect of natural and artificial ageing on electrical conductivity test (dS/cm/seed) of fenugreek

Genotypes	**Fresh**	**Natural ageing**	**Artificial ageing**	**Mean**
HM-57	295.9	351.2	432.4	**359.8**
HM-103	305.5	370.7	447.8	**374.7**
HM-273	308.8	374.3	450.7	**377.9**
RMT-361	307.6	373.6	454.3	**378.5**
FGK-49	313.4	378.1	460.2	**383.9**
FGK-80	319.1	376.6	468.1	**387.9**
Mean	**308.4**	**370.7**	**452.3**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**	
Ageing	0.004	0.002	0.001	
Genotypes	0.005	0.003	0.002	
A X G	NS	0.005	0.003	

4.3 To study the effect of priming on seed quality of marginal seed lots after natural and accelerated ageing.

4.3 (A) To study the effect of priming treatments on seed quality of natural aged seeds of fenugreek.

4.3.1 Standard germination

Effect of various priming treatments were observed on standard germination of natural aged seed of all six genotypes of fenugreek *viz*. HM-57, HM-103, HM-273, RMT-361, FGK-49 and FGK-80. It was observed that natural aged seed was decreased in standard germination continues as ageing period increased (Table 4.22).

Table 4.22 Effect of priming treatments on standard germination (%) of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	91.00 (72.53)	92.33 (73.90)	92.33 (73.90)	91.67 (73.22)	90.00 (71.55)	91.00 (72.53)	**91.39 (72.94)**
HM-103	89.00 (70.61)	91.67 (73.22)	92.33 (73.90)	91.33 (72.87)	89.33 (70.96)	91.00 (72.53)	**90.78 (72.35)**
HM-273	87.00 (68.87)	90.00 (71.63)	91.00 (72.53)	90.00 (71.55)	86.33 (68.30)	89.67 (71.24)	**89.00 (70.69)**
RMT-361	87.00 (68.87)	89.33 (70.96)	89.33 (70.91)	88.33 (70.05)	85.67 (67.74)	87.67 (69.46)	**87.89 (69.67)**
FGK-49	87.33 (69.13)	89.00 (70.61)	90.33 (71.96)	88.00 (69.78)	85.67 (67.74)	88.00 (69.72)	**88.06 (69.82)**
FGK-80	84.67 (66.94)	88.00 (69.72)	89.00 (70.61)	87.33 (69.19)	85.33 (67.46)	87.67 (69.42)	**87.00 (68.89)**
Mean	**87.67 (69.46)**	**90.06 (71.67)**	**90.72 (72.30)**	**89.44 (71.11)**	**87.06 (68.96)**	**89.17 (70.81)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.891	0.446	0.315				
Genotypes	0.891	0.446	0.315				
T X G	NS	1.093	0.773				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

** Figures in the parenthesis are arcsine value

Priming treatments given to natural aged seed (stored up to 18 months) of all the genotypes of fenugreek showed significant improvement in standard germination. All treatments enhanced

standard germination up to significant level. Among the treatments, (T_2) treatment in natural aged seed of all six genotypes was recorded significantly higher as compared to other treatments. Among the genotypes, FGK-80 showed maximum improvement (4.33 %) from 84.67 to 88.00 per cent and minimum was recorded in HM-57 (1.33 %) from 91 to 92.33 per cent.

4.3.2 Seedling length

Priming treatment to natural aged seed of all the genotypes showed significant improvement in seedling length (Table 4.23). The improvement in seedling length in natural aged seed of all six genotypes was significantly higher with (T_2) treatments. Among the genotypes, maximum improvement (3.13 cm) in genotype FGK-80 from 22.17 to 25.30 cm and minimum (2.40 cm) was recorded in HM-57 from (25.47 to 27.87 cm).

Table 4.23: Effect of priming treatments on seedling length (cm) of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	25.47	27.40	27.87	27.87	26.83	27.32	**27.13**
HM-103	24.43	26.60	27.03	26.47	25.60	26.43	**26.09**
HM-273	23.47	25.63	26.40	25.23	25.87	25.92	**25.42**
RMT-361	23.30	25.50	26.30	25.42	25.37	25.40	**25.21**
FGK-49	22.65	24.83	25.25	24.82	24.73	24.83	**24.52**
FGK-80	22.17	24.92	25.30	24.05	24.02	24.18	**24.11**
Mean	**23.58**	**25.81**	**26.36**	**25.64**	**25.40**	**25.68**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.238	0.119	0.084				
Genotypes	0.238	0.119	0.084				
T X G	0.579	0.29	0.205				

* T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.3 Seedling dry weight

Effect of various priming treatments was observed on seedling dry weight of natural aged seed of all six genotypes of fenugreek (Table 4.24). In natural aged seed it was significantly higher with (T_2) treatments in all the genotypes of fenugreek. Among the genotypes maximum improvement (0.368 mg) was recorded in genotype FGK-80 from 6.736 to 7.104 mg and minimum (0.276 mg) was recorded in HM-57 7.601 to 7.877 mg.

Table 4.24:- Effect of priming treatments on seedling dry weight (mg) of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	7.601	7.851	7.877	7.854	7.844	7.853	**7.813**
HM-103	7.153	7.542	7.545	7.529	7.518	7.523	**7.468**
HM-273	7.050	7.557	7.364	7.554	7.486	7.552	**7.427**
RMT-361	6.973	7.383	7.285	7.377	7.365	7.370	**7.292**
FGK-49	6.796	7.134	7.141	7.119	7.118	7.130	**7.073**
FGK-80	6.736	7.119	7.104	7.093	7.084	7.100	**7.039**
Mean	**7.051**	**7.431**	**7.436**	**7.421**	**7.403**	**7.421**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.015	0.008	0.005				
Genotypes	0.015	0.008	0.005				
T X G	0.037	0.019	0.013				

* T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.4 Seed vigour index-I

It was recorded that natural aged seed of all the six genotypes of fenugreek observed significant improvement in vigour index-1, as depicted in (Table 4.25). Among the treatments maximum enhancement was observed with (T_2) treatment in all genotypes of fenugreek. Among the genotypes, maximum improvement (375.1) was recorded in genotype FGK-80 from 1876.7 to 2251.8 and minimum (255.4) improvement was recorded in HM-57 2317.5 to 252.9.

Table 4.25 Effect of priming treatments on seed vigour index-I of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	2,317.5	2,530.1	2,572.9	2,554.0	2,458.4	2,442.0	**2,479.2**
HM-103	2,174.7	2,438.3	2,496.1	2,417.2	2,361.6	2,329.3	**2,369.5**
HM-273	2,041.8	2,307.0	2,402.4	2,270.6	2,237.2	2,319.9	**2,263.2**
RMT-361	2,027.2	2,269.5	2,375.9	2,237.0	2,173.0	2,235.3	**2,219.7**
FGK-49	1,978.1	2,218.6	2,255.6	2,192.1	2,118.8	2,177.0	**2,162.5**
FGK-80	1,876.7	2,192.7	2,251.8	2,100.3	2,049.4	2,120.2	**2,098.5**
Mean	**2,075.2**	**2,326.1**	**2,392.4**	**2,295.2**	**2,233.1**	**2,270.6**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	32.051	16.044	11.345				
Genotypes	32.051	16.044	11.345				
T X G	N/A	39.300	27.789				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.5 Seed vigour index-II

Seed vigour index-II was recorded in natural aged seed of all the six genotypes of fenugreek showed up to a significant level as depicted in (Table 4.26). Among the treatments, maximum enhancement was observed in (T_2) treatment. Among the genotypes, maximum improvement (61.9) was recorded in genotype FGK-80 from 570.4 to 632.3 and minimum (35.6) was in HM-57 from 691.7 to 714.6.

Table 4.26 Effect of priming treatments on seed vigour index-II of natural aged seed in fenugreek

Genotypes	Treatment						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	691.7	724.9	727.3	720.0	705.9	714.6	**714.1**
HM-103	636.6	691.4	696.7	687.6	672.1	684.2	**678.1**
HM-273	613.4	680.1	670.2	679.8	652.0	671.3	**661.1**
RMT-361	606.6	657.1	658.1	649.2	630.9	648.6	**641.8**
FGK-49	593.5	637.3	638.0	628.9	609.8	625.0	**622.1**
FGK-80	570.4	626.4	632.3	619.5	604.5	622.4	**612.6**
Mean	**618.7**	**669.5**	**675.0**	**664.2**	**645.9**	**661.0**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	7.423	3.716	2.628				
Genotypes	7.384	3.696	2.614				
T X G	N/A	9.102	6.436				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

Table 4.27 Effect of priming treatments on tetrazolium test (%) of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	91.33 (72.89)	93.67 (75.40)	94.67 (76.70)	93.67 (75.44)	90.00 (71.55)	92.33 (74.09)	**92.61 (74.35)**
HM-103	90.00 (71.55)	94.00 (75.82)	94.00 (76.05)	93.00 (74.73)	90.00 (71.58)	93.00 (74.73)	**92.33 (74.08)**
HM-273	88.00 (69.72)	90.33 (71.89)	91.33 (72.89)	90.00 (71.55)	86.67 (68.58)	89.33 (70.94)	**89.28 (70.93)**
RMT-361	88.00 (69.74)	91.00 (72.53)	92.33 (73.99)	90.67 (72.26)	87.00 (68.87)	90.33 (71.89)	**89.89 (71.55)**
FGK-49	88.00 (69.72)	91.67 (73.31)	91.67 (73.24)	91.33 (72.86)	87.00 (68.88)	91.67 (73.24)	**90.22 (71.87)**
FGK-80	85.33 (67.46)	89.67 (71.25)	90.67 (72.22)	90.00 (71.59)	85.33 (67.46)	89.67 (71.25)	**88.44 (70.21)**
Mean	**88.44 (70.18)**	**91.72 (73.37)**	**92.44 (74.18)**	**91.44 (73.07)**	**87.67 (69.48)**	**91.06 (72.69)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	1.115	0.558	0.395				
Genotypes	1.115	0.558	0.395				
T X G	NS	1.367	0.966				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

**Figures in the parenthesis are arcsine value

4.3.7 Tetrazolium test

Seed viability showed significant improvement in all the genotypes of fenugreek (Table 4.28). Within treatments, (T_2) treatment was significantly higher in natural aged seed of fenugreek. Among the genotypes, maximum improvement (5.33 percentage) was recorded in FGK-80 from 85.33 to 90.67 percentage and minimum (3.33 percentage) was recorded in HM-57 and HM-273 from 91.33 to 94.67 percentage.

4.3.6 Electrical conductivity

Electrical conductivity was recorded as dS/cm/ seed. Among the treatments, it was found better effect of (T_2) treatment in natural aged seed of all six genotypes of fenugreek (Table 4.27). Within genotypes, maximum improvement (12.66 dS/cm/ seed) was obtained in genotype FGK-80 from 376.6 to 363.3 dS/cm/ seed and minimum (5.40 dS/cm/ seed) was in HM-103.

Table 4.28 Effect of priming treatments on electrical conductivity (dS/cm/seed) of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	351.2	340.5	345.4	343.8	344.7	340.0	**344.6**
HM-103	370.7	359.0	365.3	358.9	366.1	358.6	**363.4**
HM-273	374.3	370.7	365.4	369.2	368.0	375.1	**370.4**
RMT-361	373.6	360.8	364.3	357.1	364.0	360.9	**363.8**
FGK-49	378.1	372.3	368.6	369.4	373.8	372.9	**372.5**
FGK-80	376.6	365.5	363.3	367.7	370.0	367.8	**368.9**
Mean	**370.7**	**361.2**	**356.8**	**361.0**	**364.4**	**362.5**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	4.311	2.158	1.526				
Genotypes	4.311	2.158	1.526				
T X G	N/A	5.286	3.738				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.8 Seeding emergence index

Priming treatments to natural aged seed of all the genotypes of fenugreek showed significant improvement in seedling emergence index (Table 4.29). All treatments enhanced speed of germination at significant level. In all the treatments, (T_2) treatment was recorded higher over other treatments. Among the genotypes, maximum improvement (0.481) in genotype FGK-80 from 7.465 to 7.946 and minimum (0.342) was recorded in HM-57 from 7.829 to 8.171.

4.3.9 Mean emergence time

Priming treatments to natural aged seed of all the genotypes of fenugreek observed significant improvement in mean emergence time (Table 4.30). All the treatments showed

improvement at significant level. Advancement in mean emergence time of natural aged seed significantly higher in (T_2) treatment. Among the genotypes, maximum improvement (0.546) in genotype FGK-80 from 7.075 to 6.529 and minimum (0.285) was recorded in HM-103 from 5.842 to 5.557.

Table 4.29: Effect of priming treatments on seedling emergence index of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	7.829	7.956	8.171	7.965	7.846	7.937	**7.951**
HM-103	7.639	7.923	8.040	7.851	7.835	7.854	**7.857**
HM-273	7.588	7.810	7.948	7.817	7.809	7.714	**7.781**
RMT-361	7.584	7.836	7.948	7.822	7.812	7.714	**7.786**
FGK-49	7.513	7.868	7.974	7.769	7.763	7.664	**7.759**
FGK-80	7.465	7.736	7.946	7.728	7.626	7.741	**7.707**
Mean	**7.6031**	**7.855**	**8.005**	**7.825**	**7.782**	**7.771**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.021	0.01	0.007				
Genotypes	0.021	0.01	0.007				
T X G	0.051	0.025	0.018				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

Table 4.30: Effect of priming treatments on mean emergence time of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	5.695	5.419	5.316	5.419	5.522	5.518	**5.482**
HM-103	5.842	5.675	5.557	5.679	5.771	5.762	**5.714**
HM-273	6.593	6.241	6.124	6.235	6.337	6.334	**6.311**
RMT-361	6.868	6.579	6.516	6.619	6.622	6.618	**6.637**
FGK-49	6.657	6.369	6.343	6.459	6.566	6.557	**6.492**
FGK-80	7.075	6.743	6.529	6.833	6.839	6.834	**6.809**
Mean	**6.455**	**6.171**	**6.064**	**6.207**	**6.293**	**6.271**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.020	0.010	0.007				
Genotypes	0.020	0.010	0.007				
T X G	0.050	0.025	0.018				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.10 Seedling establishment

Priming treatments to natural aged seed of all six genotypes of fenugreek observed significant improvement in seedling establishment (Table 4.31). All the treatments showed improvement at significant level but (T_2) treatment was recorded significantly higher in seedling establishment. Among the genotypes, maximum improvement (5 %) in FGK-80 from 60.67 to 65.67 per cent and minimum (2.67 %) was recorded in HM-57 and HM-273 from 65.33 to 68.00 per cent.

Table 4.31: Effect of priming treatments on seedling establishment (%) of natural aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	65.33 (53.91)	67.67 (55.33)	68.00 (55.33)	66.00 (54.31)	64.67 (53.51)	65.33 (53.91)	**66.17 (54.42)**
HM-103	63.67 (52.91)	66.67 (54.72)	67.67 (55.33)	66.00 (54.31)	66.00 (54.31)	66.33 (54.52)	**66.06 (54.35)**
HM-273	61.67 (51.73)	63.00 (52.52)	64.33 (53.31)	62.67 (52.32)	62.33 (52.12)	63.00 (52.52)	**62.83 (52.42)**
RMT-361	61.67 (51.73)	65.00 (53.71)	65.67 (54.11)	64.67 (53.51)	60.00 (50.75)	64.67 (53.51)	**63.61 (52.89)**
FGK-49	61.33 (51.53)	64.00 (53.11)	65.00 (53.71)	63.67 (52.92)	63.00 (52.52)	63.67 (52.91)	**63.44 (52.78)**
FGK-80	60.67 (51.14)	64.67 (53.51)	65.67 (54.11)	63.67 (52.91)	62.33 (52.12)	63.67 (52.91)	**63.44 (52.78)**
Mean	**62.39 (52.16)**	**65.17 (53.82)**	**66.06 (54.35)**	**64.44 (53.38)**	**63.06 (52.56)**	**64.44 (53.38)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.549	0.275	0.194				
Genotypes	0.549	0.275	0.194				
T X G	NS	0.673	0.476				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

**Figures in the parenthesis are arcsine value

3 (B) Effect of priming treatments on seed quality of artificial aged seeds

4.3.11 Standard germination

Effect of various priming treatments was observed on standard germination of artificial ageing seed of all six genotypes of fenugreek viz., HM-57, HM-103, HM-273, RMT-361,FGK-49, FGK-80 (Table 4.32). It was observed that artificial aged seed more deteriorated than natural aged seed of fenugreek.

Priming treatments given to artificial aged seed of all six genotypes of fenugreek showed significant improvement in standard germination. All treatments also showed

improvement up to significant level. Among the treatments, (T_2) treatment was higher in artificial aged seed than other treatments. Among the genotypes, HM-273 and FGK-80 showed maximum improvement (4.67 %) from 84.00 to 87.67 per cent and minimum was recorded in HM-103(2.33 %) from 85.67 to 88.00 per cen.

Table 4.32: Effect of priming treatments on standard germination (%) of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	86.33 (68.30)	88.33 (70.03)	89.67 (71.24)	89.33 (70.91)	87.00 (68.85)	87.33 69.14)	**88.00 (69.74)**
HM-103	85.67 (67.74)	87.67 (69.44)	88.00 (69.72)	88.33 (70.03)	84.33 (66.67)	87.00 (68.85)	**86.83 (68.74)**
HM-273	84.00 (66.40)	88.33 (70.03)	88.67 (70.32)	86.33 (68.30)	85.33 (67.49)	86.00 (68.01)	**86.44 (68.42)**
RMT-361	84.67 (66.95)	85.00 (67.19)	87.67 (69.48)	87.67 (69.44)	83.67 (66.15)	87.00 (68.85)	**85.94 (68.01)**
FGK-49	83.00 (65.63)	85.67 (67.74)	87.00 (68.85)	85.00 (67.19)	84.00 (66.40)	85.33 (67.46)	**85.00 (67.21)**
FGK-80	81.67 (64.65)	84.33 (66.67)	86.33 (68.30)	85.67 (67.74)	80.33 (63.66)	84.67 (66.92)	**83.83 (66.32)**
Mean	**84.22 (66.61)**	**86.56 (68.52)**	**87.89 (69.65)**	**87.06 (68.93)**	**84.11 (66.54)**	**86.22 (68.20)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.924	0.463	0.327				
Genotypes	0.924	0.463	0.327				
T X G	NS	1.133	0.801				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

**Figures in the value parenthesis are arcsine

4.3.12 Seedling length

Significant positive effect of priming treatments was observed in seedling length of artificial aged seed of all six genotypes of fenugreek (Table 4.33). Maximum improvement in seedling length was recorded in (T_2) treatments. Among the genotypes, FGK-49 showed maximum improvement (4.40 cm) from 22.43 to 26.83 cm and minimum (2.25 cm) was recorded in HM-57 from 24.82 to 27.07 cm.

4.3.13 Seedling dry weight

Significant improvement was observed in seedling dry weight of artificial aged seed of all six genotypes of fenugreek after priming treatments (Table 4.34). All treatments applied in artificial aged seed was significantly higher but (T_2) treatment was best in all over treatments. In case of genotype, maximum significant improvement (0.723 mg) was recorded

in genotype FGK-80 from 6.435 to 7.158 mg and minimum (0.060 mg) was recorded in HM-273 from 6.797 to 6.857 mg.

Table 4.33 Effect of priming treatments on seedling length (cm) of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	24.82	26.41	27.07	26.00	25.25	26.00	**25.92**
HM-103	23.80	26.68	27.73	26.27	26.17	27.05	**26.28**
HM-273	22.32	25.33	26.63	25.35	24.87	25.97	**25.08**
RMT-361	23.47	26.23	26.88	26.17	25.80	26.23	**25.80**
FGK-49	22.43	26.40	26.83	25.70	25.30	26.83	**25.58**
FGK-80	21.43	24.88	25.67	24.23	23.83	24.25	**24.05**
Mean	**23.04**	**25.99**	**26.80**	**25.62**	**25.20**	**26.06**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.485	0.243	0.172				
Genotypes	0.485	0.243	0.172				
T X G	N/A	0.595	0.421				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

Table 4.34 Effect of priming treatments on seedling dry weight (mg) of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	7.067	7.439	7.44	7.438	7.427	7.436	**7.375**
HM-103	6.990	7.158	7.160	7.156	7.152	7.158	**7.129**
HM-273	6.797	6.851	6.857	6.853	6.844	6.855	**6.843**
RMT-361	6.833	7.184	7.189	7.176	7.178	7.184	**7.124**
FGK-49	6.560	6.88	6.892	6.885	6.884	6.885	**6.831**
FGK-80	6.435	7.155	7.158	7.151	7.145	7.15	**7.032**
Mean	**6.78**	**7.111**	**7.116**	**7.110**	**7.105**	**7.111**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.028	0.014	0.010				
Genotypes	0.028	0.014	0.010				
T X G	0.680	0.340	0.024				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.14 Seed vigour index-I

It was recorded that artificial aged seed of all the six genotypes of fenugreek showed significant improvement in vigour index-I (Table 4.35). Maximum improvement was observed in (T_2) treatment in all over treatments. Within the genotypes, maximum

improvement (485.6) was recorded in HM-273 from 1875.2 to 2360.8 and minimum (285.0) was recorded in HM-57 from 2142.4 to 2427.5.

Table 4.35: Effect of priming treatments on seed vigour index-I of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	2,142.4	2,332.5	2,427.5	2,322.4	2,196.5	2,270.4	**2,282.0**
HM-103	2,038.8	2,339.4	2,440.5	2,319.8	2,206.1	2,353.4	**2,283.0**
HM-273	1,875.2	2,238.7	2,360.8	2,188.6	2,122.6	2,233.5	**2,169.9**
RMT-361	1,986.7	2,229.9	2,356.8	2,294.6	2,159.2	2,282.3	**2,218.2**
FGK-49	1,862.2	2,260.4	2,333.9	2,184.6	2,124.9	2,290.2	**2,176.0**
FGK-80	1,750.7	2,098.5	2,216.4	2,076.0	1,915.0	2,053.2	**2,018.3**
Mean	**1,942.7**	**2,249.9**	**2,356.0**	**2,231.0**	**2,120.7**	**2,247.2**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	48.73	24.394	17.249				
Genotypes	48.73	24.394	17.249				
T X G	NS	59.752	42.251				

T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.15 Seed vigour index-II

It was recorded that artificial aged seed of all the six genotypes showed significant improvement in vigour index-II by applied priming treatments (Table 4.36). In all over treatments, maximum improvement was observed in (T_2) treatment. Within the genotypes, maximum improvement (92.5) was recorded in genotype FGK-80 from 525.5 to 618.0 and minimum (31.1) was in HM-103 from 598.9 to 630.0.

Table 4.36: Effect of priming treatments on seed vigour index-II of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	610.2	657.1	667.1	664.5	646.2	649.4	**649.1**
HM-103	598.9	627.5	630.0	632.1	603.1	622.8	**619.1**
HM-273	570.9	605.2	608.0	591.6	584.0	589.6	**591.5**
RMT-361	578.5	610.6	630.2	629.1	600.5	625.0	**612.3**
FGK-49	544.5	589.4	599.6	585.2	578.3	587.5	**580.8**
FGK-80	525.5	603.4	618.0	612.6	574.0	605.4	**589.8**
Mean	**571.4**	**615.5**	**625.5**	**619.2**	**597.7**	**613.3**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	7.128	3.568	2.523				
Genotypes	7.128	3.568	2.523				
T X G	17.460	8.740	6.180				

T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.16 Tetrazolium test

Significant improvement was observed in seed viability of artificial aged seed in all six genotypes of fenugreek after priming (Table 4.37). Among the treatments, (T_2) treatment was significantly higher in all the genotypes. Within the fenugreek genotypes, maximum significant improvement (0.33 %) was recorded in genotype FGK-80 from 84.00 to 89.33 per cent and minimum (1.33 %) was in HM-273 from 86.00 to 87.33 per cent.

Table 4.37: Effect of priming treatments on tetrazolium test (%) of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	86.33 (68.30)	90.33 (71.89)	91.67 (73.31)	89.67 (71.35)	89.67 (71.36)	90.33 (71.89)	**89.67 (71.35)**
HM-103	86.00 (68.01)	88.67 (70.33)	89.67 (71.28)	88.33 (70.03)	89.00 (70.64)	88.67 (70.33)	**88.39 (70.10)**
HM-273	86.00 (68.01)	86.00 (68.01)	87.33 (69.15)	86.33 (68.30)	87.00 (68.87)	87.00 (68.87)	**86.61 (68.53)**
RMT-361	84.33 (66.70)	86.33 (68.30)	87.33 (69.19)	85.67 (67.74)	86.67 (68.58)	87.00 (68.85)	**86.22 (68.22)**
FGK-49	85.00 (67.22)	88.00 (69.72)	89.00 (70.64)	87.67 (69.44)	88.00 (69.75)	88.33 (70.01)	**87.67 (69.46)**
FGK-80	84.00 (66.40)	88.67 (70.32)	89.33 (70.94)	87.00 (68.85)	86.33 (68.28)	87.33 (69.14)	**87.11 (68.99)**
Mean	**85.28 (67.44)**	**88.00 (69.76)**	**89.06 (70.75)**	**87.44 (69.28)**	**87.78 (69.58)**	**88.11 (69.85)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	1.012	0.507	0.358				
Genotypes	1.012	0.507	0.358				
T X G	NS	1.241	0.877				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

**Figures in the parenthesis are arcsine value

4.3.17 Electrical conductivity

Significant improvement was observed in electrical conductivity by applied priming treatments (Table 4.38). In all over treatments, maximum improvement was observed in (T_2) treatment. Within the genotypes, maximum improvement (41.1 ds/cm/seed) was recorded in genotype FGK-80 from 458.1 to 417.0 ds/cm/seed and minimum (23.3 ds/cm/seed) was in HM-273 from 451.7 to 427.4 ds/cm/seed.

Table 4.38: Effect of priming treatments on electrical conductivity (ds/cm/seed) of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	449.1	428.1	423.0	433.1	439.0	427.3	**433.3**
HM-103	447.8	430.0	421.8	435.3	438.7	431.8	**434.2**
HM-273	450.7	433.1	427.4	435.5	436.8	428.0	**435.2**
RMT-361	454.3	427.2	423.6	426.9	442.6	434.0	**434.8**
FGK-49	460.2	435.0	430.1	432.3	443.1	433.6	**439.1**
FGK-80	458.1	426.3	417.0	424.2	435.1	423.9	**430.8**
Mean	**453.4**	**430.0**	**423.8**	**431.2**	**439.2**	**429.8**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	4.13	2.067	1.462				
Genotypes	4.137	2.067	1.462				
TXG	N.S.	5.064	3.581				

* T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.18 Seedling emergence index

Significant improvement was observed in seedling emergence index of artificial aged seed of all six genotypes of fenugreek after priming (Table 4.39). With in the treatments, (T_2) treatment was significantly higher over other treatments. Among the genotypes, maximum improvement (0.235) was recorded in HM-103 from 7.943 to 8.178 and minimum (0.139) was in FGK-80 from 7.878 to 8.017.

Table 4.39: Effect of priming treatments on seedling emergence index of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	7.992	8.179	8.185	8.183	8.174	8.186	**8.150**
HM-103	7.943	8.167	8.178	8.170	8.172	8.175	**8.134**
HM-273	7.781	7.929	7.938	7.931	7.932	7.930	**7.907**
RMT-361	7.882	8.023	8.047	8.027	8.030	8.037	**8.008**
FGK-49	7.820	7.977	7.999	7.978	7.982	7.986	**7.957**
FGK-80	7.878	8.007	8.017	8.003	8.006	8.010	**7.987**
Mean	**7.883**	**8.047**	**8.061**	**8.049**	**8.049**	**8.054**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.005	0.003	0.002				
Genotypes	0.005	0.003	0.002				
T X G	0.013	0.007	0.005				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.19 Mean emergence time

Mean emergence time in artificial aged seed of all six genotypes of fenugreek showed significant improvement (Table 4.40). Among the treatments, (T_2) treatment was significantly higher in all the genotype of fenugreek. Within genotypes, maximum significant improvement (0.298) was recorded in genotype FGK-80 from 6.953 to 6.655 and minimum (0.421) was in HM-57 from 7.236 to 6.815.

Table 4.40: Effect of priming treatments on mean emergence time of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	7.236	6.824	6.815	6.832	6.844	6.819	**6.895**
HM-103	6.975	6.674	6.652	6.659	6.862	6.655	**6.746**
HM-273	6.582	6.246	6.238	6.25	6.449	6.249	**6.336**
RMT-361	6.777	6.46	6.444	6.457	6.655	6.46	**6.542**
FGK-49	6.921	6.461	6.447	6.463	6.753	6.458	**6.584**
FGK-80	6.953	6.678	6.655	6.682	6.883	6.678	**6.755**
Mean	**6.907**	**6.557**	**6.542**	**6.557**	**6.741**	**6.553**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.008	0.004	0.003				
Genotypes	0.008	0.004	0.003				
T X G	0.019	0.009	0.007				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

4.3.20 Seedling establishment

Significant improvement was observed on seedling establishment of artificial aged seed of all six genotypes of fenugreek after priming (Table 4.41). Within treatment, (T_2) treatment was significantly higher in all the genotypes. Among the genotypes, maximum significant improvement (11.67 %) was recorded in genotype FGK-80 from 56.00 to 67.67 per cent and minimum (9.33 %) was recorded in HM-103 from 60.67 to 69.00 per cent.

Table 4.41: Effect of priming treatments on seedling establishment (%) of artificial aged seed in fenugreek

Genotypes	Treatments						Mean
	T0	T1	T2	T3	T4	T5	
HM-57	62.00 (51.92)	71.67 (57.83)	69.67 (56.56)	70.33 (56.98)	65.67 (54.13)	69.00 (56.16)	**68.06 (55.60)**
HM-103	60.67 (51.14)	70.00 (56.77)	69.00 (56.15)	68.33 (55.74)	63.67 (52.91)	66.00 (54.34)	**66.28 (54.51)**
HM-273	58.00 (49.58)	69.33 (56.35)	68.00 (55.53)	67.00 (54.92)	61.67 (51.73)	65.00 (53.72)	**64.83 (53.64)**
RMT-361	56.00 (48.43)	64.33 (53.31)	66.33 (54.52)	64.33 (53.31)	59.67 (50.55)	60.67 (51.15)	**61.89 (51.88)**
FGK-49	57.67 (49.39)	67.33 (55.12)	68.33 (55.74)	66.33 (54.52)	61.33 (51.53)	62.33 (52.13)	**63.89 (53.07)**
FGK-80	56.00 (48.43)	67.00 (54.92)	67.67 (55.33)	66.67 (54.72)	59.33 (50.36)	60.67 (51.14)	**62.89 (52.48)**
Mean	**58.39 (49.82)**	**68.28 (55.72)**	**68.17 (55.64)**	**67.17 (55.03)**	**61.89 (51.87)**	**63.94 (53.11)**	
Factors	**C.D.**	**±SE(d)**	**±SE(m)**				
Treatments	0.781	0.391	0.276				
Genotypes	0.781	0.391	0.276				
T X G	NS	0.957	0.677				

*T_0= Control, T_1= Thiram, T_2= GA_3, T_3=PEG T_4= KNO_3, T_5= $CaCl_2$

**Figures in the parenthesis are arcsine value

CHAPTER-V

DISCUSSION

The seeds of fenugreek are used as condiment and seasoning agent for garnishing and flavoring dishes. The seeds are also medicinally important as they are used in the treatment of several diseases. Seed has been important for agriculture ever since the plants were domesticated. The quality of seed is mainly measured by its genetic purity and capacity to develop into a healthy plant. International Seed Testing Association (ISTA, 2003) has proposed a number of standardized vigour and viability test to check their potential level of activities and performance of the seed during germination and seedling emergence. The quality of the seed and storage capacity are also correlated to each other. This effort will provide an idea about the seed viability and vigour under ambient storage conditions.

The quality of seeds is deteriorated by many factors and a number of parameters are available to assess the quality of seed. These parameters could be used reliably to predict crop establishment under field conditions. Deterioration of seeds during storage is well known, however, the extent of loss is governed by a number of intrinsic and extrinsic factors. The results obtained from this investigation have been discussed in this chapter in the light of information available and work done by previous researchers.

5.1 Effect of natural ageing on quality seed of fenugreek

5.1.1 Physiological parameters

The change in seed viability under ambient storage conditions is a function of complex interaction of genetic constitutions and environmental conditions. In the present investigation, the different genotypes showed variability in standard germination. The standard germination significantly decreased (Figure 5.1) as the periods of ageing increased in all the genotypes of fenugreek. Maximum standard germination (95.06 %) was recorded at initial month of storage and minimum (87.67 %) was recorded after the 18 months of storage. Among the genotypes, maximum standard germination (94.52 %) was recorded in genotype HM-57 followed by HM-103 whereas, minimum (90.67 %) was observed in FGK-80. Akhter *et al*. (1992) observed that reduction in germination percentage was related to chromosomal aberrations that occur due to long term storage conditions. Reduction in germination percentage as time increased can be due to reduction in α-amylase and carbohydrate contents (Bailly, 2004) or denaturation of protein (Nautiyal *et al*., 1985). Similar findings were recorded in sorghum by Kannababu *et al*., (2015); in Indian mustard by Rai *et al*. (2017) and Verma *et al*. (2003); in onion by Kumar, (2004); in coriander by Deshraj *et al*. (2002) and Kumar, (2007 and 2010); in wheat by Singh, (2009) and in four vegetable crops seed (tomato,

carrot, onion and cucumber) by Alhamdan *et al*. (2011). So, it can be concluded that natural ageing has adverse effect on standard germination of fenugreek.

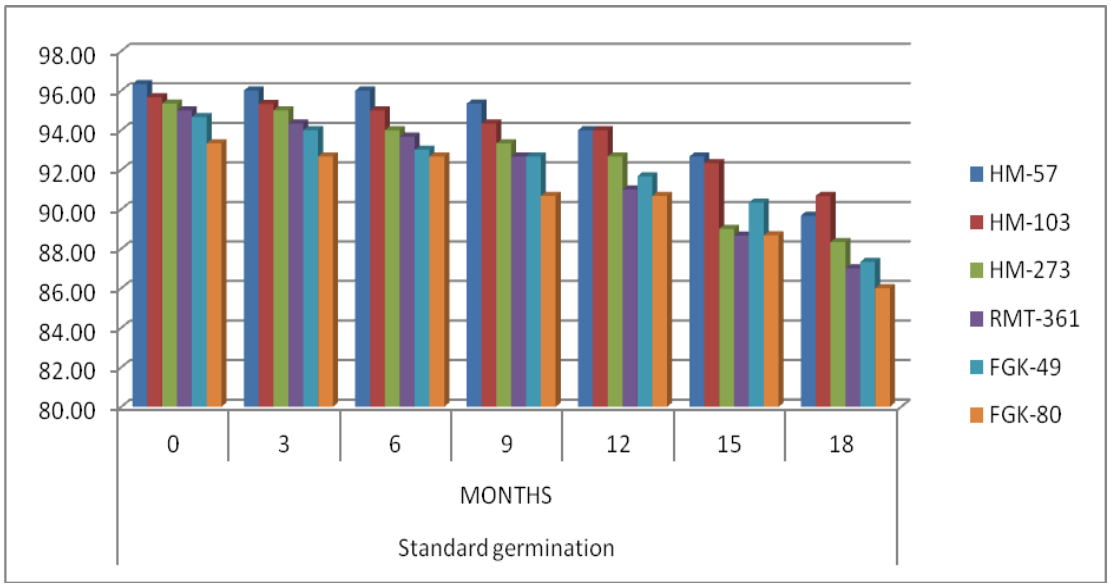

Figure 5.1 Effect of natural ageing on standard germination of fenugreek

Seedling length (cm) in all the genotypes decreased significantly with the rise of ageing periods (Table 4.2). However, maximum seedling length (27.34 cm) was observed at the initial month of storage while minimum seedling length (23.65 cm) was recorded after the 18 months of storage. Among all the six genotypes, HM-57 recorded highest seedling length (26.91 cm) whereas; minimum seedling length (24.21 cm) was recorded in FGK-80. Similar findings were observed in Indian mustard by Rai *et al*. (2017) and Verma *et al*. (2003); in mung bean and urd bean by Singh *et al*. (2003); in chick pea by Kapoor *et al*. (2010); in turnip seeds by Khan *et al*. (2005); in barley by Tabatabaei (2015) and Eisvand (2010).

Seedling dry weight (mg) significantly decreased in all the genotypes due to the passage of time (Table 4.3). Maximum seedling dry weight (7.838 mg) was recorded in genotype HM-57 whereas, minimum seedling dry weight (7.031 mg) was reported in genotype FGK-80. With the passage of time, seedling dry weight was reduced from 7.546 to 7.051 after the 18 months of storage. These results are in accordance with Goodarzian *et al*. (2014) in wheat; in soybean seeds by Mohammadi *et al*., (2011); in Indian mustard by Rai *et al*. (2017) and Verma *et al*. (2003); in okra by Nagarajan *et al*. (2004); in wheat by Singh (2009); in onion by Kumar, (2004) and in oat by Maurya *et al*. (2006).

Seed vigour index-I decreased with the increase of natural ageing period in all the six genotype of fenugreek (Table 4.4). Maximum seed vigour index-I (2599.2) was observed at the initial month of storage, whereas minimum (2075.2) was observed after the 18 month of storage. Among the different fenugreek genotypes, maximum seed vigour index-I (2546.8) was recorded in genotype HM-57 whereas minimum seed vigour index-I (2194.5) was recorded in genotype FGK-80. Seed vigour index-II decreased with the increase of natural

ageing in all the six genotypes of fenugreek. Maximum seed vigour index-II (717.5) was recorded at initial month of storage whereas minimum seed vigour index-II (618.7) was reported after 18 month of storage. Among the different genotypes of fenugreek, maximum seed vigour index-II (741.2) was recorded in genotype HM-57 whereas minimum seed vigour index-II (636.6) was recorded in FGK-80. Similar findings for vigour index-I and vigour index-II were reported in pea by Rajkumar *et al*. (2004); in bitter gourd by Kanwar *et al*. (2014); in maize by Basu *et al*. (2004); in rapeseed and mustard by Suma *et al*. (2013), Rai *et al*. (2017) and Verma *et al*. (2003); in chickpea by Kapoor *et al*. (2010); in turnip by Khan *et al*. (2005); Kannababu *et al*. (2015) in sorghum; in garden pea by Pandita *et al*. (2002) and in onion by Dahiya *et al*. (2006) and Suthar *et al*. (2006).

Tetrazolium test is the most important method for evaluating seed quality. It was developed by Lakon (1942). Tetrazolium salt stains all living embryo red, thus enabling trained researchers to determine the seed viability. The present investigation resulted that the viability of fenugreek seeds decreased after increasing the ageing duration (Figure 5.2). Maximum seed viability (96.00 %) was observed at the initial month of storage whereas minimum seed viability (88.44 %) was recorded after the 18 months of storage. Among the different genotypes of fenugreek, maximum seed viability (95.14 %) was recorded in genotype HM-57 whereas, minimum seed viability (90.76 %) was observed in genotype FGK-80. Seeds lost their viability during storage due to the limited water uptake were reported during imbibition (Balesevic *et al*., 2005). These results are similar in accordance with Verma *et al*. (2003) and Rai *et al*. (2017) in Indian mustard; in cotton by Lather *et al*. (2010); Kapoor *et al*. (2010) in chickpea; in tomato seeds by Santos *et al*. (2007) and in coriander by Kumar (2010) and Deshraj (2002).

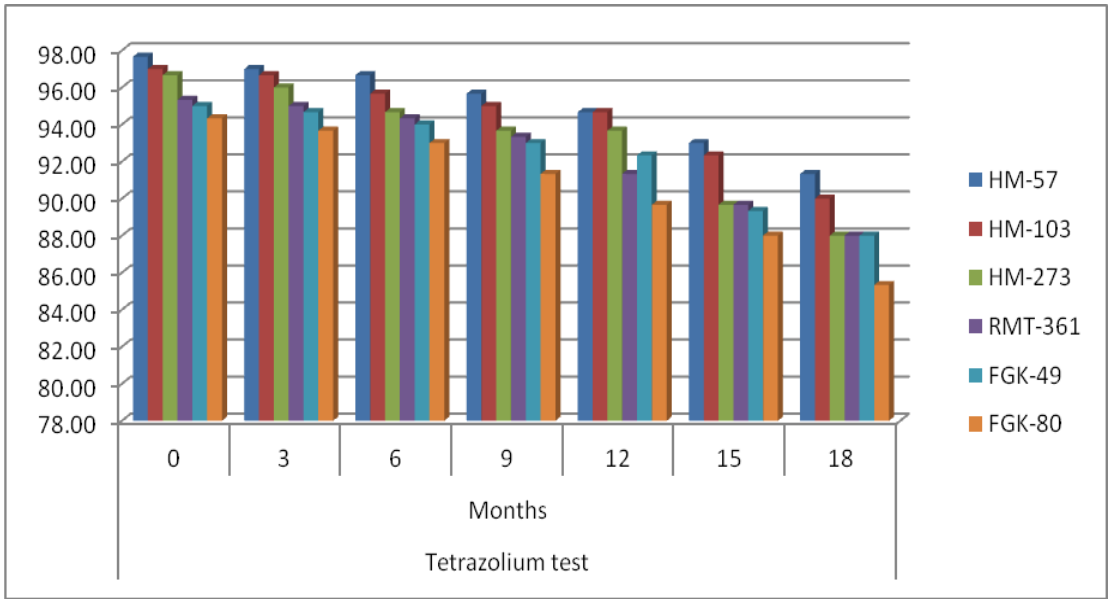

Figure 5.2 Effect of natural ageing on tetrazolium test of fenugreek

5.1.2 Biochemical Parameters

Catalase and peroxidase activity (mg protein^{-1} min^{-1}) decreased as the advancement of natural ageing in all the six genotypes of fenugreek (Figure 5.3). Among the different genotypes, maximum catalase (0.242) and peroxidase activity (0.490) was recorded for the genotype HM-57 whereas minimum catalase (0.197) and peroxidase activity (0.426) in the genotype FGK-80. Among the different ageing durations, maximum catalase (0.261) and peroxidase activity (0.564) was recorded at the initial month of storage while minimum catalase (0.177) and peroxidase activity (0.356) was observed after the 18 months of storage. Rajjou *et al*., (2008) suggested that natural ageing induced deterioration increase the extent of protein oxidation, loss of functional properties of protein and enzymes. Based on research, it is a evident that many enzymes could be seriously degraded because of seed ageing and that others failed to activate normal levels during germination. These results are also in accordance with the finding of Bhanuprakash *et al*. (2010) in bell pepper; Verma *et al*. (2003) and Rai *et al*. (2017) in Indian mustard; in sunflower by Pallavi *et al*. (2003); Singh *et al*. (2015) in fenugreek; in peanut by Sung and Jeng (1994); in onion by Demirkaya and Sivritepe (2010); in sunflower by Balesevic *et al*. (2005); in alfalfa by Cakmak *et al*. (2010).

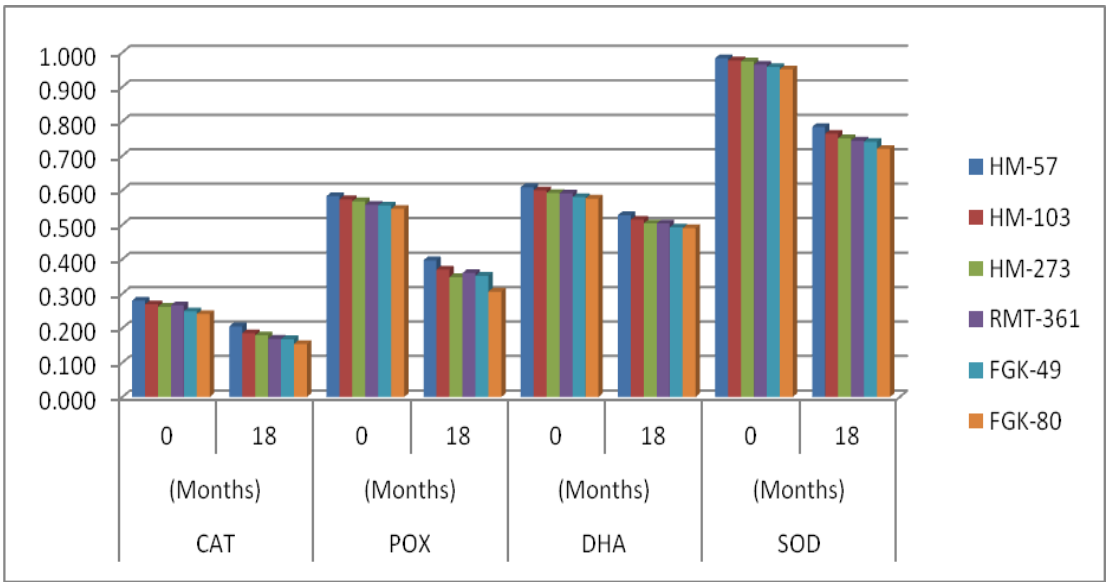

Figure 5.3: Effect of natural ageing on catalase, peroxidase, dehydrogenase and superoxide dismutase activities test of fenugreek

The permeability of membrane has been reported to increase upon seed storage which is expressed by increased loss of electrolytes, sugars, amino acid and phenols in the seed leachates during imbibitions (Deswal and Sheoran, 1993). Investigation on electrical conductivity (dS/cm/Seed) of seed leachates assessed for their storability and quality has also given good idea of seed quality. The electrical conductivity of seed leachates was found higher (370.7) after the 18 months of storage whereas lower electrical conductivity of seed leachates (308.4) was reported at initial month of storage. Minimum electrical conductivity of

seed leachates (323.5) was found in genotype HM-57 and maximum (347.8) was recorded in genotype FGK-80. Similar findings were recorded in coriander by Kumar *et al*. (2017); in ground nut by Swara *et al*. (2002); Khan *et al*. (2005) reported in onion; in sweet pepper by Kaewnaree *et al*. (2011); in soybean by Mohammadi *et al*. (2011); in karanj by Kumar *et al*. (2011); in radish by Jain *et al*. (2006) and Verma *et al*. (2003) in Indian mustard.

The optical density of formazan (OD g^{-1} ml^{-1}) and superoxide dismutase activity (mg $protein^{-1}$ min^{-1}) was decreased with the advancement of ageing duration in all the six genotypes of fenugreek (Figure 5.4). Among the different ageing durations, maximum optical density of formazan (0.591) and superoxide dismutase activity (0.968) was recorded with the initial month of storage while minimum optical density of formazan (0.505) and superoxide dismutase activity (0.751) was recorded after the 18 months of storage. Among the different genotypes, HM-57 was found maximum optical density of formazan (0.569) and superoxide dismutase activity (0.883). Similar results were found in soybean, maize and mustard by Dey and Mukherjee (1986); in karanj by Sahu *et al*. (2017); in coriander by Kumar *et al*. (2017); in Indian mustard by Rai *et al*. (2017) and Verma *et al*. (2003); Pallavi *et al*. (2003) in sunflower and in wheat by Chauhan *et al*. (2011).

5.1.3 Field parameters

Seedling emergence index declined as a period of natural ageing increased in all the different genotypes of fenugreek. Maximum seedling emergence index was recorded (8.578) at the initial month of storage whereas minimum seedling emergence index (7.603) was recorded after the 18 months of storage. Among the different fenugreek genotypes HM-57 was found to have higher seedling emergence index (8.337) as compared to other genotypes. The present investigation confirms the finding of Khajeh-Hosseini (2010) in rapeseed and mustard; in mungbean by Verma *et al*. (2006); and in wheat by Soltani *et al*. (2008 and 2009).

Mean emergence time (MET) increased in all the six genotypes of fenugreek with the passage of time. Significant differences were observed for the effect of naturally aged fenugreek seed on mean emergence time. Among the different ageing durations, maximum mean emergence time (6.455) after the 18 month of storage whereas minimum mean emergence time (4.499) was observed at the initial month of storage. Among all the six genotypes, minimum mean emergence time was observed for HM-57 and maximum (5.914) was recorded in the genotype FGK-80. Similar findings were observed in fenugreek by Singh *et al*., (2015) and Rai *et al*. (2017) in Indian mustard.

Seedling establishment decreased as the periods of natural ageing increased in all the six genotypes of fenugreek (Figure 5.4). Among the different ageing durations, maximum seedling establishment (72.61 %) was observed at the initial month of storage whereas minimum (62.39 %) was recorded after the 18 month of storage. Among the different fenugreek genotypes, maximum seedling establishment (71.00 %) was observed in the

genotype HM-57 while minimum (67.00 %) was observed in the genotype FGK-80. Similar findings were observed in fenugreek by Singh *et al*. (2015) and Rai *et al*. (2017) in Indian mustard.

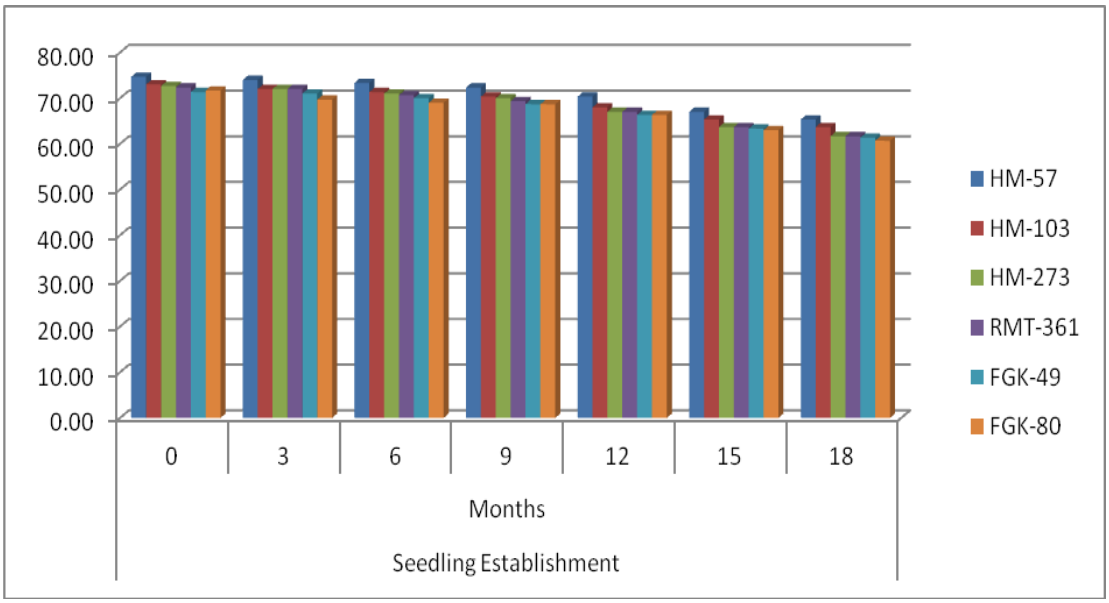

Figure 5.4: Effect of natural ageing on seedling establishment of fenugreek

5.2 Artificial ageing on seed quality of fenugreek

In artificial ageing test, seed exposure to high temperature and high relative humidity leads to the loss of vigour and eventually viability and that is an excellent method to determine the vigour changes during seed storage (Tian *et al*., 2008).

5.2.1: Physiological parameters

The results of the present investigation revealed that the germination and vigour of the seeds decreased after artificial ageing (Figure 5.4). Effect of ageing was found more in artificially aged seed as compared to naturally aged seed. Maximum reduction (11.66 %) was recorded in genotype FGK-80 from (93.33 to 81.67 %) and minimum (10.00 %) was in HM-57 from (96.33 to 86.33 %). Same trends were recorded for seedling length and seedling dry weight. Vigour index-I & II indicated more degradation in artificial aged seed as compared to natural aged seed. Maximum reduction (701.3) was recorded in genotype FGK-80 and minimum (610.7) was recorded in HM-57. Same trend of vigour index-II was recorded in artificially aged seeds in all genotype of fenugreek. Maximum reduction of seed viability was observed in artificial aged seed as compared to natural aged seed. Maximum reduction (11.33 %) was observed in genotype FGK-80 from 94.33 to 83.00 per cent and minimum (10.00 %) in RMT-361 from 95.33 to 85.33 per cent. Among different genotypes, artificial aged seed revealed that ageing of seed is characterized by the loss of germination, reduced seedling dry weight and poor seedling length development (Lekic, 2003 and Tatic *et al*., 2012).

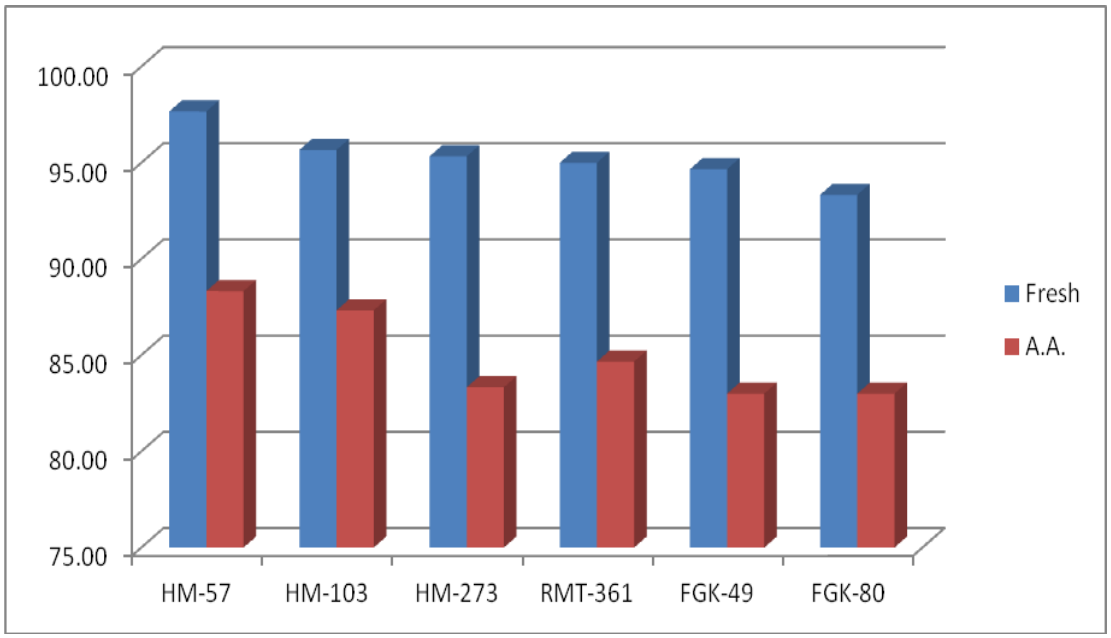

Figure 5.5: Effect of artificial ageing on standard germination of fenugreek

These results are in accordance with Kaewnaree *et al*. 2011 in sweet peper; in sunflower by Hussein *et al*. 2012 and vijay *et al*. 2015; in rice by Kapoor *et al*. 2011, Bobak *et al*. 2015, Somasundaram and Bhaskaran 2017; in sunflower by Kapilan 2015, Hussein *et al*. 2012 and in cotton by Iqbal *et al*. 2002 and Sudharani and Padmasri 2014; in *Dendrocalamus brandisii* by Lakhsmi *et al*. 2014; in rapeseed and mustard by Abdolahi 2012; in radish by Jain *et al*. 2006.

5.2.2 Biochemical parameters

Catalase and peroxidase activities were decreased after natural and artificial ageing in all six genotypes of fenugreek (Figure-5.6). Maximum reduction (0.123) in catalase activity was found in genotype FGK-80 from 0.241 to 0.118 whereas minimum reduction (0.155) was observed in HM-57 from 0.280 to 0.185. Maximum reduction (0.300) in peroxidase activity was observed in FGK-80 from 0.546 to 0.246 while minimum in (0.282) HM-57 from 0.583 to 0.301. Same trend of reduction was observed in dehydrogenase & superoxide dismutase activities in all the six genotypes of fenugreek. Maximum reduction (0.126) in dehydrogenase activity was observed in FGK-80 from 0.576 to 0.450 and minimum (0.107) was in HM-57 from 0.609 to 0.502. In Superoxide dismutase activity, maximum reduction (0.323) was found in genotype FGK-80 from 0.951 to 0.628 whereas, minimum reduction (0.287)) was observed in HM-57 from 0.983 to 0.696. Similar results were found in rice by Kapoor *et al*. 2011; by Radha *et al*. 2014 in maize; in cotton by Goel *et al*. 2003; in barley by Tabatabei 2015; in sunflower by Balesevic *et al*. 2005; in alfalfa by Cakmak *et al*. 2010.

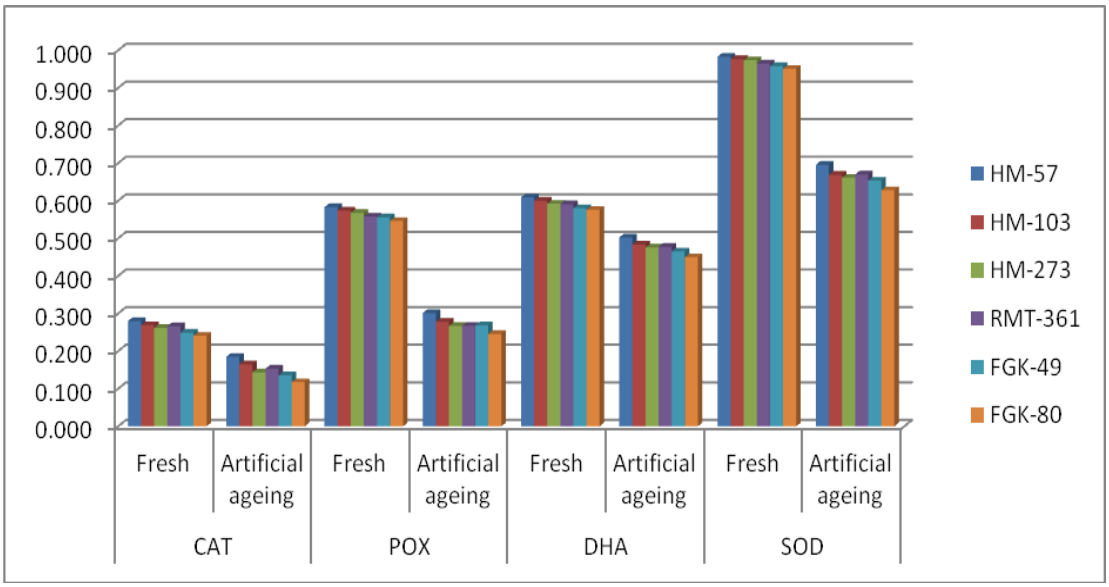

Figure 5.6: Effect of artificial ageing on catalase, peroxidase, dehydrogenase and superoxide dismutase activities test of fenugreek

5.3: Effect of priming treatments on seed quality of naturally and artificially aged seed of fenugreek

Uniform and rapid seedling emergence is a vital pre-requisite to increase yield, quality and finally enhanced economic benefits. Rapid, uniformity and percentage of field emergence of direct seeded crops have a major effect on yield and quality (Wurr and Fellow, 1983). Uniformity and rate of seedling emergence are innate to seed quality and environmental conditions.

Different pre-sowing seed treatments have been used to decrease the time between sowing and seedling emergence to enhance synchronization of field emergence in annual as well as perennial crops. Seed priming have been related to the repair and build-up of nucleic acid, increase in the synthesis of protein, osmotic adjustment mechanism, the repair of membrane and reduced lipid peroxidation, resulting from enhanced anti oxidative activities.

The significant differences were observed of different priming treatments on standard germination and other quality parameters of naturally and artificially aged seeds of different genotypes of fenugreek. Seed priming with different priming treatments were found effective to improve the quality in marginal seed of fenugreek. Seed priming with GA_3 (50 ppm) was found best priming treatment for enhancing seed quality followed by hydropriming and dry dressing with thiram, hydration with PEG (6000) and hydration with $CaCl_2$ (0.2 %) as compared to control.

5.3.1 Physiological parameters

Standard germination is a test indicating the capability of a seed to produce normal seedling under ambient sowing conditions, whereas, vigour index determines the actual germinability of seed under normal as well as adverse climatic conditions. Maximum standard

germination (90.72 and 87.89 %), seedling length (26.36 and 26.80 cm), seedling dry weight (7.436 and 7.116 mg), vigour index-I (2392.4 and 2356.0), vigour index-II (675.0 and 625.5) and seed viability (92.44 and 89.06 %) were observed with priming treatment 'T_2' in both naturally and artificially aged seed of fenugreek respectively (Figure 5.7). This may be due to the fact that gibberellins promote germination and play an important role in mobilization of endosperm reserve during germination of seeds (Weiss and Ori, 2007). The gibberellins also promote the indication of a number of cell wall hydrolysis and released proteins (Bradford *et al*., 2000). The treatment of family Graminae seeds with GA_3 induces proteins in aleuronic layer and rapid hydrolysis of starch in the endosperm and appearance of free phosphate in the seed. Results of present investigation are in conformity with the finding of Rahman *et al*. (1996) and Brar *et al*. (2015), who had suggested the increased standard seed germination and greater vigour index with the supply of biofertilizers. Seed priming with biofertilizers resulted in increased cytokinin production, which actively involves the cell division (Suma *et al*., 2014). Similar findings were observed in maize by Ahmad *et al*., (2012); in soybean by Ahmadvand *et al*. (2012); in bromus seeds by Tavili *et al*. (2012); in *Capsicum chinense* by Adebisi *et al*. (2015); in onion by Selvarani *et al*. (2011); Pandita and Nagarajan (2004) in bittergourd; Singh *et al*. (2017) in coriander; Hammouda *et al*. (2017) in *Cucurbita pepo*; in sunflower by Poonam *et al*. (2006); Pandey *et al*. (2017) in cucumber.

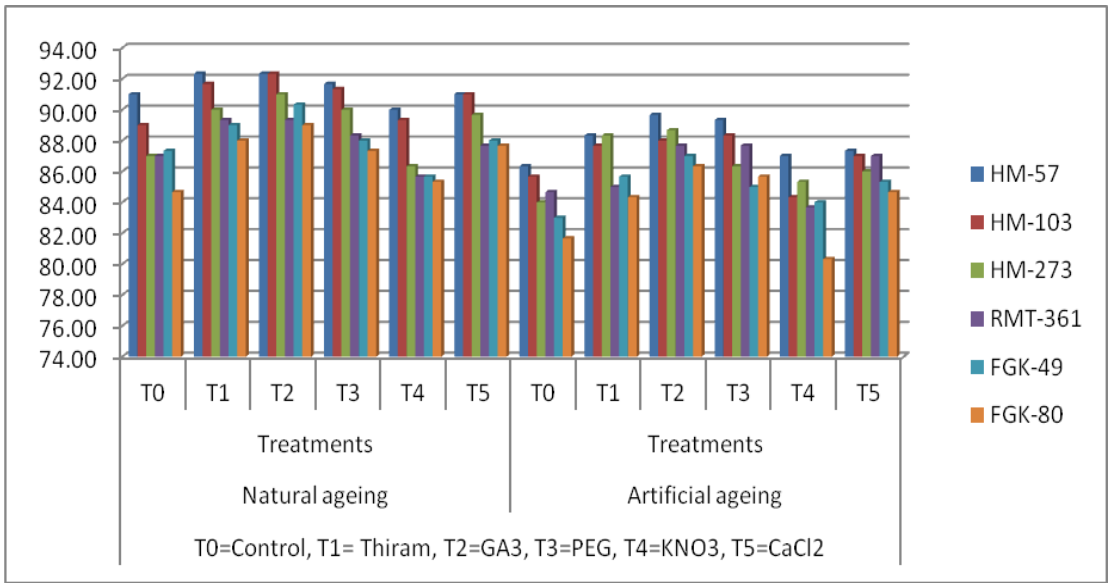

Figure 5.7: Effect of priming treatments on standard germination of naturally and artificially aged seed of fenugreek

A combination of standard germination along with seedling length and seedling dry matter gives a broad evaluation of seed vigour of fenugreek seeds. Vigour index offers a seed to be characterized into different classes of seed quality which will have more numerical value than specific value or percentage and more specially it directly, affected the planting value of the seed. The vigour index defined based on seedling length and seedling dry weights

have been classified as vigour index-I and Vigour index-II, respectively. The experiment of seed priming revealed that there is a significant enhancement in naturally aged seed of all the six genotypes of fenugreek.

5.3.2 Biochemical parameters

The electrical conductivity of seed leachates have been found to be higher in naturally and artificially aged seeds of fenugreek, indicating deterioration of cell permeability and degradation of food reserves in seed (Delouche and Baskin, 2016). In the present investigation, significantly increased electrical conductivity of seed leachates of all the six genotypes was reported with the advancement of natural and artificial ageing, whereas, seed priming treatments reduced the electrical conductivity in all the six genotype of fenugreek (Figure 5.8). Priming treatment 'T_2' (GA_3 50 ppm) was found best for decreasing the value of electrical conductivity in all the different genotypes of fenugreek.

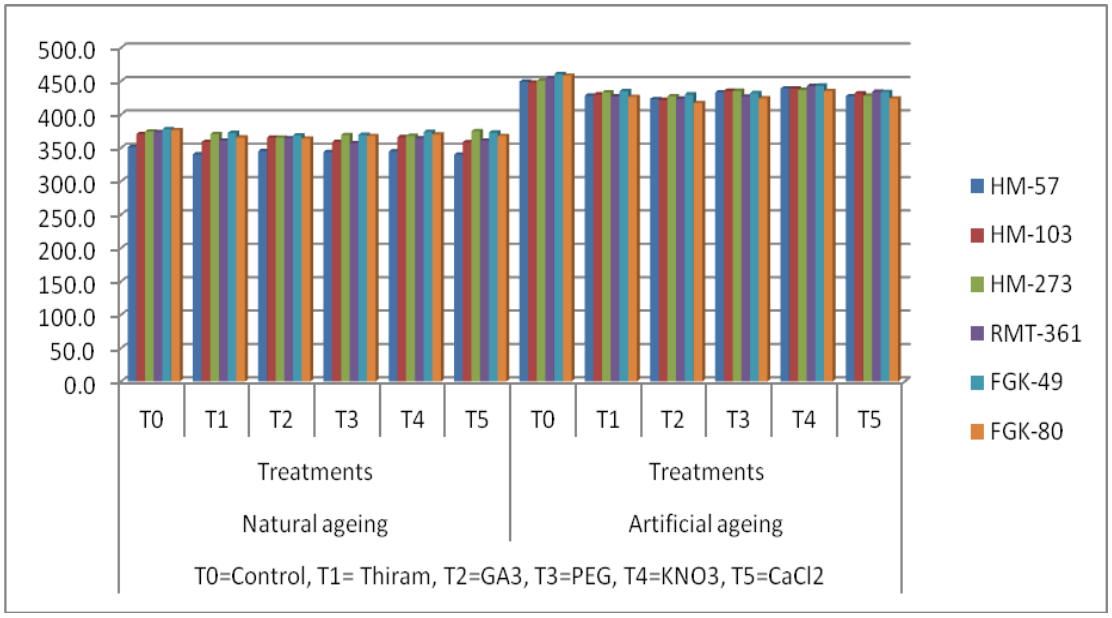

Figure 5.8: Effect of priming treatments on electrical conductivity of naturally and artificially aged seed of fenugreek

Maximum value of electrical conductivity (370.7 and 453.0 dS/cm/seed) was found in both naturally and artificially aged seed of fenugreek respectively whereas minimum value of electrical conductivity of seed leachates (356.8 and 423.8 dS/cm/seed) was observed with priming treatment 'T_2' in both naturally and artificially aged seeds of fenugreek. The electrical conductivity of naturally and artificially aged seed was decreased with various seed priming treatments. These results are same in accordance with Kalsa *et al*. (2011) in vetch; in brinjal by Satishkumar (2005); Yogananda *et al*. (2004); in soybean by Ahmadvand *et al*. (2012); Hammouda *et al*. (2017) in *Cucurbita pepo*; in sunflower by Poonam *et al*. (2006); Pandey *et al*. (2017) in cucumber and Singh *et al*. (2015) in fenugreek.

5.3.3 Field parameters

Seed priming treatments have been applied for seedling emergence index, mean emergence time and seedling establishment to reduce the time between seed sowing and seedling emergence (uniform and rapid germination) and to improve synchronization of emergence in many crops, so that good plant population and higher yield can be obtained. Maximum seedling emergence index (8.005 and 8.061), seedling establishment (66.06 %) (68.17 %), whereas lowest mean emergence time (6.064 and 6.542) were observed with priming treatment 'T_2' in both naturally and artificially aged seed of fenugreek respectively (Figure 5.9). The positive effect of seed priming on seedling emergence index and seedling establishment may be due to the improved hydration of all parts of seed and thus decreasing the damage of embryonic axis (Ramadevi and Gopalkrishan, 2001). Similar findings were observed in fenugreek by Singh *et al*. (2015) in fenugreek and in coriander by Singh *et al*. (2017).

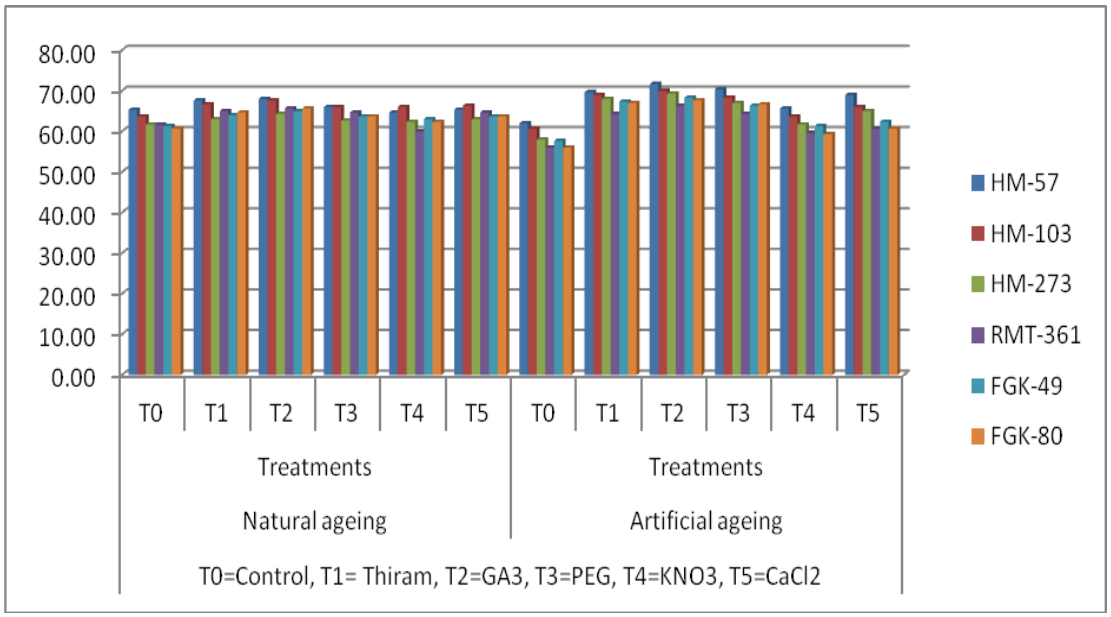

Figure 5.9: Effect of priming treatments on seedling establishment of naturally and artificially aged seed of fenugreek

CHAPTER-VI

SUMMARY AND CONCLUSION

Fenugreek is an important spice crop of many tropical and sub-tropical countries of the world. The high relative humidity and high temperature of the tropics and sub-tropics are most favorable conditions for seed deterioration during storage. Retention of seed quality, its viability and vigour during storage has been great concern to seed producers. With the increasing awareness of cultivators about the use of good quality seed, there is need to have some reliable parameters to evaluate seed quality. During the past decade, seed viability, vigour and other biochemical parameters have gained wide importance in seed quality programme.

In the first experiment, six genotypes of fenugreek were stored in cotton bag under ambient condition and studied physiological, biochemical and field parameters after each 3 months interval up to 18 months. In the second experiment, seeds of six genotypes were subjected to artificial ageing and compared with 18 months naturally stored seed. In third experiment, all marginal seed lots of both natural and artificial aged were subjected to different seed priming treatments *viz*., hydration for 16 hours followed by dry dressing with Thiram (0.25 %) and hydration with GA_3 (50 ppm), PEG (6000), KNO_3 (0.5 %), $CaCl_2$ (2%) and drying at room temperature .

The important achievements of these experiments are here as under:

- During the periods of natural ageing, standard germination, seedling length, seedling dry weight, vigour index-I, vigour index-II, viability by T.Z test, seedling emergence index, mean emergence time and seedling establishment decreased significantly with the passage of storage under natural condition.
- Activity of all anti-oxidant enzymes *viz*., catalase, peroxidase, dehydrogenase, superoxide dismutase decreased during natural ageing also.
- After natural ageing, maximum standard germination was retained by genotype HM-57 followed by HM-103 under laboratory conditions, while maximum deterioration was recorded in genotype FGK-80.
- In field parameter like seedling emergence index and seedling establishment decreased with natural ageing.
- Quality of seeds was more degraded in artificial ageing as compared to natural ageing.
- At the end of storage period of natural ageing, activity of all these enzymes were recorded higher than artificial ageing which revealed that the activity of these enzymes were badly affected by artificial stress conditions.

- Electrical conductivity of seed leachates and mean emergence time of all the genotypes of fenugreek increased with the periods of natural and artificial ageing, which indicated that the loss of membrane integrity of seed coat took place at faster rate with artificial ageing.
- Among different genotypes under study, FGK-80 had fast reduction of physiological and biochemical parameters, while, HM-57 was found to be a good storer genotypes.
- All seed priming treatments enhanced the seed quality in relation to all physiological parameters in naturally and artificially aged seed of different genotypes of fenugreek and these treatments enhanced better seed quality in artificial ageing as compared to natural ageing.
- Among all priming treatments, hydration with GA_3 (50 ppm) was reported superior followed by thiram for enhancing the seed quality of all the seed lots of different genotypes in fenugreek under both conditions (natural and artificial).

CONCLUSION

- The seeds of all the genotypes of fenugreek lost their viability and vigour with the advancement of natural ageing under ambient storage conditions. Among genotypes of fenugreek under study, HM-57 had comparatively better storability, followed by HM-103 whereas FGK-80 was recorded poor storer.
- The enzymes activities *viz*., CAT, POD, DHA and SOD decreased significantly with the advancement of natural storage periods in all the genotypes of fenugreek. The maximum performance of standard germination and enzymes activities were observed in fresh seed of all genotypes.
- Seed priming with different treatments were found effective to enhance the seed quality in marginal seeds of naturally and artificially aged. Performance of marginal seed was observed better over all the other seed lot.
- Artificial aged seeds were more degraded as compared to naturally aged and enhancement was observed more in artificial aged seed as compared to naturally aged seed due to priming treatments.
- Among seed priming treatments, GA_3 was found better performing treatment for improving the seed quality followed by thiram @ 0.25% hydration and dry dressing with PEG (6000) and $CaCl_2$ (2%) respectively where as KNO_3 (0.5 %) showed negative results.
- Among the genotypes, FGK-80 and FGK-49 were found better responsive to GA_3 followed by thiram whereas, genotype HM-57 showed less response to GA_3 as compared to other genotypes.

Therefore, from present investigation, it is concluded that standard germination, vigour indices, biochemical test and electrical conductivity test could be used as reliable

predictors of seed quality due to quickness, accuracy and easiness in their execution. Further, various seed priming treatments can be used for improving seed quality of marginal seed lots under both conditions (natural and artificial). In overall, the priming technology was found effective and beneficial for enhancing the physiological, biochemical and storage potential of fenugreek seeds.

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ABSTRACT

Title of Dissertation	:	**Seed quality assessment in natural and artificially aged seed of fenugreek (*Trigonella foenum-graecum* L.)**
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Major Subject	:	Seed Science & Technology
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Key words: Fenugreek, Thiram, GA_3, PEG, $CaCl_2$, KNO_3 Dehydrogenase, Standard germination, Tetrazolium, Accelerated ageing, Electrical conductivity, Seedling establishment.

The present study entitled **"Seed quality assessment in natural and artificially aged seed of fenugreek (*Trigonella foenum-graecum* L.)"** was carried out in the Department of Seed Science & Technology at CCS Haryana Agricultural University, Hisar (Haryana). The experiments were laid out in factorial Complete Randomized Design (CRD) and Randomized Block Design (RBD) for laboratory and field parameters, respectively on six genotypes of fenugreek *viz*., HM-57, HM-103, HM-273, RMT-361, FGK-49, FGK-80. The present investigation was carried out in three different experiments to meet out the objectives of study. In the first experiment, To assess the seed quality during natural ageing at 3 months interval up to 18 months and check physiological and biochemical parameters *viz*., standard germination, seedling length, seedling dry weight, vigour index-I & II, viability test (Tz %) catalase (CAT) peroxidase (POX), superoxide dismutase (SOD), dehydrogenase enzyme activity parameters were decreased significantly with the advancement of natural ageing of seeds whereas, electrical conductivity of seeds leachates increased with the advancement of time. The field parameters *viz*., seedling emergence index and seedling establishment were also decreased significantly with the advancement of time whereas, mean emergence time increased with the passage of time. In the second experiment, to study the physiological and biochemical change after accelerated ageing of all six genotypes of fenugreek. Effect of artificial ageing was more as compared to natural ageing to degradation fresh seed. In the third experiment effect of priming on marginal seed of natural and artificial aged seed of all six genotypes of fenugreek. Seed priming treatments *viz*., T_0: Control, T_1: hydro-priming followed by dry dressing with thiram @ 0.25%, T_2: Hydration with GA_3 (50 ppm), T_3: Hydration with PEG (6000), T_4: Hydration with KNO_3 (0.5%), T_5: 2% $CaCl_2$ were analyzed to identify the suitable priming treatment. Among various priming treatments 'T_2'- hydration with GA_3 @ 50 ppm followed by 'T_1'-Thiram performed best to enhance all the seed vigour and viability characteristics and to lower down the electrical conductivity of naturally and artificially aged seed of fenugreek. The seed quality improvement through seed priming was noticed more in marginal seed *i.e.* artificial aged seed. Among the different genotypes, maximum enhancement was observed in FGK-80 followed by FGK-49 whereas, minimum enhancement was observed in genotype HM-57 during the study. In conclusion, the present study revealed that fenugreek seed lose its viability and vigour with the advancement of storage time and seed priming with GA_3 @ 50 ppm and thiram can be used as an effective tool to enhance vigour and viability of seed.

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I, hereby, declare that all the information given in the resume is true to the best of my knowledge.

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I, **Sunil Kumar,** Admn. No**. 2014A46D**, undertake that I give copy right of my thesis entitled, **"Seed quality assessment in natural and artificially aged seed of fenugreek (*Trigonella foenum-graecum* L.)"** to the CCS Haryana Agricultural University, Hisar.

I also undertake the patent if any, arising out of the research work conducted during the programme shall be filed by only with due permission of the competent authority of CCS Haryana Agricultural University, Hisar.

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