SEED QUALITY ASSESSMENT IN NATURALLY AGED SEEDS OF FENUGREEK (*Trigonella foenum graecum* L.)

BY

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CERTIFICATE-I

This is to certify that this thesis entitled, **"Seed quality assessment in naturally aged seeds of fenugreek (*Trigonella foenum graecum* L.)"** submitted for the degree of **Master of Science (Agriculture)** in the subject of **Horticulture-Vegetable Science** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar is a bonafide research work carried out by **Ms. Smita Kumari, Admission No. 2011A70M** under my supervision and that no part of the thesis has been submitted for any other degree.

The assistance and help received during the course of investigation has been fully acknowledged.

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CERTIFICATE-II

This is to certify that this thesis entitled, "**Seed quality assessment in naturally aged seeds of fenugreek (*Trigonella foenum graecum* L.)"** submitted by **Ms. Smita Kumari Admission No. 2011A70M** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfillment of the requirement for the degree of **Master of Science (Agriculture)** in the subject of **Horticulture-Vegetable Science**, has been approved by the Student's Advisory Committee after an oral examination.

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CHAPTER - I

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.), popularly known as 'metha or methi' belonging to the tribe Trigonellae and family Fabaceae, is an important leafy vegetable and seed spice grown during *Rabi* (winter) season in India. It performs well under moderately saline soil conditions where no other grain legume is profitable (Habib *et al.,* 1971). India is the major producer of fenugreek followed by Morocco, Pakistan, Egypt, Ethiopia and Mediterranean countries. In India, it is mainly grown in the states of Rajasthan, Gujarat, Punjab, Haryana, Uttar Pradesh, Maharasthra, Tamil Nadu and Andhra Pradesh, where during 2010-2011, it was grown over an area of 81.2 thousand hectares with production of 118.43 thousand tones and productivity of 12.6q/ha (Anonymous, 2011). Out of this more than 80% area and production of the country is contributed by Rajasthan alone. In Haryana, fenugreek is grown as *Rabi* crop both under rain-fed as well as irrigated conditions on a limited acreage and has remained commercially underexploited.

Nutritionally fenugreek is a vital source of essential minerals, vitamins, and dietary fibers. This multipurpose crop is utilized in one or the other form as leafy vegetable, fodder and condiment purpose. Fenugreek seeds are used in perfumery, pickles, curry powders, mixed spices and condiments and also have both feed and medicinal value (Kirthikar and Basu, 1975). Seeds are effectively used against diabetes (Jain *et al.,* 1987), anti-fertility (Sethi *et al.,* 1990) and the digestive disorders (Sharma *et al.,* 1991). Besides this, the fenugreek seeds possess 'diosgenin', an important steroid mainly used the synthesis of sex hormones and oral contraceptives.

Fenugreek plants have a well developed tap root and a spreading, fibrous root system, carrying small and flattened nodules containing *Rhizobium* bacteria usually the *Rhizobium meliloti*. Spraying the growing fenugreek plants with a solution containing the N-fixing bacteria *Klebsiella* gave significantly increased herbage and seed yield (Mukhopadhyay and Sen, 1997). Normally plants have single stems but cultivars may have three to six basal branches giving a multi-stemmed appearance. The stem is green to purple, smooth, erect, stiff up to 140 cm tall but usual height is 60-90cm. The leaves are alternate, pinnate, with primary pinnae odd-numbered (3-19), trifoliate and 2-5 cm long. Per 100g of its leaves contain the water 87g, protein 5g, fat 0.2g, carbohydrates 5g, fiber 1.4g, Ca 150mg and P 50mg.

Seed is an important component and its quality plays a crucial role in agricultural production and ultimately in the national economy. The quality of seed is mainly measured by its genetic purity and capacity to develop into a healthy plant. Thus, good quality seed is pre-requisite to enhance the production and productivity. Availability of viable and vigorous seed at the planting time is important for achieving targets of agricultural production because good quality seed acts as catalyst for realizing the potential of other inputs. Since, the total

cultivable area is shrinking due to ever growing population; therefore, the increased agricultural productivity remains the only option. Plant breeders have developed a number of high yielding varieties and to harvest their potential, quality seed has been recognized as an important and cheapest input.

Seed deterioration of fenugreek is a serious problem in tropical and sub-tropical countries like India, where its seeds are stored in places usually without a proper control of humidity and temperature. The main factors influencing seed deterioration and viability loss in storage are the temperature and seed moisture content (Barton, 1964; James, 1967 and Roberts, 1972). Lower temperature and humidity result in delayed seed deteriorative process and ageing, thereby lead to extended viability period. Seed ageing is generally marked by reduction in vigour (Agrawal and Sinha, 1980; Saxena, 1987; Trawatha *et al*., 1995; Gupta and Aneja, 2004), viability, rate and capacity of germination (Chhetri *et al*., 1993; Arefi and Abdi, 2003), increased solute leakage (Agrawal, 1990; Kalpana and Rao, 1995; and Basra *et al*., 2003) and susceptibility to stresses and reduced tolerance for storage under adverse conditions (Duffus and Slaughter, 1980).

The importance of quality seed cannot be ignored since this determines the quantity as well as quality production. Further, due to high commercial value of seed spices, the quality of seeds becomes still more important. The seed stored in tropical conditions absorb moisture from humid atmosphere and thus high moisture content, which at high ambient temperature along with higher respiration rate results in rapid deterioration and loss of germination ability. As the seeds get aged, they respire and germinate at slower rate than the fresh seeds and become more prone to diseases, chromosomal abnormalities and increased proportion of abnormal seedlings. Since the viability of carryover seed lots deteriorates rapidly, therefore, its prior assessment is important to plant only the viable seeds in the coming season.

For increasing awareness among farmers about the use of quality seeds, there is also a need to have some reliable parameters that evaluate the seed quality before it is sown in the field. Hence, the present study was planned to investigate the seed quality status of the naturally aged seed *vis-a-vis* the freshly harvested seeds, so that the information generated may be used by the farmers, researchers and seed industry. Keeping the above facts in view, the present experiment entitled "Seed quality assessment in naturally aged seeds of fenugreek (*Trigonella foenum-graecum* L.)" was carried out in the laboratories and research farm of Department of Seed Science and Technology CCS Haryana Agricultural University, Hisar with the following objectives:

1. To study the effect of natural ageing on seed quality of fenugreek
2. To determine the association of various seed quality parameters with field emergence

CHAPTER - II

REVIEW OF LITERATURE

Seed is the basic input in agriculture and seed viability and vigour are the most important attributes of the seed quality, which are controlled both genetically and environmentally. Seeds attain maximum viability and vigour at physiological maturity and thereafter they begin to age with gradual decline in the parameters of potency (Harrington, 1972). In tomato and chillies, Tekrony and Egli (1995) found that maximum accumulation of seed vigour occurred after physiological maturity. However, the stage at which maximum quality during seed development is attained has not been resolved (Rasyad *et al*., 1990, Pieta Filho and Ellis, 1991). Physiological quality of a seed lot is routinely evaluated by the standard germination test, which states that germination is the emergence and development from the seed embryo of those essential structures which are indicative of the ability to produce a normal plant under favourable conditions (AOSA, 1991). The germination test, however, does not correspond to the performance of the seed lots when they are sown under field conditions and does not provide complete evaluation of seed lot deterioration or quality (McDonald, 1980) and also does not differentiate the strong and weak seedlings.

Viability depends upon the history of seed during its development (Austin, 1972). Seed deterioration, a natural process, is expressed as loss of quality, viability and vigour during ageing or adverse conditions. It is an irreversible degenerative process that occurs during storage. The rate of deterioration is however, influenced by the seed moisture content and the temperature of the storage, that lead to more rapid deterioration (Demir and Ellis 1992).Seed deterioration in turn leads to reduction in seed quality, field stand establishment and the performance (Christiansen and Rowland, 1981). Seed deterioration is an even unavoidable process even under ideal conditions and ultimately the viability is lost and seeds die.

The climatic conditions of India greatly accelerate the seed ageing phenomenon under ambient storage environment, causing consequent deterioration and loss of seed viability (Basu, 1976). Liklotchev *et al*. (1984) concluded that the physiological changes in seeds subjected to accelerated ageing were same as natural ageing, with only difference being the rate. During storage, the viability is affected by various factors such as genetic, pre-harvest climatic conditions, seed type, seed structure, seed health, temperature, relative humidity of atmosphere, seed moisture content and seed treatment etc. Environmentally, high temperature and high relative humidity during storage enhance the seed deterioration (Abbas and Lovato, 1999). High temperature and high humidity accelerate the seed ageing phenomenon. Economically, seed deterioration is a major problem in agriculture production (McDonald,

1999). As the seed age, they come to germinate more slowly than fresh seeds, respire slower and become more susceptible to disease, chromosomal abnormalities and increased proportion of morphologically abnormal seedlings. Therfore, it is of prime importance to have an accurate idea about the planting value of the seed before sowing. Seed lots of varieties may vary in seed viability and vigour, therefore it is necessary to have accurate knowledge about the maintaince of seed quality of each variety along with other important agronomical characters from harvesting till planting in the field. The literature collected on various aspects in relation to the present investigation in fenugreek and its related crops have been under the following sections.

2.1 Test weight

Seed weight is most often influenced by stresses that occur during the grain-filling period of the plant. Factors that decrease the rate or duration of grain fill can result in lower test weight at harvest which cause adverse effect on seed quality parameters. Sampaio *et al*. (1999) reviewed standard germination and vigour tests indicated that larger seeds were most vigorous. Whittington and Fierlinger (1972) reported that variation in time to germinate in tomato could be modified by selection probability at the expense of seed size and embryo size at the time of emergence. Seed vigour in lettuce varied according to seed weight (Smith *et al*., 1973). The hypocotyl and shoot length, vigour index and DNA production were also significantly correlated with seed weight (Paul and Ramaswamy, 1979). In the accelerated aging test, the test weight had significant effect on sprouting percentage and dry weight of seedling, while it has no significant effect on seedling length in *Salvia* (Afshari *et al*., 2011).

2.2 Standard germination

Standard germination test is an excellent measure of seed viability. Germination test is an acceptable measure of seed quality and it provides information about emergence capacity of seed lots under favourable conditions (ISTA, 2003). Seed testing procedures including laboratory germination test have been described for most of the agricultural and horticultural crops. Germination characteristics are mechanism for determining the timing of seedling emergence, which is crucial to the fate of seedlings (Nishitkimi and Yagi 1996). The potential factors influencing the timing of germination may range from intrinsic ones such as dormancy, seed weight and pericarp, to extrinsic condition such as light regime, GA_3, KNO_3, and soil moisture content.

Kumar (2001) recorded maximum germination of fenugreek seeds in between paper substrata at 25^{o}C. Singh (2012) also reported that 25^{o}C was the optimum temperature for germination in fenugreek seeds. Jethani (1982) revealed that the 15^{o}C was the optimum temperature for germination in the coriander seeds for between paper media which was the best than other media (top of the paper and sand). Abba and Lovatto (1999) found that the normal seedling percentage dropped more under accelerated ageing than the other treatment

(cold treatment). Pandita *et al*., 2002 reported that seed germination and speed of germination were significantly reduced, as the ageing process progressed from 8 to 32 months in garden pea. In various plant species the field emergence has been reported positively correlated with standard germination tests (Verma *et al*., 1998 in barley; Fessel *et al*., 2001 in lettuce and Kasraie *et al*., 2001 in sorghum). The percentage germination of seeds increased with increasing seed size in carrot (Austin and Longdon, 1967). Narwal (1995) observed that germination gradually declined during further course of ambient storage in okra. Deka *et al*. (1993) observed high genotypic variation in storage potential of tomato seeds of different varieties.

All the physiological parameters *viz*., germination percentage, viability percentage, seedling length and seedling dry weight, vigour index, field emergence index, seedling establishment and DHA activity were decreased significantly with the progress of ageing period (Kumar, 2004 in onion; Deshraj, 2002; Kumar, 2007 and Kumar 2010 in coriander, and Singh, 2012 in fenugreek). Mavi *et al.* (2010) recorded the relative emergence of seed lots of three cucurbit crops under stress conditions and reported that, mean emergence time was higher (*i.e.* germination was slower) in physiologically older seed in commercial seed lots of watermelon (10 lots), melon (10 lots) and cucumber (9 lots). There was a general decrease in standard germination percentage for all vegetables (carrot, cucumber, onion and tomato) seeds when storage was prolonged (Alhamdan *et al*., 2011).

2.3 Seedling length

The length of seedling after a specified period is the product of time taken to germination i.e. initiation of growth and subsequent rate of growth. Measuring plumule growth as a vigour test was used for cereals and sugarbeet by Germ (1960). It had also been used by Woodstock (1969) for corn. Austin and Longdon (1967) observed that large seeds gave better seedling than small seeds in carrot. The largest seedling arose from the heaviest seeds and the smallest from the lightest seeds in celery (Thomas *et al*., 1979).

Nagarajan *et al*., (2004) reported that seedling length decreased with the accelerated ageing period in okra. The seedling length decreased with increasing accelerated ageing period in cotton (Khan *et al*., 1998), mung bean (Maity *et al*., 2000), mustard (Verma *et al*., 2003) and sunflower (Pallavi *et al*., 2003). Khan *et al*., (2005) studied that seedling length after accelerated ageing decreased as compared to fresh seeds in turnip. Kumar (2007) revealed that seedling length was positive and significant correlation with seed vigour index-I in coriander. Jyothi *et al*., (2008) observed that seedling length become decrease with the advancement of ageing period in chilli. All the physiological parameters *viz*., germination percentage, viability, root length, shoot length and consequently the vigour index in all varieties of chickpea were significantly decreased with accelerated ageing (Kapoor *et al*., 2010).

2.4 Seedling dry weight

The measurement of seedling dry weight as vigour test has been suggested by the AOSA vigour testing committee (Woodstock, 1976). Currah and Salter (1973) observed 25 percent increase in seedling weight and seed yield by grading of carrot seeds.

Paul and Ramaswamy (1979) reported in cowpea that dry weight was significantly correlated with all seedling vigour measurement. According to Doijode (1988), dry weight of seedling increased with the maturity of seeds in chilli. At ripe stage of harvest, seeds also possessed high storability there by suggesting longevity and vigour were associated with the stage of harvest. Demir and Ellis (1992) found maximum seed quality (as defined in terms of percentage normal germination, mean germination time, seedling size and percentage germination after seed storage) occurred after maximum seed dry weight was produced but they did not record stage of ripeness of fruit. As per the findings of Verma *et al* (2003) the dry matter (weight) of seedlings is decreased as the ageing period increased in mustard. Nagarajan *et al*. (2004) observed that with increasing of ageing period the dry weight of seedling decreased in okra. Deshraj (2002) revealed that the seedling dry weight was positively and significantly associated with seed vigour indices and tetrazolium test in coriander.

2.5 Seed vigour indices

Vigour index offers the possibility of categorizing seed lots into various classes of seed quality. Seed mass is frequently investigated as indicator of seed vigour, Although large seed produce larger seedlings. Importance of seed size in relation to seedling vigour has been reported in clover (Black, 1958) and oats (Landenmark, 1972). Positive and significant association of germinability and seedling characters viz. root length, shoot length and dry matter of seedling among themselves and positive correlation in prediction of seed quality and field establishment in pigeonpea has been investigated (Ram *et al*., 1991 and Kharb, 1992).

A combination of standard germination test value with seedling length and dry matter production provides a broad evaluation of seed vigour. Abdul Baki and Anderson (1973a, 1973b) established vigour levels in soyabean by germinating the seeds in standard germination test for five days and then normal germination and length by hypocotyls was determined. Seed ageing is generally marked by reduction in vigour (Trawatha *et al*., 1995 in soyabean; Agrawal and Sinha, 1980 in okra and Gupta and Aneja, 2004 in soyabean). Saxena *et al*., (1987) reported that the per cent germination, fresh and dry weight, seedling length and dehydrogenase activity declined with increase in stoage period in cabbage, radish, cauliflower, onion and pea seeds and the leachates showed positive relationship with loss of viability. Basu *et al.* (2004) observed that the vigour index-I and vigour index-II decreased as the duration of storage period increase in maize. Khare and Satpute (1999) reported that germination and seedling vigour were significantly influenced by seed size and bold seeded

genotypes had higher vigour index in pigeon pea. Ilbi and Eser (2006) emphasized that seed vigour is an important seed quality parameter, because standard germination test does not consistently predict the field performance of seed lot. Estimate of vigour not only provides indication of field emergence value but also gives storage value of seed lot. Vadivelu *et al*. (1989) observed genotypic variation in respect of seed weight, germination and vigour in tomato.

2.6 Accelerated ageing test

Seed storage in ambient condition is a problem especially in the humid tropical region. Temperature, length of storage, seed moisture and initial viability are the important factors influencing the viability of seeds during storage (Tyagi, 1992). Safe storage conditions were defined as those, which maintained seed quality without loss of viability for the years (Harrington, 1958). Ageing tests are used to measure seed vigour and are conducted by exposing seeds to high temperature and relative humidity (Delouche and Baskin, 1973). The most widely accepted single crieteria of seed deterioration is reduced germinability however; many tests for measuring the loss in vigour have been developed based on the physiological effect of ageing (ISTA, 1993; Tekrony, 1993; Woltz and Tekrony, 2001; Jatoi *et al*., 2004; Khan *et al*., 2007; Malik and Shamet, 2009). Among them the most important method is accelerated ageing which is done by subjecting the seeds to elevated temperature and high relative humidity (moisture content). It provides a simple and good method for studying sequence and relationship of process of deterioration over short periods. The ageing of the seeds is indicated by delayed germination, slower growth and increased susceptibility to environmental stresses, eventually leading to loss of viability (Byrd and Delouche, 1971., Parrish and Leopold, 1978., McDonald and Nelson, 1986). Seeds contain a number of solutes, including amino acids which have been shown to leak preferentially from nonviable compared to viable seeds (Priestley, 1986). Amino acid leakage has been studied in several species with respect to seed deterioration. Amino acids were detected in leachate from *Brassica pekinensis* and *Allium tuberosum* and a general negative relationship was observed between germination and leakage (Zheng, 1991). Doijode (1990) studied relative storability of seeds of several tomato varieties and recorded germination percentage varying from 25 to 75% after accelerated ageing treatment for eight days.

Kumar and Verma (2008) was conducted accelerated ageing test under 40^{o}C and 100% relative humidity for 48 h in fenugreek and after that stressed seeds were tested for standard germination and observed that decreased in standard germination was more in 4 and 3 years old seeds as compared to freshly harvested seed lot. Similar result was also observed by Singh (2012) in fenugreek. Agarwal and Sinha (1980) found that seed germination, seedling length and dry weight decreased during early period of ageing in 4 and 3 years old seeds in okra. Nagarajan *et al*. (2004) reported that seed deterioration in terms of viability and vigour was faster in seed lots of high initial moisture content compared to seed lots of low

initial moisture content in okra. Dahiya *et al*. (1994) observed that seed storability, seedling establishment and other quality parameters could be predicted by accelerated ageing test. Yadav (1995) reported that time period of ageing had significant deterioration effect on germinability in carrot. Rajkumar *et al*. (2004) revealed that germination rate and vigour index decreased with progressive ageing in pea. Kehinde *et al* (2013) revealed that after 3 months of storage under ambient conditions, viability and vigour reduced from 41.67-28.25% and 2.37-0.66% respectively in amaranthus.

2.7 Tetrazolium test (%)

The tetrazolium test (TZ) is one of the most reliable for the evaluation of seed quality and vigour. TZ is reduced in living cells and produces a coloured insoluble salt, formazan, which makes it possible to distinguish the living embryos by their red coloration from the dead embryos that retain their original colour (Deswal and Chand, 1997; Pina-Rodrigues *et al*., 2004). It was developed by Lakon (1942) who relied on the action of TZ molecule to react with hydrogen atoms released of dehydrogenase enzyme activity in living tissues. The tetrazolium test has gained wide acceptance not only as a rapid technique for estimating quality but also a powerful tool for assessing vigour. On the basis of topographical pattern of staining, the tetrazolium test as a measure of seed viability has been used (Moore, 1973). The evaluation of germinability and non germianability was carried out on the basis of the topographical staining pattern. McDonald (2002) proposed that for more accurately portray the seed quality of native species, and especially those seeds with deep dormancy, it is necessary to conduct a tetrazolium test prior to the germination test to identify the viability of the seed.

Kumar and Verma (2008) recorded good results in fenugreek by soaking the seeds in water for 16 h at room temperature and were kept in 0.5% tetrazolium chloride solution at 30^{o}C for two hours. Sahoo and Swain (1994) standardized the tetrazolium test in some umbelliferous spice seeds and found that 36 h in pre-soaking for coriander and cumin and 24 h soaking was needed for fennel. The seeds were immersed in 0.5 to 0.7 percent solution of tetrazolium chloride at 30^{o}C for 2h (fennel and cumin) or 4h for coriander. Deswal and Chand (1997) observed that the direct soaking of preconditioned ricebean (*Vigna umbellata*) seeds in 1.0 percent tetrazolium solution at 40^{0}C for 3h produced good colour intensity. ISTA (1999) recommended TZ as an important indirect method for testing seed viability of some selected species where the germination test took more than 60 days to complete. Agrawal and Sinha (1980) in okra and Saxena (1987) in some vegetable seeds reported loss of seed viability and vigour with increase in period of storage. Perez-Camacho *et al.*(2008) has studied the effect of deterioration caused by natural ageing on husk tomato seed, var. CHF1- Chapingo and revealed that viability decreased from 84.5 to 50.8 % after three years of storage.

2.8 Electrical conductivity test

Electrical conductivity in general is the property of a solution to allow the passage of electric current between electrodes having an electric potential difference. This is directly proportional to the amount of electrolytes in the solution and therefore, can be used to measure the amount of electrolytes in the solution. Seed deterioration is always correlated with the increased EC of seed leachates and this is being used as one of the method for evaluating seed deterioration. The conductivity test was first adopted for seed testing by Parsley (1958) to measure cotton seed viability.

The conductivity test has been found to be correlated with vigour in seeds of pea (Matthews and Bradnock, 1967 and Scott and Close 1976). Powell and Mathews (1977) reported increased electrolytes leakage from pea seeds prior to the development of dead areas in seeds and loss in viability. Thus, damage to membrane system prior to loss in viability was considered to be an important event. Dadlani and Agarwal (1983) reported that correlation coefficient of germination percentage with leaching of water soluble sugars from carrot seeds was negative and highly significant. Torres *et al*. (1999) observed that electrical conductivity increases with natural ageing in coriander. Dahiya *et al*. (1999) observed a negative correlation of standard germination with electrical conductivity test in sunflower. The electrical conductivity increased significantly with progress of ageing period (Kumar, 2004 in onion; Desraj, 2002, Kumar, 2007 and 2010 in coriander; and Singh, 2012 in fenugreek). Electrical conductivity of seed leachates due to membrane deterioration increased as the ageing duration increased, which plays a role in seed quality in turnip (Khan *et al.,* 2005). Kaewanaree *et al.* (2011) demonstrated that cell membrane damage decreases the ability of carrier proteins and caused increased electrolytes leakage in sweet pepper after accelerated ageing. Krishnasamy and Ramarajpalaniappan (1989) observed that association of electrical conductivity of seed leachates with seed quality parameters like germination, root length, vigour index and field emergence potential was significant in tomato.

2.9 pH exudate test

The test based on seed exudates, most commonly used for pea seeds as a vigour test (Matthews and Bradrock, 1968), measures the conductivity of exudates after seeds have been allowed to imbibe for a certain period of time. The test based on conductivity of the exudates may give a good correlation with germination depending on cultivar, species, temperature, initial seed moisture content and seed vigour (Tao, 1978; Miranda 1981). A close relationship between pH of seed exudates and its germination was observed in soyabean (Peske and Amaral, 1986). Peske *et al*.(1990) reported the pH exudates test as a measure of rapidity of germination and viability. Tyagi (1993) reported that seed exudates pH test in calorimetric method predict the germinability of individual seed based on colour change in the seed exudates from colorless to rose colour. Kumar and Verma (2008) revealed positive and significant correlation of pH with field emergence in fenugreek.

2.10 Dehyhrogenase activity test

Dehyhrogenase activity is also known as tetrazolium reduction ability. The activity of dehydrogenase enzyme is directly correlated with the seed vigour. To estimate the activities of these enzymes, Kittock and Law (1968) gave an indirect method. i.e. by colorimetric estimation of formazan (a product in reaction of tetrazolium solution with dehydrogenase enzyme). High seed quality in terms of viability and vigour is essential for seedling establishment in field as well as for good crop production. The level of fruit maturity affects both germination and vigour of seed and determines the longevity of seed (Pollock and Roose, 1972). Anderson and Baker (1983) reported a decrease in dehydrogenase activity with advancement of storage.

It is of great importance to harvest a seed crop at the stage which allow maximum yield and the best quality, as the stage of maturity was found to influence both viability and longevity of seeds (McAlister, 1943). Palanisamy and Karivaratharaju (1991) reported that dehydrogenase enzyme activity of seeds decreased with the advancement in the storage. Subbarao (1984) also found decrease in activity of dehydrogenase as well as amylase during ageing in brinjal. Kharb *et al*. (1994) reported that dehydrogenase activity test is positively correlated with field emergence in pigeon pea. Narwal (1995) observed that the absorbance decreased dramatically in all the varieties of okra after six month of ambient storage. According to Verma *et al*. (2003) dehydrogenase activity was reduced as the ageing progressed and was found lowest after four year of storage in Brassica species. Kumar and Verma (2008) in fenugreek showed that dehydrogenase activity test found to be positive and significant correlated with field emergence. Dehydrogenase activity was higher up to two years of old seed and there after it declined among all the varieties of fenugreek under study (Singh, 2012).

2.10 Field emergence

Emergence rate index has been measured in different ways and given different names such as germination rate (Magrine, 1962), speed of germination (Lawrence, 1963) and germination value/ peak value (Djavanshir and Pourbeik, 1976). Matthews (1981) also reported that the vigour tests are indicative of field emergence of soyabean, cotton, wheat and rice. Deshraj (2002) reported that standard germination, vigour index-I, dehydrogenase activity test and respiration rate were found positively correlated with emergence rate index but electrical conductance was negatively correlated with emergence rate index in coriander. Verma *et al*. (1999) reported that all the vigour and viability tests were positively correlated with emergence rate index in barley. Yadav and Dhankar (2001) reported that field emergence was positively associated with standard germination, vigour index-I, vigour index-II and accelerated ageing in okra.

CHAPTER - III

MATERIAL AND METHODS

The present study entitled "Seed quality assessment in naturally aged seeds of fenugreek (*Trigonella foenum-graecum* L.)" was conducted during 2012-2013 at the Vegetable Research Farm and Laboratories of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar. The detailed of procedure followed, criteria used for treatment evaluation and methods adopted during entire course of investigation are presented in this chapter.

3.1 Experimental Material

Seed material comprised of four seed lots of eleven genotypes of fenugreek *viz*., HM-247, HM-257, HM-273, HM-292, HM-444, HM-548, HM-552, RMt-1, UM-305, Hisar Mukta and Hisar Suvarna were procured from the Department of Vegetable Science, CCS Haryana Agricultural University, Hisar. Four seed lots of each genotype included the freshly harvested seed, one year old, two year old and three year old seeds stored under ambient conditions were taken.

3.2 Methods

The details of methods followed for recording various observations for all the genotypes for different seed lots are given below:

3.2.1 Standard Germination test (%)

One hundred seeds of each genotype of variable seed lots in three replicates were placed in between sufficiently moistened rolled towel papers (BP) and kept at 25^{o}C in seed germinator. The final seed germination count was taken on 12th day and only normal seedlings were considered for percent germination in accordance to the rules of International Seed Testing Association (ISTA, 1999).

3.2.2 Seedling length (cm)

Seedling length was measured on ten randomly selected normal seedlings taken from three replications of standard germination test and recorded in centimeter. At last average of ten seedlings was taken for final calculation.

3.2.3 Seedling fresh weight (g)

Seedling fresh weight was measured on ten randomly selected normal seedlings taken from three replications of standard germination test and average seedling fresh weight of each genotype was calculated. It was assessed after the final count of the standard germination.

3.2.4 Seedling dry weight (mg)

Seedling dry weight was assessed after the final count of the standard germination. Ten normal seedlings of each genotype replicated thrice were dried in a hot oven for 24h at

80±1°C. The dried seedlings of each replication were weighed and average seedling dry weight of each genotype was calculated.

3.2.5 Seedling vigour index

Seedling vigour indices were calculated as per suggested by Baki and Anderson (1973):

1. Vigour index-I = Standard germination (%) × Average seedling length (cm)
2. Vigour index-II= Standard germination (%) × Average seedling dry weight (mg)

3.2.6 Tetrazolium Test (%)

Fifty seeds of each seed lot replicated thrice were soaked in 50 ml water for 16h at 25°C to activate dehydrogenase enzymes. After removal of seed coat, the seeds were stained in 0.5% tetrazolium solution (2,3,5 – triphenyl tetrazolium chloride) for 4h at 38^{0}C, in Petri plates. After that solution was poured off and seeds were washed in tap water and examined. The completely red seed stained were considered as normal viable seeds and expressed in percentage.

3.2.7 Electrical conductivity (µS/cm/50seed)

Fifty normal and uninjured seeds in three replications of each seed lot were soaked in 75 ml deionized water in 100 ml beakers. Seeds were immersed completely in water and beakers were covered with foil, and kept at 25°C for 24h. The electrical conductivity of the seed leachates was measured by using conductivity meter and expressed in µS/cm/50seed.

3.2.8 Accelerated ageing (%)

Sufficient number of seeds in a single layer from each genotype was taken on wire mesh tray fitted in plastic boxes having 40 ml of distilled water. The boxes were placed in ageing chamber after closing their lids. The seeds were aged at 40±1° C temperature and about 100 percent RH for 48 hour and tested for germination in three replications of 100 seeds for each genotype. The number of normal seedlings were counted on 7th day and expressed in percentage.

3.2.9 Dehydrogenase activity test ($ODg^{-1}ml^{-1}$)

Two gram seeds of each seed lot replicated thrice were ground to pass through a 20 mesh screen to obtain 200mg flour. The flour was soaked in 5ml of 0.5% tetrazolium solution at 38°C for 3-4 h. Then it was centrifuged at 10000 rpm for 3 minutes and the supernatant was poured off. The formazan was extracted with 10 ml acetone for 16 hours followed by centrifugation and then absorbance of the solution was determined by Systronic spectrophotometer 169 at 480 nm. Enzyme activity was measured by $ODg^{-1}ml^{-1}$.

3.2.10 pH of seed lechates (%)

The pH exudates test is based on the principle that as seed deterioration progresses, the cell membrane becomes less rigid and more water permeable, allowing the cell contents, specially acidic and hydrogen ion to escape into solution with water resulting in the lower pH.

The pH exudates test is a calorimetric method that predicts the germinability of individual seed based on colour change in the seed exudates from colourless to rosy colour. Three replications of 100 seeds of each seed lot were placed in a hundred cells of plastic trays, 100 seeds per tray and one seed per cell and 2ml of distilled water was added to each cell. The seeds were then allowed to imbibe for 30 minutes at 25^0C. At the end of imbibition period 25µl of phenolphthalein solution and 50µl of Na_2Co_3 solution were added to soaking water in each cell. The colour change was noticed. Rosy colour indicated viable seeds where as no colour change indicated the seeds to be dead and expressed as percentage.

3.2.11 Field emergence index

Hundred seeds in three replicates of each four seed lots of each genotype including the freshly harvested, one year old, two year old and three year old seed stored under ambient conditions were sown in a randomized block design, during rabi season, 2012-2013 at the Research Farm of Department of Vegetable Science, CCS Haryana Agricultural University, Hisar. The number of seedlings emerged daily were counted up to 14 days after sowing. The field emergence index was estimated as follows:

$$FEI = \frac{\text{Number of seedlings emerged}}{\text{Days to first count}} + \frac{\text{Number of seedlings emerged}}{\text{Days to final count}}$$

3.2.12 Field emergence (%)

The seedling establishment was determined by counting the total number of seedlings when the emergence was completed or when there was no further addition in the total emergence *i.e.* on 14^{th} day.

3.3 Statistical Analysis:

The experiment was conducted in a completely Randomized Design for laboratory parameters and, for field parameters the same was designed in a Randomized Block. The angular transformation was applied to the percent data and the transformed data were subjected to the statistical analysis on the basis of the model described by Ostle and Mensing (1975).

ANOVA for RBD

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Mean Squares (MS)	F- value
Replication	(r-1)	SSr	MSr	MSr/MSe
Treatments	(t-1)	SSt	MSt	MSt/MSe
Error	(r-1)(t-1)	SSe	MSe(EMS)	
Total	(rt-1)	Total SS		

Where,

r	=	number of replications
t	=	number of treatments (genotypes)
SSt	=	Sum of square due to treatment combination
SSe	=	Error sum of square
MSt	=	Mean square due to treatments
MSe	=	Error mean square

The standard error of difference (SEd), standard error of means (SEm), critical difference (CD) and coefficient of variation (CV) were calculated as follows:

$$SE(m) = \sqrt{\frac{MSe}{r}}$$

$$SE(d) = \sqrt{\frac{2\,MSe}{r}}$$

CD (5%) = SEd × t value at error degrees of freedom at 5%

$$CV\ (\%) = \frac{\sqrt{MSe}}{\overline{X}} \times 100$$

Where, r = number of replications; and $\overline{X}$ = overall mean (grand total /n)

The correlation coefficients (r) between laboratory and field parameters were estimated as per standard formulae as given below:

$$r = \frac{Cov(x, y)}{\sigma x.\ \sigma y} \qquad \text{or} \qquad r = \frac{Cov(xy)}{\sqrt{Varx.Vary}}$$

Where,

r	=	Correlation cofficient
Cov(x, y)	=	Covariance between characters x and y
σx	=	Standard deviation of character x
σy	=	Standard deviation of character y

CHAPTER - IV

RESULTS

The most important single factor affecting crop production is the quality of seed and that is why testing of seeds before sale or distribution for sowing has now become an essential pre- condition. The accurate assessment of seed quality will enable the farmers to make economic decisions regarding cost of seeds, earliness of planting, quantity of seed to plant, uniformity of plant stand and net returns. With the increasing awareness among farmers about the use of quality seeds, there is an urgent need to have some reliable parameters to evaluate seed quality before sowing. In order to assess the quality of seed of different fenugreek genotypes stored under ambient conditions, in terms of field performance, the seeds were subjected to a number viability and vigour tests. All the seed lots of eleven fenugreek genotypes studied in the present investigation showed considerable variability in their storage behavior under ambient conditions prevailing at Hisar. Features of special interests obtained in the results are described below:

4.1 Analysis of Variance

The results of analysis of variance (ANOVA) for different seed quality parameters of natural aged seeds of all the genotypes are presented in Table-4.1(a), (b) and (c). The mean sum of squares due to variety, ageing and their interaction were found significant for all the parameters studied. It revealed that there is a sufficient level of variability present in the material studied. The interaction among genotypes and ageing period was found significant for all the characters under study.

4.2 Test weight (g)

Significant differences were found among all the genotypes and ageing periods for test weight Table 4.2. Test weight was recorded maximum in Hisar Mukta (16.99 g) followed by HM-444 and minimum seed weight was recorded for UM-305 (11.84 g) in freshly harvested seed. Maximum test weight was found in freshly harvested seed lots. Test weight decreased with advancement of ageing period in all the eleven genotypes. Significant decrease was observed in one year, two year and three year seed lots as compared to fresh seed lot. The results indicated that the genotype Hisar Mukta (15.64g) recorded highest mean seed weight where as UM-305 recorded lowest (10.85g). Maximum (3.36g) decrease in test weight was recorded for Hisar Mukta and minimum (0.73) in HM-548 from fresh seed lot to three year old seed lot.

4.3 Standard Germination (%)

The results obtained for standard germination based on normal seedling percentage are presented in Table 4.3. Standard germination decreased with the ageing period in all the genotypes.

Table 1 (a): Analysis 0f variance (ANOVA) for various seed quality laboratory parameters in fenugreek

	D.F.	T.W. (g)	S.G.T. (%)	S.L. (cm)	F.W (g)	D.W. (mg)	V.I-I	V.I-II	Tz test (%)	A.A test (%)	DHA ($ODg^{-1}ml^{-1}$)	E.C (µS/cm/seed)	pH exudates
Variety	10	10.238**	613.122**	24.025**	0.617**	7.350**	895, 376.649**	191,988.684**	623.227**	1,778.333**	0.092**	0.011**	630.56
Ageing	3	0.628**	26.428**	3.110**	0.025**	0.419**	52, 649.816**	8,882.100**	27.007**	77.919**	0.001**	0.000**	29.080
Variety X Ageing	30	7.051**	22.133**	9.369**	0.086**	0.443**	114, 484. 604**	7,161.501**	22.329**	139.675**	0.034**	0.002**	24.125
Error	88	0.017	2.959	0.010	0.002	0.002	1, 115.381	147.659	2.867	2.955	0.000	0.000	2.756

$P \leq 0.05$

D.F. = Degree of freedom; T.W. = Test weight; S.G.T. = Standard germination test; S.L. = Seedling length; F.W = Fresh wt; D.W. = Dry weight;
V.I-I = Vigour index-I; V.I-II = Vigour index-II; Tz test = Tetrazolium test (viability test); A.A. = Accelerated ageing test; DHA = Dehydrogenase activity test
E.C. = Electrical conductivity test; pH = pH exudates test.

Table 1 (b): Analysis of variance (ANOVA) for field emergence index

Source	Degree of freedom	M.S.S	S. E. (m)
Replication	2	1.279	
Variety	10	542.016	0.121
Ageing	3	1.424	0.073
Variety X Ageing	30	4.546	0.243
Error	86	0.177	

Table 1 (c): Analysis of variance (ANOVA) for field emergence

Source	Degree of freedom	M.S.S	S. E. (m)
Replication	2	12.69	
Variety	10	1697.486	0.427
Ageing	3	46.959	0.257
Variety X Ageing	30	74.809	0.853
Error	86	2.185	

In freshly harvested seed, all the genotypes showed maximum germination percentage, however, the germination gradually declined during the further course of ambient storage. Maximum mean standard germination (94.12%) was observed in freshly harvested seed lot while, minimum (71.84%) was observed in three year aged seed lot. Among different genotypes, maximum loss of germination was observed in Hisar Mukta (26.40) from freshly harvested seed lot to three year ambient stored seed lot.

Table 4.2: Effect of natural ageing (seed lots) on test weight (g) of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	12.31	11.63	11.22	10.47	11.41
HM-257	13.33	12.61	11.22	10.92	12.02
HM-273	13.44	12.24	11.80	10.89	12.09
HM-292	13.25	12.55	11.18	10.76	11.93
HM-444	13.67	12.13	11.00	10.88	11.92
HM-548	12.17	11.96	11.85	11.22	11.80
HM-552	12.49	12.34	11.16	10.37	11.59
RMt-1	12.46	11.43	10.50	9.69	11.02
UM-305	11.84	11.22	10.84	9.49	10.85
Hisar Mukta	16.99	16.33	15.60	13.62	15.64
Hisar Suvarna	11.88	11.25	10.58	10.25	10.99
Mean	13.06	12.24	11.65	10.78	

C.D. (p = 0.05) for genotypes = 0.105, ageing = 0.063, Genotypes x ageing = 0.209

Table 4.3: Effect of natural ageing (seed lots) on germination (%) of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	94.75 (75.41)	92.86 (72.91)	83.00 (64.11)	72.89 (56.91)	85.66 (67.84)
HM-257	94.00 (75.34)	92.43 (72.77)	84.35 (65.89)	70.33 (54.37)	85.25 (67.09)
HM-273	92.00 (72.70)	89.00 (68.13)	84.66 (65.95)	70.00 (54.11)	83.91 (65.22)
HM-292	95.89 (77.72)	93.00 (74.29)	85.00 (66.53)	74.00 (59.27)	86.91 (69.70)
HM-444	93.66 (73.87)	91.33 (71.25)	82.00 (63.18)	69.72 (52.23)	84.16 (65.13)
HM-548	96.83 (79.62)	94.00 (75.34)	87.00 (67.91)	76.67 (62.81)	88.58 (71.22)
HM-552	93.71 (73.85)	90.57 (70.47)	83.76 (64.61)	71.53 (55.81)	84.75 (66.19)
RMt-1	93.66 (73.56)	91.66 (71.47)	83.33 (64.39)	72.33 (56.67)	85.50 (66.22)
UM-305	91.33 (71.23)	88.67 (67.28)	80.53 (60.47)	72.76 (56.91)	83.25 (63.97)
Hisar Mukta	94.33 (75.54)	92.00 (72.70)	81.31 (61.24)	67.93 (51.78)	83.83 (65.32)
Hisar Suvarna	95.00 (77.11)	91.00 (71.03)	82.69 (63.31)	73.83 (58.42)	85.69 (67.72)
Mean	94.12 (75.45)	91.48 (71.60)	83.39 (64.25)	71.84 (56.30)	

C.D. (p = 0.05) for genotypes = 1.398, ageing = 0.843, Genotypes x ageing = 2.796

Fig. 1: Effect of natural ageing (see lots) on standard germination (%) of fenugreek genotypes

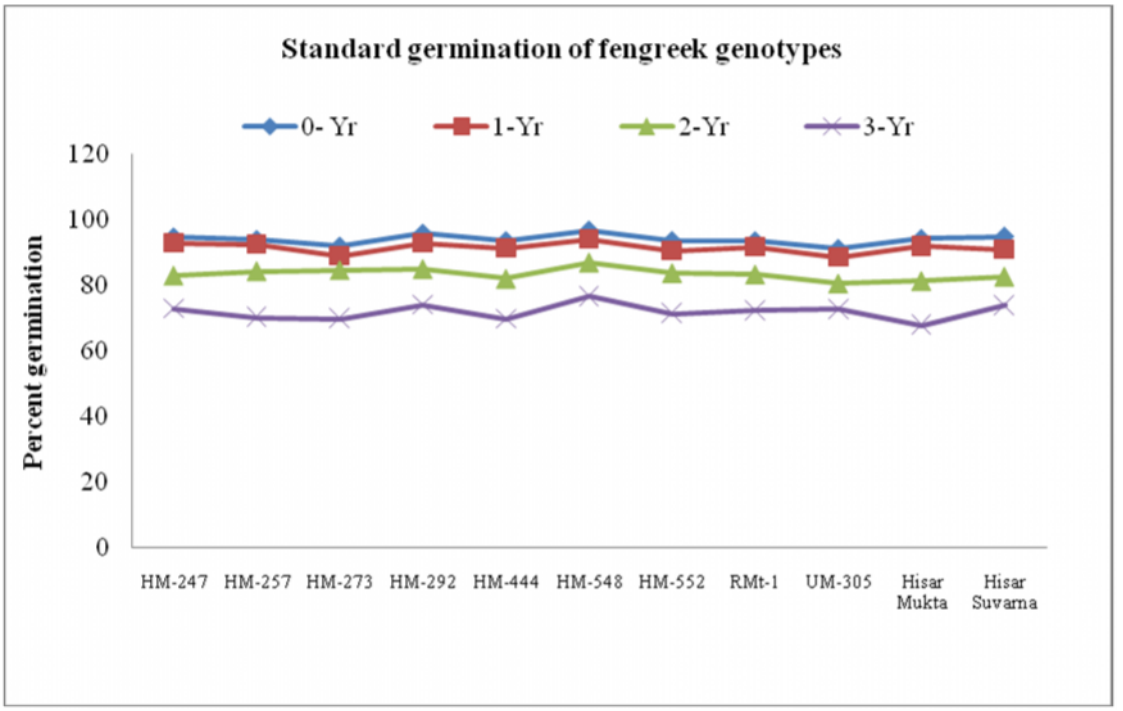
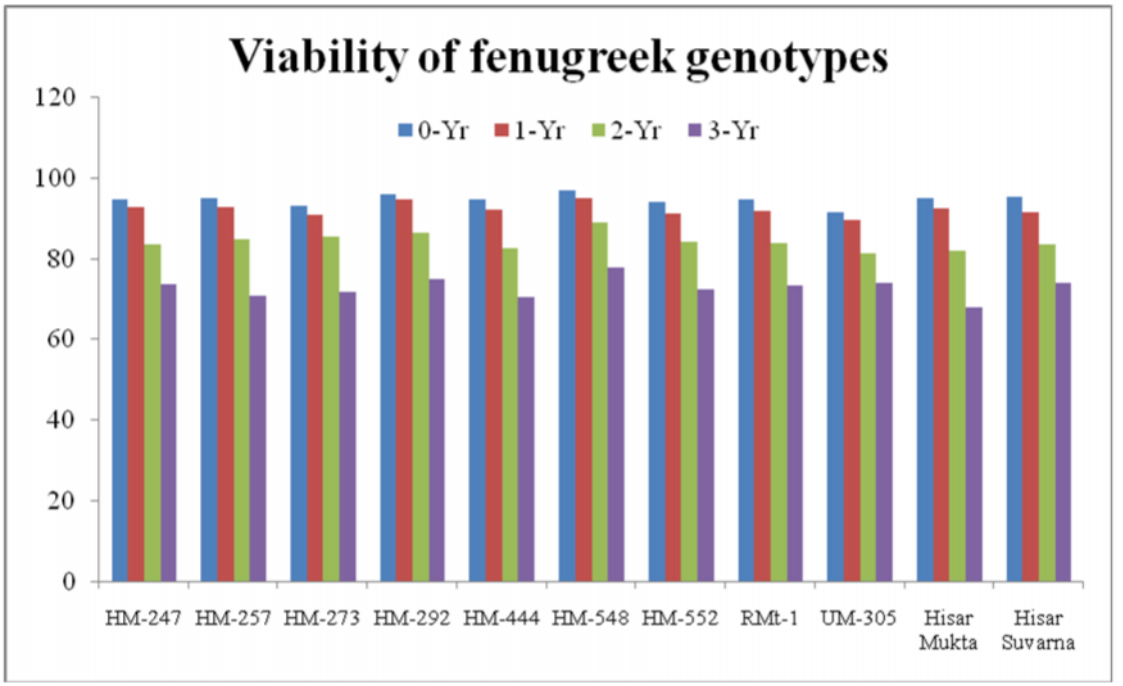


Fig. 2: Effect of natural ageing (see lots) on viability (%) of fenugreek genotypes



The range of standard germination of seed among different genotypes varied from 96.83 (HM-548) to 91.33% (UM-305) in freshly harvested seed lot, 94.00 to 88.66 % in one year old seed lot, 87.00 to 80.33% in two year old seed lot and 76.67 (HM-548) to 67.93% (Hisar Mukta), respectively. Maximum mean standard germination (88.58%) was observed in HM-548 while minimum (83.25%) for UM-305. Significant critical difference 1.398 and 0.843 was observed within all genotypes and within storage periods, respectively (Fig 1).

4.4 Seedling length (cm)

All the genotypes recorded maximum seedling length Table 4.4 at the commencement of storage and thereafter, it declined as the period of ambient storage advanced. Seedling length in all the eleven genotypes decreased significantly with the advancement of ageing period. Seedling length showed a variation in freshly harvested seed of different genotypes from 19.29 to 23.41cm with a general mean of 20.67cm, respectively. The maximum average value for seedling length was recorded for genotype HM-548 (21.88cm) followed by HM-292 (20.85cm) and minimum (17.29cm) in Hisar Mukta. The maximum (4.42cm) effect of ageing on seedling length was reported in Hisar Mukta and minimum (2.57cm) effect in HM-292 from fresh seed lot to three year old seed lot. Seedling length in all the eleven genotypes decreased significantly with advancement of ageing period. The genotypes x ageing interaction were also found significant as this decreasing response of genotypes was not similar for all the aged seed lots. So, decrease in seedling length with the passage of time. The seedling length for all the genotypes decrease significantly with an increased significantly with an increase in ambient storage period but the trend was inconsistent.

Table 4.4: Effect of natural ageing (seed lots) on seedling length (cm) of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	21.57	20.61	19.66	18.94	20.19
HM-257	20.29	19.53	18.78	16.34	18.73
HM-273	19.29	18.37	17.33	16.67	17.91
HM-292	22.23	21.25	20.28	19.66	20.85
HM-444	19.31	18.26	17.21	16.22	17.75
HM-548	23.41	22.35	21.28	20.49	21.88
HM-552	20.94	17.52	18.18	16.59	18.30
RMt-1	20.47	18.98	17.49	16.30	18.31
UM-305	19.75	18.48	17.21	16.53	17.99
Hisar Mukta	19.55	18.25	16.25	15.13	17.29
Hisar Suvarna	20.55	19.54	18.54	17.26	18.97
Mean	20.67	19.38	18.38	17.28	

C.D. (p = 0.05) for genotypes = 0.083, ageing = 0.050, Genotypes x ageing = 0.166

4.5 Seedling fresh weight (g)

Seedling fresh weight of different genotypes of different seed lots has been presented in Table 4.5. Seedling fresh weight of different genotypes decreased significantly with the advancement of ageing period. Highest mean seedling fresh weight was observed in HM-548 (2.06g) followed by HM-292 (2.03g) and lowest in Hisar Mukta (1.56g). The seedling fresh weight of fresh seeds was observed maximum (2.12g) and decreased significantly with the advancement of ageing period.

Table 4.5: Effect of natural ageing (seed lots) on fresh weight (g) of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	2.16	2.02	1.91	1.73	1.95
HM-257	2.17	2.12	1.95	1.72	1.99
HM-273	2.19	2.14	1.89	1.52	1.93
HM-292	2.21	2.17	1.96	1.76	2.03
HM-444	2.10	2.06	1.67	1.51	1.84
HM-548	2.25	2.21	2.00	1.79	2.06
HM-552	2.21	2.14	1.96	1.61	1.98
RMt-1	2.13	2.00	1.84	1.65	1.90
UM-305	1.99	1.95	1.91	1.33	1.79
Hisar Mukta	1.97	1.69	1.49	1.11	1.56
Hisar Suvarna	1.99	1.98	1.91	1.53	1.85
Mean	2.12	2.04	1.86	1.57	

C.D. (p = 0.05) for genotypes = 0.032, ageing = 0.019, Genotypes x ageing = 0.064

4.6 Seedling dry weight (mg)

Seedling dry weight of different genotypes is presented in Table 4.6. All the eleven genotypes differed significantly with respect to their seedling dry weight. In general, seedling dry weight decreased in all the genotypes as the storage period proceeded. Highest mean seedling dry weight was observed in HM-548 (7.59mg) followed by HM-292 (7.48mg) and lowest in UM-305 (6.67mg). The seedling dry weight of fresh seed was observed highest (7.89) in freshly harvested seed lot and lowest (6.01mg) for three year old seed lot. Significant critical difference 0.033 and 0.020 was observed within all genotypes and within storage periods, respectively. The seedling dry weight was affected by the seed aged significantly with increase in storage age of the seeds; however, the decrease in seedling dry weight was not consistent with seed lot as well as for the genotypes studied.

4.7 Seed Vigour index-I

Seed vigour index-I expressed as a product function of standard germination percentage and the seedling length is depicted in Table 4.7. All the genotypes recorded the maximum vigour index in

Table 4.6: Effect of natural ageing (seed lots) on dry weight (mg) of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	7.93	7.51	6.66	5.68	6.94
HM-257	7.98	7.83	6.82	6.16	7.20
HM-273	7.99	7.63	6.56	5.92	7.02
HM-292	8.05	7.94	7.14	6.80	7.48
HM-444	8.00	7.84	6.82	6.14	7.20
HM-548	8.14	8.02	7.26	6.93	7.59
HM-552	7.90	7.53	6.64	5.95	7.00
RMt-1	7.97	7.74	6.49	5.84	7.01
UM-305	7.52	7.38	6.33	5.46	6.67
Hisar Mukta	7.82	7.65	6.54	5.54	6.89
Hisar Suvarna	7.54	7.36	6.34	5.73	6.74
Mean	7.89	7.67	6.69	6.01	

C.D. (p = 0.05) for genotypes = 0.033, ageing = 0.020, Genotypes x ageing = 0.065

freshly harvested seed lot and the genotype HM-548 showed highest value (2263.61). The overall mean value showed that HM-548 (1946.9) had highest vigour index-I followed by HM-292 (1820.74), where as Hisar Mukta (1467.67) exhibited lowest seedling vigour there by suggested that genotype HM-548 was more vigorous than other genotypes. Results indicated that seedling vigour declined significantly in all the genotypes with the passage of seed storage time.

Table 4.7: Effect of natural ageing (seed lots) on vigour index-I of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	2034.77	1910.32	1631.78	1376.80	1738.41
HM-257	1907.57	1804.04	1584.06	1149.24	1611.23
HM-273	1775.29	1635.52	1467.27	1166.90	1511.25
HM-292	2127.30	1977.02	1723.80	1454.84	1820.74
HM-444	1809.33	1668.35	1411.76	1129.99	1504.86
HM-548	2263.61	2101.05	1851.94	1571.15	1946.94
HM-552	1954.40	1588.48	1521.06	1183.42	1561.84
RMt-1	1938.14	1739.99	1457.50	1179.03	1578.67
UM-305	1804.13	1638.86	1382.80	1201.42	1506.80
Hisar Mukta	1851.05	1679.00	1321.67	1018.98	1467.67
Hisar Suvarna	1952.56	1778.90	1532.91	1265.73	1632.53
Mean	1947.10	1774.68	1535.14	1245.22	

C.D. (p = 0.05) for genotypes = 27.142, ageing = 16.367, Genotypes x ageing = 54.284

4.8 Seed vigour index-II

Seed vigour index-II expressed as a product function of standard germination percentage and the seedling dry weight is given in Table 4.8. The significant differences were observed for seed vigour index for genotype, ageing periods and their interaction levels. Vigour index –II ranged from 787.19 (HM-548) to 686.83 (UM-305) in freshly harvested seed. A perusal of the data indicated that seedling vigour index decreased in all the genotypes with the passage of seed storage time. However, in three year old seed lot, the maximum value of seed vigour index-II was recorded in HM-548 (508.45) followed by HM-292 (501.42), which were statistically at par. Among different seed lots, the vigour index-II was found maximum (743.40) in freshly harvested seed lot where as minimum (430.02) was observed in three year aged seed lot. The maximum average value for seed vigour index (670.43) was observed for genotype HM-548 followed by HM-292 (654.45) and minimum in UM-305 (561.80).

Table 4.8: Effect of natural ageing (seed lots) on vigour index-II of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	748.70	695.00	553.33	413.23	602.56
HM-257	750.43	723.59	575.71	433.72	620.87
HM-273	735.08	679.07	555.41	414.63	596.05
HM-292	770.44	739.04	606.90	501.42	654.45
HM-444	749.96	716.06	559.52	427.75	613.32
HM-548	787.19	754.19	631.91	508.45	670.43
HM-552	737.02	683.02	555.55	424.91	600.12
RMt-1	754.50	710.11	540.83	422.90	607.09
UM-305	686.83	654.36	508.78	397.25	561.80
Hisar Mukta	740.92	704.41	531.92	373.25	587.62
Hisar Suvarna	716.30	670.06	524.66	412.80	580.96
Mean	743.40	702.63	558.60	430.03	

C.D. (p = 0.05) for genotypes = 9.876, ageing = 5.955, Genotypes x ageing = 19.751

4.9 Tetrazolium test (Viability %)

Tetrazolium test is being used as quick seed viability evaluation method, where the seed viability is determined on the basis of topographical staining of the live and storage tissue of the seeds. The data on effect of ambient storage on seed viability based on tetrazolium chloride test is presented in Table 4.9. In one year old seed lot, the maximum

viability was found in HM-548 (95.00 %) followed by HM-292 (94.67 %) which were statistically at par. Among different seed lots, the viability based on tetrazolium test was found maximum (94.67) in freshly harvested seed lot whereas minimum (72.79) was observed for three year aged seed lot. The maximum mean value (89.750) was observed in HM-548 followed by HM-292 (88.00) and minimum in Hisar Mukta (884.42). Results also indicated that the number of viable seeds decreased significantly with their increased age, and the pattern was not similar for all the genotypes (Fig 2).

Table 4.9: Effect of natural ageing (seed lots) on viability (%) of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	94.67 (75.16)	93.00 (73.16)	83.67 (64.61)	73.67 (58.77)	86.25 (68.04)
HM-257	95.23 (77.38)	93.00 (73.16)	85.00 (66.16)	71.00 (55.63)	86.00 (68.08)
HM-273	93.33 (73.85)	91.00 (71.03)	85.67 (66.76)	71.67 (55.93)	85.41 (66.89)
HM-292	96.00 (78.22)	94.67 (75.54)	86.33 (67.14)	75.00 (61.27)	88.00 (70.54)
HM-444	94.67 (75.54)	92.33 (72.77)	82.67 (63.97)	70.67 (54.52)	85.08 (66.70)
HM-548	97.00 (80.92)	95.00 (77.11)	89.00 (68.13)	78.00 (63.61)	89.75 (72.44)
HM-552	94.00 (75.34)	91.33 (71.23)	84.33 (65.89)	72.33 (56.67)	85.50 (67.28)
RMt-1	94.67 (75.54)	92.00 (72.70)	84.00 (65.17)	73.33 (58.42)	86.00 (67.96)
UM-305	91.67 (71.89)	89.67 (62.65)	81.33 (62.65)	74.00 (59.27)	86.17 (68.17)
Hisar Mukta	95.00 (77.11)	92.67 (72.93)	82.00 (63.18)	68.00 (51.98)	84.42 (66.30)
Hisar Suvarna	95.33 (77.33)	91.67 (71.47)	83.67 (64.61)	74.00 (59.27)	86.17 (68.17)
Mean	94.67 (76.25)	92.31 (72.56)	84.33 (65.30)	72.79 (56.93)	

C.D. (p = 0.05) for genotypes = 1.376, ageing = 0.830, Genotypes x ageing = 2.752

4.10 Dehydrogenase Activity Test ($ODg^{-1}ml^{-1}$)

The range of optical density of formazan was examined with the help of spectrophotometer. The results of this test showed that the dehydrogenase enzyme of the seeds decreased after natural ageing Table 4.10. Among freshly harvested seed lot, the maximum value of dehydrogenase activity was found in HM-247 (0.803) followed by HM-548 (0.801), which were statistically at par. The results revealed that highest mean intensity of formazon was produced in the seeds of genotype HM-247 (0.615) followed by the seeds of genotype HM-548 (0.610) and lowest for the genotype HM-444 (0.385). Data further indicated that all the eleven genotypes did not perform well after three years of ambient storage conditions. The results also revealed that dehydrogenase activity decreased significantly as the storage period proceeded but response was not uniform (Fig 3).

Table 4.10: Effect of natural ageing (seed lots) on dehydrogenase ($ODg^{-1}ml^{-1}$) of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	0.803	0.605	0.538	0.515	0.615
HM-257	0.678	0.514	0.491	0.470	0.538
HM-273	0.559	0.502	0.469	0.446	0.494
HM-292	0.764	0.580	0.548	0.513	0.601
HM-444	0.525	0.415	0.319	0.298	0.385
HM-548	0.801	0.602	0.534	0.502	0.610
HM-552	0.533	0.482	0.425	0.349	0.447
RMt-1	0.715	0.493	0.454	0.432	0.523
UM-305	0.712	0.598	0.517	0.498	0.581
Hisar Mukta	0.618	0.494	0.321	0.291	0.431
Hisar Suvarna	0.707	0.528	0.486	0.476	0.549
Mean	0.674	0.527	0.463	0.435	

C.D. (p = 0.05) for genotypes = 0.007, ageing = 0.004, Genotypes x ageing = 0.014

4.11 Electrical conductivity (µS/cm/50seed)

The measurement of electrical conductivity is very important for judging vigourness of seeds. The electrical conductivity test of solution reflected the amount of leachates extruded from the seeds and the data is presented in Table 4.11. The range of electrical conductivity of seed varied from 0.08 (HM-548) to 0.16 (Hisar Suvarna) in freshly harvested seed lot, 0.11 (HM-548) to 0.20 (Hisar Mukta) in one year old seed lot, 0.14 (HM-548) to 0.22 (Hisar Mukta) in two year old seed lot and 0.16 (HM-548) to 0.29 (Hisar Mukta) and average mean of genotypes under study varied from 0.14 to 0.21.

All the genotypes lost their membrane integrity significantly and gradually with advancement of storage period. The freshly harvested seeds showed minimum (0.12) electrical conductivity, while three year aged seed showed maximum (0.23). The data on electrical conductivity of leachates indicated that the highest membrane integrity was present in the HM-548 (0.08) followed by UM-305 (0.09) in frehly harvested seed lot. The maximum (0.21) electrical conductivity was recorded in Hisar Mukta and minimum (0.14) in HM-548. The maximum increase in electrical conductivity was recorded in three year aged seed lot in all eleven genotypes followed by two and one year aged seed lots, respectively. The genotype Hisar Mukta showed highest value of electrical conductivity and thus it was rated as poor storer. The electrical conductivity for all the genotypes increased significantly with an increase in ambient seed storage period but the trend was not consistent (Fig 4).

Fig.3: Effect of natural ageing (seed lots) on dehydrogenase ($ODg^{-1}ml^{-1}$) of fenugreek genotypes

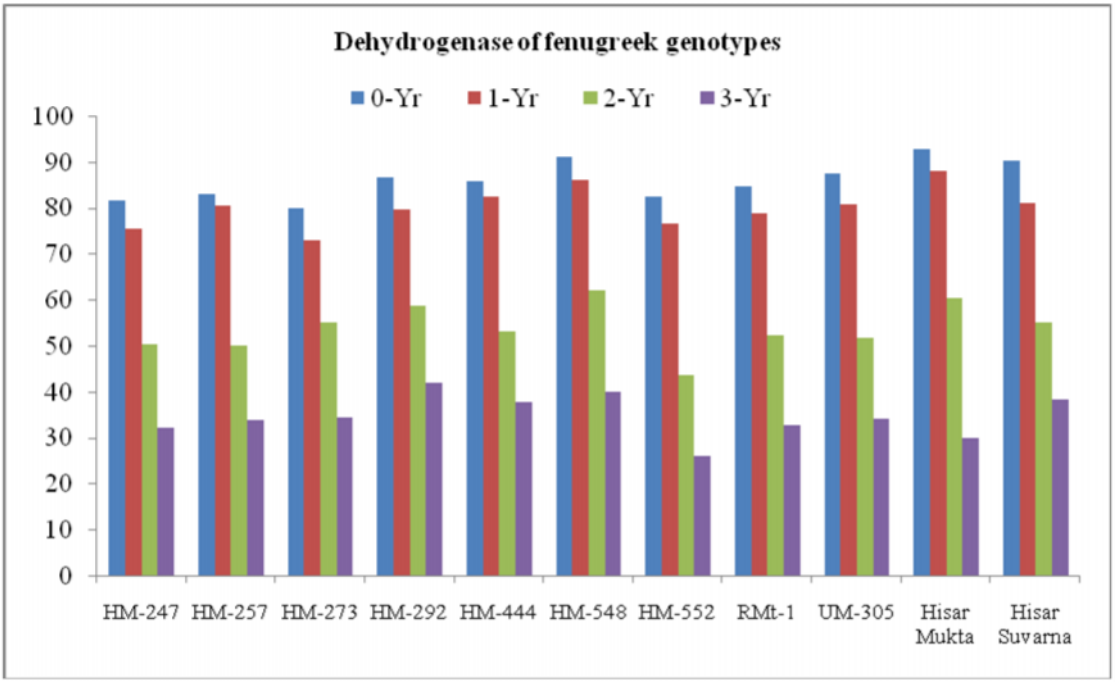


Fig.4: Effect of natural ageing (seed lots) on electrical conductivity (µS/cm/seed) of fenugreek genotypes

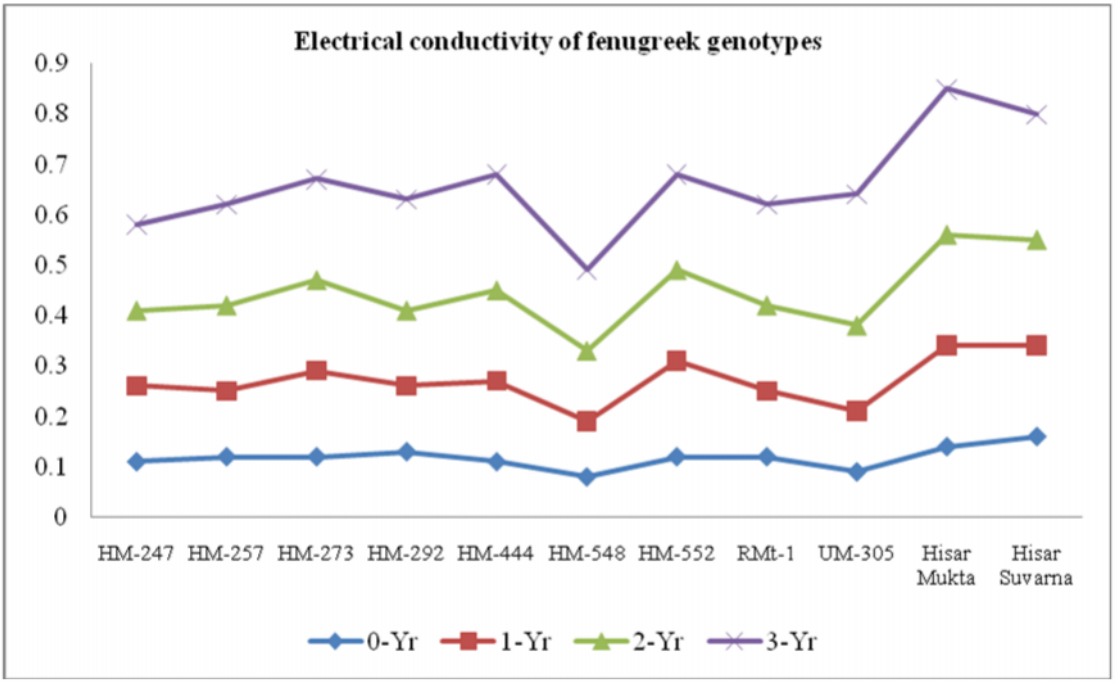


Table 4.11: Effect of natural ageing (seed lots) on electrical conductivity (µS/cm/seed) of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	0.11	0.15	0.15	0.17	0.15
HM-257	0.12	0.13	0.17	0.20	0.15
HM-273	0.12	0.17	0.18	0.20	0.17
HM-292	0.13	0.13	0.15	0.22	0.16
HM-444	0.11	0.16	0.18	0.23	0.17
HM-548	0.08	0.11	0.14	0.16	0.14
HM-552	0.12	0.19	0.18	0.19	0.17
RMt-1	0.12	0.13	0.17	0.20	0.15
UM-305	0.09	0.12	0.17	0.26	0.16
Hisar Mukta	0.14	0.20	0.22	0.29	0.21
Hisar Suvarna	0.16	0.18	0.21	0.25	0.15
Mean	0.12	0.15	0.16	0.23	

C.D. (p = 0.05) for genotypes = 0.009, ageing = 0.006, Genotypes x ageing = 0.019

4.12 Accelerated ageing test

Different seed lots of different genotypes of fenugreek were subjected to accelerated ageing treatment and the on percentage of normal seedlings as per ISTA rules are presented in Table 4.1.

The range of percentage of normal seedlings in different genotypes varied from 92.67 (Hisar Mukta) to 80.00 (HM-273) in freshly harvested seed, 86.00 (HM-548) to 73.00 (HM-273) in one year old seed lot, 62.00 (HM-548) to 43.67 (HM-552) in two year seed lot and 42.00 (HM-292) to 26.00 (HM-552) in three year old seed lot. The mean value ranged from 69.75 (HM-548) to 57.17 (HM-552). The genotype HM-548 (69.75) and Hisar Mukta (67.75) recorded significantly high percentage of normal seedlings because these genotypes strongly resisted the accelerated ageing up to certain period, hence could be classified as a good storer. All the genotypes showed a declining trend as the period of ambient storage advanced (Fig 5).

4.13 pH of seed lechates (%)

The pH exudates test is based on the principle that as the seed deterioration progresses, the cell membrane becomes less rigid and more water permeable, allowing the cell contents, specially acidic and hydrogen ion to escape into solution with water resulting in the lower pH. The pH exudates test is a calorimetric method that predicts the germinability of individual seed based on colour change in the seed exudates from colourless to rosy colour.

Table 4.12: Effect of natural ageing (seed lots) on accelerated ageing of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	81.67	75.33	50.33	32.00	59.83
HM-257	83.00	80.33	50.00	33.67	61.75
HM-273	80.00	73.00	55.00	34.33	60.58
HM-292	86.67	79.67	58.67	42.00	66.75
HM-444	85.67	82.33	53.00	37.67	64.67
HM-548	91.00	86.00	62.00	40.00	69.75
HM-552	82.33	76.67	43.67	26.00	57.17
RMt-1	84.67	78.67	52.33	32.67	62.09
UM-305	87.33	80.67	51.67	34.00	63.42
Hisar Mukta	92.67	88.00	60.33	30.00	67.75
Hisar Suvarna	90.33	81.00	55.00	38.33	66.17
Mean	85.94	80.15	53.82	34.61	63.63

C.D. (p = 0.05) for genotypes = 1.397, ageing = 0.842, Genotypes x ageing = 2.794

Genotypes and storage periods had produced significant effect on pH exudates Table 4.13. The pH exudates test showed variation ranging from 73.24 to 94.51% from three year old seed lot to freshly

Table 4.13: Effect of natural ageing (seed lots) on pH of seed leachates of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	81.67	75.33	50.33	32.00	59.83
HM-257	83.00	80.33	50.00	33.67	61.75
HM-273	80.00	73.00	55.00	34.33	60.58
HM-292	86.67	79.67	58.67	42.00	66.75
HM-444	85.67	82.33	53.00	37.67	64.67
HM-548	91.00	86.00	62.00	40.00	69.75
HM-552	82.33	76.67	43.67	26.00	57.17
RMt-1	84.67	78.67	52.33	32.67	62.09
UM-305	87.33	80.67	51.67	34.00	63.42
Hisar Mukta	92.67	88.00	60.33	30.00	67.75
Hisar Suvarna	90.33	81.00	55.00	38.33	66.17
Mean	85.94	80.15	53.82	34.61	63.63

C.D. (p = 0.05) for genotypes = 1.381, ageing = 0.849, Genotypes x ageing = 2.763

Fig.5: Effect of natural ageing (seed lots) on accelerated ageing (%) of fenugreek genotypes

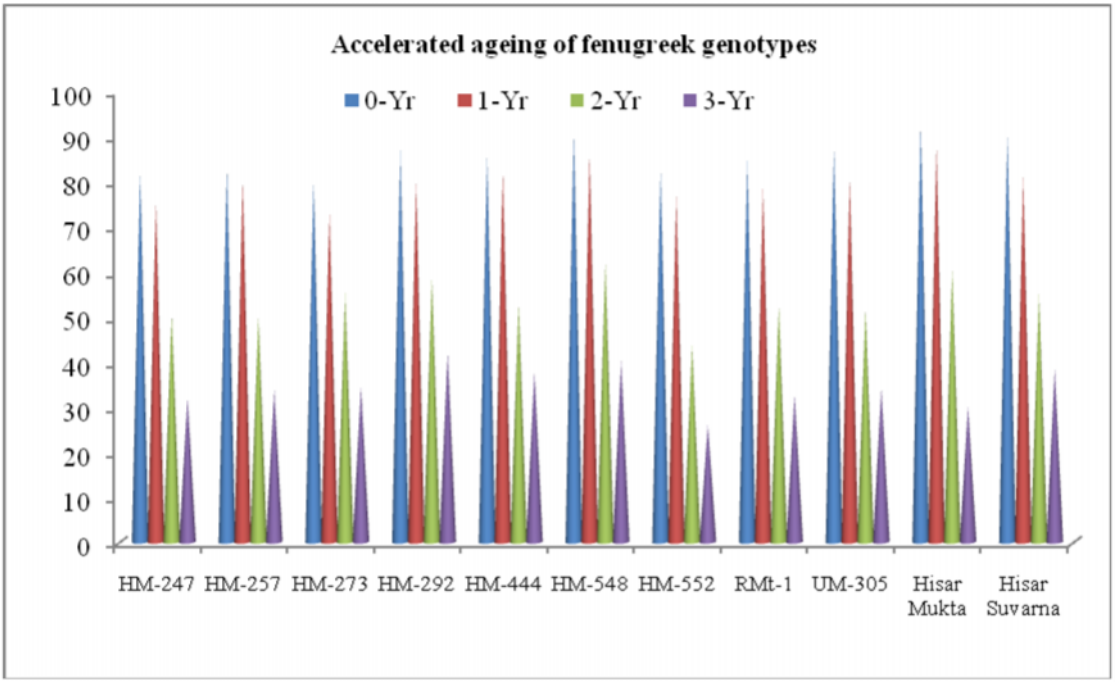
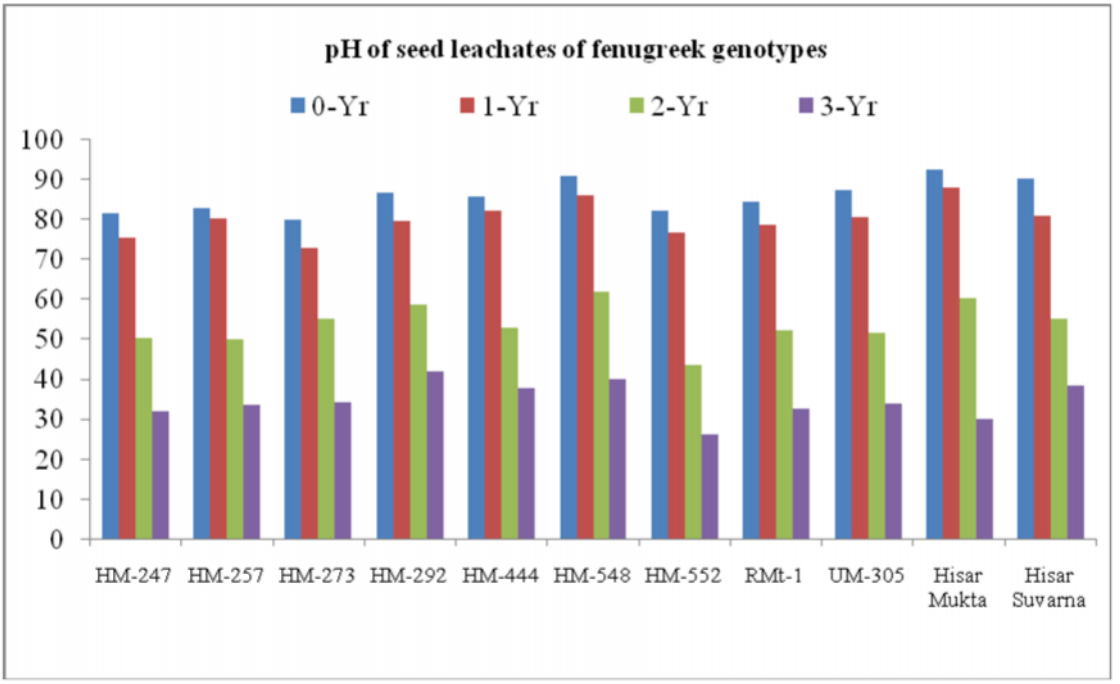


Fig 6: Effect of natural ageing (seed lots) on pH of seed leachates of fenugreek genotypes



harvested seed lot. The genotype HM-548 recorded highest mean value (89.73%) followed by HM-292 and pH was lowest in Um-305(84.24%). However, in third year seed lot, highest pH recorded in HM-548 (77.57%) followed by HM-292 (76.66%), which was statistically at par. It means these two genotypes are good storer and can be used in breeding process. The results further indicated that pH exudates declined significantly in all the genotypes with the passage of storage time, which was 97.00 to 91.78% in freshly harvested seed lot, 94.67 to 91.67% in one year seed lot, 89.67 to 81.88% in two year seed lot and 77.57 to 71.34% in three year seed lot (Fig 6).

4.14 Field emergence index

Under field conditions the speed of seedling emergence was calculated as field emergence index and the results have been presented in Table 4.14. The genotype, seed lot and their interactions were found significant. Irrespective of the genotype, the freshly harvested seeds germinated with faster speed as compared with one, two and three year's old seeds. The results also revealed that the genotype HM-548 emerged with fastest speed (9.20) followed by HM-292 (8.40). The genotype HM-273 emerged at a slowest speed (5.95). Speed of emergence decreased as period of natural ageing increased in all the genotypes. Results also indicated that the number of viable seeds decreased significantly with their increased age, and the pattern was not similar for all the genotypes.

Table 4.14: Effect of natural ageing (seed lots) on field emergence index of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	10.60	8.40	7.76	4.70	7.87
HM-257	8.88	8.15	5.31	2.48	6.21
HM-273	8.91	7.01	4.93	2.95	5.95
HM-292	11.31	8.75	8.10	5.45	8.40
HM-444	8.75	8.07	5.47	2.69	6.25
HM-548	12.49	9.20	8.93	6.18	9.20
HM-552	9.14	8.72	4.88	2.18	6.23
RMt-1	9.10	8.49	5.80	3.30	6.67
UM-305	9.20	8.58	5.77	3.23	6.70
Hisar Mukta	10.40	7.02	4.24	2.32	6.00
Hisar Suvarna	8.95	7.43	7.04	4.81	7.06
Mean	9.70	8.07	6.11	3.57	

C.D. (p = 0.05) for genotypes = 0.342, ageing = 0.206, Genotypes x ageing = 0.684

4.15 Field emergence (%)

Significant differences in field emergence were found among different seed lots of different genotypes of fenugreek Table 4.15. Field emergence ranged from 92.67% (Hisar Mukta) to 80.00% (HM-273) in freshly harvested seeds. Among different seed lots, the field emergence was found maximum (85.94%) in freshly harvested seed lot where as minimum (54.3) was observed in three year aged seed lot. The maximum average value (77.25%) was observed in HM-548 followed by Hisar Mukta (75.25%) and minimum in HM-552 (64.66%). Data indicated that seedling field emergence decreased in all the genotypes with the passage of seed storage time. The data also indicated that the genotype Hisar Mukta reduced sharply from freshly harvested seed to three year old seed.

Table 4.15: Effect of natural ageing (seed lots) on field emergence of fenugreek genotypes

Genotypes	Age of the seed lots assessed (in years)				
	0	1	2	3	Mean
HM-247	85.67 (66.76)	82.33 (63.32)	63.00 (47.71)	57.67 (40.07)	72.16 (56.37)
HM-257	83.00 (64.11)	80.33 (60.37)	60.00 (44.09)	53.67 (36.51)	69.25 (52.37)
HM-273	80.00 (60.30)	73.00 (58.08)	65.00 (49.23)	54.33 (37.42)	68.08 (51.99)
HM-292	86.67 (67.39)	79.67 (64.62)	68.67 (52.19)	62.00 (46.19)	74.25 (59.36)
HM-444	81.67 (61.31)	75.33 (61.39)	60.33 (44.21)	52.00 (34.89)	67.33 (50.18)
HM-548	91.00 (71.03)	86.00 (67.03)	72.00 (56.34)	60.00 (44.09)	77.25 (63.42)
HM-552	82.33 (63.21)	76.67 (62.81)	53.67 (36.51)	46.00 (27.91)	64.66 (48.59)
RMt-1	84.67 (65.95)	78.67 (63.92)	62.33 (46.63)	52.67 (35.33)	69.58 (53.43)
UM-305	87.33 (67.19)	80.67 (60.51)	61.67 (45.51)	54.00 (37.14)	70.91 (54.53)
Hisar Mukta	92.67 (72.93)	88.00 (67.99)	70.33 (54.41)	50.00 (32.19)	75.25 (61.37)
Hisar Suvarna	90.33 (70.31)	81.00 (61.11)	65.00 (49.23)	58.33 (41.29)	73.66 (58.41)
Mean	85.94 (66.91)	80.15 (60.13)	64.27 (48.51)	54.3 (37.42)	

C.D. (p = 0.05) for genotypes = 1.291, ageing = 0.746, Genotypes x ageing = 2.616

Simple correlation coefficient among field and laboratory parameters

Correlation coefficient analysis was employed to find out the association among various seed viability and vigour parameters. The correlation between field and laboratory parameters of freshly harvested seed lot is given in Table 4.16. It is evident that all the parameters were significantly correlated with each other except test weight, seedling length and standard germination. Test weight showed positive and significant correlation with seedling length (0.992), standard germination (0.992) and negative correlated with the electrical conductivity (-0.273). Seedling length showed positive and significant correlation only with standard germination (0.994) and negative correlation with electrical conductivity (-0.235). Dry weight showed positive significant association with all the parameters except electrical conductivity (-0.997).

Table (4.16): Simple correlation coefficient between various seed quality parameters in lab and field (freshly harvested seed)

Kind of tests	TW	SL	SG	SFW	SDW	VI-I	VI-II	TZ	AA	DHA	EC	pH	FEI	FE
TW														
SL	0.992**													
SG	0.992**	0.994**												
SFW	0.270	0.240	0.254											
SDW	0.271	0.238	0.254	0.999**										
VI-I	0.237	0.205	0.220	0.998**	0.999**									
VI-II	0.199	0.166	0.181	0.996**	0.997**	0.999**								
TZ	0.263	0.228	0.243	0.997*	0.999**	0.998**	0.997**							
AA	0.221	0.185	0.199	0.995**	0.997**	0.998**	0.998**	0.998**						
DHA	0.241	0.204	0.218	0.995**	0.997*	0.997**	0.996**	0.998**	0.999**					
EC	-0.273	-0.235	-0.249	-0.995**	-0.997**	-0.996**	-0.994**	-0.998**	-0.998**	-0.999**				
pH	0.274	0.237	0.251	0.995**	0.997**	0.996**	0.994**	0.998*	0.998**	0.999**	-1.000**			
FEI	0.089	0.122	0.108	0.932**	0.933**	0.944**	0.957**	0.936**	0.951**	0.945**	-0.934**	0.933**		
FE	0.092	0.123	0.122	0.931**	0.932**	0.944**	0.956**	0.935**	0.951**	0.945**	-0.933**	0.933**	0.937**	

*Significant at 5% (p = 0.05)
** Significant at 1% (p = 0.01)
TW = Test Weight, SL = Seedling length, SFW = Seedling fresh weight, SDW = Seedling dry weight, VI-I = Vigour index-I, VI-II = Vigour index-II, TZ = Tetrazolium test, AA = Accelerated ageing test, DHA = Dehydrogenase activity test, EC = Electrical conductivity test, pH = pH exudates test, FEI = Field emergence index, FE = Field emergence.

Table (4.17): Simple correlation coefficient between various seed quality parameters in lab and field (one year old seed)

Kind of tests	TW	SL	SG	SFW	SDW	VI-I	VI-II	TZ	AA	DHA	EC	pH	FEI	FE
TW														
SL	0.993**													
SG	0.992**	0.993**												
SFW	0.292	0.264	0.279											
SDW	0.288	0.258	0.274	0.999**										
VI-I	0.222	0.192	0.208	0.996**	0.998*									
VI-II	0.217	0.186	0.202	0.996**	0.997**	1.000**								
TZ	0.285	0.252	0.268	0.996**	0.999**	0.996**	0.996**							
AA	0.267	0.232	0.247	0.995**	0.998**	0.997*	0.997**	0.999**						
DHA	0.272	0.237	0.252	0.994**	0.996**	0.995**	0.995**	0.998*	0.999**					
EC	-0.305	-0.269	-0.284	-0.994**	-0.996**	-0.992**	-0.992**	-0.997**	-0.998**	-0.999**				
pH	0.314	0.278	0.293	0.994**	0.996**	0.992**	0.991**	0.997**	0.998**	0.999**	-1.000**			
FEI	0.088	0.119	0.104	0.923**	0.926**	0.949**	0.951**	0.928**	0.936**	0.934**	-0.922**	0.918**		
FE	0.091	0.120	0.118	0.923**	0.925**	0.949**	0.951**	0.927**	0.935**	0.934**	-0.921**	0.917**	0.931**	

*Significant at 5% (p = 0.05)
** Significant at 1% (p = 0.01)
TW = Test Weight, SL = Seedling length, SFW = Seedling fresh weight, SDW = Seedling dry weight, VI-I = Vigour index-I, VI-II = Vigour index-II,
TZ = Tetrazolium test, AA = Accelerated ageing test, DHA = Dehydrogenase activity test, EC = Electrical conductivity test, pH = pH exudates test,FEI = Field emergence index, FE = Field emergence.

Table (4.18): Simple correlation coefficient between various seed quality parameters in lab and field (two year old seed)

Kind of tests	TW	SL	SG	SFW	SDW	VI-I	VI-II	TZ	AA	DHA	EC	pH	FEI	FE
TW														
SL	0.988**													
SG	0.989**	0.990**												
SFW	0.276	0.240	0.261											
SDW	0.307	0.267	0.290	0.998*										
VI-I	0.199	0.160	0.181	0.995**	0.994**									
VI-II	0.167	0.127	0.149	0.992**	0.989**	0.999**								
TZ	0.200	0.157	0.178	0.993**	0.992**	0.999**	0.998*							
AA	0.234	0.190	0.211	0.994**	0.995**	0.997**	0.995**	0.998**						
DHA	0.230	0.185	0.207	0.993**	0.993**	0.996**	0.994**	0.997**	0.999**					
EC	-0.270	-0.223	-0.245	-0.993**	-0.995**	-0.993**	-0.991**	-0.995**	-0.998**	-0.999**				
pH	0.307	0.260	0.281	0.993**	0.996**	0.989**	0.986**	0.991**	0.996**	0.997**	-0.999**			
FEI	0.086	0.127	0.107	0.929**	0.919**	0.956**	0.966**	0.957**	0.947**	0.949**	-0.936**	0.921**		
FE	0.089	0.127	0.121	0.929**	0.918**	0.956**	0.965**	0.956**	0.946**	0.949**	-0.935**	0.921**	0.918**	

*Significant at 5% (p = 0.05)
** Significant at 1% (p = 0.01)
TW = Test Weight, SL = Seedling length, SFW = Seedling fresh weight, SDW = Seedling dry weight, VI-I = Vigour index-I, VI-II = Vigour index-II,
TZ = Tetrazolium test, AA = Accelerated ageing test, DHA = Dehydrogenase activity test, EC = Electrical conductivity test, pH = pH exudates test,
FEI = Field emergence index, FE = Field emergence.

Table (4.19): Simple correlation coefficient between various seed quality parameters in lab and field (three year old seed)

Kind of tests	TW	SL	SG	SFW	SDW	VI-I	VI-II	TZ	AA	DHA	EC	pH	FEI	FE
TW														
SL	0.984**													
SG	0.984**	0.986**												
SFW	0.249	0.206	0.232											
SDW	0.323	0.275	0.302	0.995**										
VI-I	0.166	0.120	0.146	0.995**	0.987**									
VI-II	0.158	0.111	0.137	0.994**	0.985**	1.000**								
TZ	0.192	0.141	0.167	0.994**	0.989**	0.998**	0.998*							
AA	0.210NS	0.158	0.183	0.994**	0.991**	0.996**	0.996**	0.998**						
DHA	0.179	0.126	0.152	0.991**	0.985*	0.996**	0.996**	0.997**	0.999**					
EC	-0.216	-0.161	-0.187	-0.993**	-0.990**	-0.994**	-0.994**	-0.997**	-0.999**	-0.999**				
pH	0.323	0.266	0.292	0.989**	0.995**	0.981**	0.981*	0.988**	0.992**	0.989**	-0.994**			
FEI	0.086	0.134	0.110	0.938**	0.912**	0.965**	0.967**	0.959**	0.955**	0.964**	-0.954**	0.914**		
FE	0.090	0.134	0.126	0.938**	0.911**	0.965**	0.967**	0.958**	0.954**	0.964**	-0.953**	0.913**	0.927**	

*Significant at 5% (p = 0.05)
** Significant at 1% (p = 0.01)
TW = Test Weight, SL = Seedling length, SFW = Seedling fresh weight, SDW = Seedling dry weight, VI-I = Vigour index-I, VI-II = Vigour index-II, TZ = Tetrazolium test, AA = Accelerated ageing test, DHA = Dehydrogenase activity test, EC = Electrical conductivity test, pH = pH exudates test, FEI = Field emergence index, FE = Field emergence.

Field emergence showed positive and significant correlation with seedling fresh weight (0.931), seedling dry weight (0.932), vigour index-I (0.944), vigour index-II (0.956), tetrazolium test (0.935), accelerated ageing test (0.951), dehydrogenase activity test (0.945), pH exudates (0.933) and field emergence index (0.937) and negative and significant correlation with electrical conductivity (-0.933).

The correlations between field and laboratory parameters of one year old seed lot are presented in Table 4.17. Field emergence showed positive and significant correlation with fresh weight (0.923), dry weight (0.925), vigour index- I (0.949), vigour index-II (0.951), tetrazolium test (0.927), accelerated ageing test (0.935), dehydrogenase activity test (0.934) and and pH exudates (0.917) and field emergence index (0.931) and negative and significant correlation with electrical conductivity (-0.921). Test weight showed positive and significant correlation with seedling length (0.993) and standard germination (0.992). All the parameters showed negative and significant correlation with electrical conductivity.

The correlations between field and laboratory field and laboratory parameters of two year old seed lot are presented in Table 4.18. It is evident that pH exudates showed positive and significant correlation with all the parameters except electrical conductivity. Test weight and seedling length predicted positive and significant relationship with standard germination (0.989) and (0.990) respectively.

The correlations between field and laboratory parameters of three year old seed lot are presented in Table 4.19. The data indicated that dehydrogenase activity test was positively and significantly correlated with fresh weight (0.991), dry weight (0.985), vigour index-I (0.996), vigour index-II (0.996), tetrazolium test (0.997), accelerated ageing test (0.999), pH exudates (0.989), field emergence index (0.964) and field emergence (0.964) and it show negative correlation with electrical conductivity (-0.999).

It was predicted from all the correlations between various laboratory parameters of seed quality and field emergence was having higher values in freshly harvested seed lot as compared to other seed lot. So the lots which showed maximum values were more vigorous. However, same trend of correlation was observed among different field and laboratory parameters for all the seed lots of different genotypes.

CHAPTER - V

DISCUSSION

The vigour and viability of seeds are at the highest level at the time of optimum harvest which undergo a continuous gradual decline in its quality during the course of seed storage particularly under ambient storage conditions till the seeds become completely non-viable. Deterioration of seed during storage is governed by a number of intrinsic and extrinsic factors. Intrinsic factor includes all such variations in seed metabolism which occur due to the differences in environmental and seraphic conditions during plant growth particularly, during development and growth of seed, whereas the extrinsic factors include relative humidity, temperature and availability of oxygen in the storage environment. Due to these factors, the rate and extent of decline in seed quality with respect to viability and vigour varies considerably among different cultivars of same species and even different seed lots of the same variety. In the present investigations, eleven fenugreek genotypes were studied with a view to have substantial information on seed viability and vigour status of these cultivars for a healthy and bumper crop.

Although a number of viability and vigour tests have been attempted to evaluate the viability and vigour status of the cultivars, based on physical, physiological and biochemical parameters of the different seed lots. Efforts were also made to find out a most practicable test which could give satisfactory reproducible results with regards to field performance of seed. Studies were also made to work out correlation among different tests to determine the association of various seed quality parameters with field emergence and to assess the efficacy of different seed quality parameters as a predictor of field emergence (*viz*.,Laboratory vs. Field emergence).

The change in the seed viability under ambient storage conditions is a function of a complex interaction of genetic constitution and environmental conditions. In the present studies also considerable variability was found in seed viability (estimated by standard germination and Tz-test) of different varieties. In both cases, with slight variations, HM-548 followed by HM-292 retained higher viability (germination) percentage even after three years of ambient storage while the varieties UM-305, HM-444 and Hisar Mukta recorded significantly lower viability percentage. James (1967) described this variation for seed longevity among genotypes to be genetically controlled. The germination and viability of the seeds was found maximum in all the varieties at the initial stage of testing i.e. before storage and thereafter, it declined gradually as the period of ambient storage advanced. The present results also corroborate with the findings of Kumar (2001) and Singh (2012) in fenugreek where loss of seed viability and vigour increased with increase in period of storage.

The correlation studies revealed highly significant and positive correlation between these two tests and field emergence, suggesting that these two tests can be successfully used as predictor of field emergence of fenugreek seeds. Similar results were reported by Kumar (2007 and 2010) in coriander.

The germination test fails to account for the progressive nature of seed deterioration. Seeds are merely classified as either germinable or non-germinable with no distinctions between strong and weak seedlings or seed storage potential. These weaknesses have encouraged interest in vigour testing to provide information about the quality of seed not revealed by standard germination test. In 1979, Association of Official Seed Analyst's Vigour Committee defined seed vigour as "those properties which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions (McDonald, 1980). Among the factors that influence seed vigour are genetic constitution, environment during seed development and seed storage environment.

In the present studies also, the variability in the fenugreek seeds of different genotypes of three years seeds lots were observed for seed vigour based on seedling length, dry matter production and seedling vigour index. Seedling length (root + shoot), seedling fresh weight and seedling dry weight decreased as the ageing period increased in all the eleven genotypes of fenugreek. Decrease in seedling length was found maximum in Hisar Mukta (4.42cm) followed by HM-552 (4.35cm) and minimum in HM-548 (2.92cm) while, seedling fresh and dry weight are found maximum in Hisar Mukta and minimum in Hisar Suvarna. In three year old seed, seedling length much more less than freshly harvested seed lot. Similar findings were reported by Nagrajan *et al.* (2004) in okra, Verma *et al.* (2003) in mustard and Kumar (2007) in coriander.

The seeds of all the varieties recorded maximum seedling vigour before storage (freshly harvested seed). With increased period of storage, a progressive decline in seed quality and vigour was observed as exemplified by decreasing seedling length, dry matter production and vigour indices irrespective of varieties. After three years of storage, HM-548 was found strongest in seed vigour in terms of longest seedling length and seedling vigour index followed by HM-292 while HM-444, UM-305 and Hisar Mukta was recorded as poorest performer with respect to these parameters. The genotype HM-548 showed high seed vigour index-I and was at par with HM-292 which showed highest vigour index-II indicating that these two vigorous genotypes can be used in further breeding improvement programmes for better results. Singh (2012) studied storage potential of fenugreek genotypes and observed decline in germination, seedling length, dry matter production and seedling vigour index with increase in the period of storage. The reduction in the physical and physiological manifestation of vigour during storage could be attributed to the irreversible deteriorative changes occurring in them as a result of ageing (Palanisamy and Karivaratharaju, 1991).

Furthermore, root length, seedling dry weight and seedling vigour index exhibited significant positive correlation among themselves and with germination percentage in field.

The accelerated ageing technique has given useful information on the relative storability of normal seed of different varieties in these studies. The seeds of HM-548 followed by Hisar Mukta produced maximum percentage of normal seedlings after accelerated ageing at all the storage periods. Although, in general, there was gradual decline in all the varieties as the storage period progressed but the percentage of normal seedlings was drastically reduced in HM-552 after three years of ambient storage. Hence these varieties were proved poor storer under ambient storage. Deka et al. (1993) and Doijode (1990) also observed the genotypic differences of tomato to accelerated ageing treatment confirming the results of present investigation.

This trend of results is similar to the quantitative trend of seed quality tests without ageing treatment. This fact is evident from critical review of correlation among accelerated ageing, standard germination test, Tz test, seedling vigour index under laboratory conditions and germination percentage (field emergence) in the field conditions where highly significant and positive correlations were indicated. The present results are in accordance with those of Ram (1987) in green gram and Karuna and Aswathaiah (1990) in beetroot and carrot. The work's and information available in this area particularly for vegetable seeds are limited. Hence, for more accurate prediction of storability, storage life and field performance of seeds, a detailed work needs to be undertaken to develop close relation between vigour levels and storability.

Despite an increasing amount of research on various aspects of seed deterioration and vigour, the relative importance of the factors at the biochemical level, which may limit seed performance, are still very poorly understood. Investigations on electrical conductivity (EC) of seed lechates assessed for their storability and quality have also given good idea of seed quality. The genotypes which withstood the stress were found to maintain their integrity as determined by the low electrolyte leakage in the seed steep water. In the present study, the amount of electrolytes leached in to the seed steep water was negatively associated with seed vigour thereby implying the well knit relationship between these tests and their negative relationship to the germination, seedling vigour index and field emergence indicating the relationship of both biochemical and physiological tests in studying the seed vigour under storage. The seeds of variety HM-548 were recorded good storer in the present investigation with respect to this parameter. In general, there was a gradual increase in EC in seed steep water as the storage period advanced irrespective of the variety. Kumar (2010) also observed increase in leaching of electrolytes in coriander seed with increased period of storage. The results of the present study clearly implicate cellular membranes in seed deterioration. The loss of germinability during storage was dependent on corresponding loss of membrane

integrity as demonstrated by increased leakage of electrolytes and metabolites from the seeds. These results also corroborate with the work done by Vadivelu *et al*. (1989) in tomato and Kumar (2004) in onion.

The dehydrogenase enzyme activity, a measure of vigour (Kittock and Law, 1968) and α-amylase enzyme, important for energy production and protein synthesis during germination, exhibited a progressive decrease with advancement of storage period in the present study. The activity was found at high order in freshly harvested seeds tested just before storage. The varieties, HM-247 followed by HM-548, recorded higher enzyme activity among all the varieties irrespective of the storage period. Similar findings were reported by Kumar and Verma (2008) in fenugreek and Kumar (2010) in coriander who observed that dehydrogenase and α-amylase enzyme activity of seeds decreased with advancement in storage period. Kumar (2001) also studied a decline in the activity of dehydrogenase with ageing duration in fenugreek. He obtained a significant positive correlation with the enzyme activity, germination and vigour of seeds. Further documentation of evidence to the present investigation is by Subbarao (1984) who also observed decrease in the activity of dehydrogenase as well as amylase enzyme during ageing in brinjal.

Depletion of food reserves is one of the oldest theories on deterioration; however, it has not survived critical scrutiny in the present studies also. Slight variations in seed weight index of each variety during various storage periods may be attributed to the genetic constitution of seed and relative humidity of storage environment. It showed association with certain laboratory tests up to some extent with field emergence but failed in the prediction of percent germination germination in field conditions. However, varieties with higher seed weight index i.e. HM-548 and HM-292 were found superior in terms of various viability and vigour paremeters of seed quality. Similar results were also reported by Whittington and Fierlinger (1972) and Palanisamy and Ramasamy(1985) in tomato.

The results revealed that, the genotype HM-548 was observed to be superior for all the above paramaters except for test weight and DHA test. Superiority of this genotype over others might be due to different genetic makeup as seed vigour was influenced by genetic factors. Similarly, among all the four years seed lots, freshly harvested seed lot showed maximum vigour and viability for all the parameters except electrical conductivity, where it showed lowest value among all the seed lots because it had negative correlation with freshly harvested seed lot and less amount of seed leachates exudated.

CHAPTER - VI

SUMMARY AND CONCLUSION

Fenugreek (*Trigonella foenum-graecum* L.) is one of the most important seed spice crop of the country. The inadequate availability of quality seeds in sufficient quantities of the improved cultivars is one of the constraints in improving the productivity of fenugreek. Quality seed is a basic input for realizing higher yields per unit area. The quality seed not only offers the highest economical and social returns among all the inputs but also ensures the optimum utilization of all the inputs *viz.*, fertilizer, irrigation, pesticides etc. Availability of viable and vigour seeds at planting time is important for achieving targets of agricultural production because good quality seeds act as a catalyst for realizing the full potential of other inputs.

Therefore, the present study entitled "**Seed quality assessment in naturally aged seeds of fenugreek**" was carried out at the Vegetable Research Farm and Laboratories of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar during 2012-2013 with a view to study the comparative efficacy of seed viability and vigour tests as predictor of standard germination and seedling establishment in the field and to evaluate different genotypes of fenugreek for various seed quality parameters. The salient findings of the present investigation are summarized below:-

- The viability and germination of seeds declined to varying degrees in different genotypes of fenugreek during ambient storage up to three years. Genotypes HM-548 was observed as best genotype with respect to these parameters followed by HM-292 and genotype UM-305 and Hisar Mukta were found to be poorest among all the eleven genotypes.
- The vigour of seeds as indexed by seedling length, seedling dry weight, seedling vigour index-I & II, accelerated ageing, electrical conductivity of of seed leachates, dehydrogenase enzyme activity also declined to varying degrees in different genotypes of fenugreek during ambient storage and genotypes HM-548, HM-292 and HM-247 were found superior over other genotypes.
- Two years old seed lots and three year old seed lots showed significant reduction for all the parameters under study irrespective of genotypes.
- Electrical conductivity increased with the advancement of storage period.
- Genotypes HM-548 and HM-292 showed better performance under laboratory as well as field conditions than other genotypes.
- The standard germination, seedling dry weight, seedling vigour index-I & II, accelerated ageing, dehydrogenase enzyme activity and Tz-test were found

significantly and positively correlated with field emergence index except test weight and seedling length in all the seed lots.

- ❖ Electrical conductivity was negatively correlated with field emergence index in all the seed lots.

On the basis of above mentioned experimental findings, it can be concluded that seeds all the genotypes under studies except Hisar Mukta and HM-444 can be stored for three years with considerable seed viability and vigour under ambient conditions. The laboratory tests viz., standard germination, vigour index-I, vigour index-II, dehydrogenase enzyme activity and tetrazolium test emerged as reliable predictors of seed quality and field emergence in fenugreek.

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ABSTRACT

Title of Thesis	:	**Seed quality assessment in naturally aged seeds of fenugreek (*Trigonella foenum graecum* L.)**
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Keywords: Fenugreek, natural ageing, seed quality, germination, ambient conditions, vigour, viability, accelerated ageing , electrical conductivity, field emergence, correlation.

Seed quality plays an important role in the crop establishment and overall performance of the crop .The present investigation was carried out on fenugreek seeds having eleven genotypes *viz.,* HM-247, HM-257, HM-273, HM-292, HM-444, HM-548, HM-552, RMt-1, UM-305, Hisar Mukta, Hisar Swarna. Four seed lots of each genotype included the freshly harvested, one year old, two year old and three year old seeds stored under ambient conditions. All the eleven genotypes of fenugreek having diversity in seed size and colour were taken to evaluate the seed viability/vigour parameters, to determine the inter-relationship between laboratory and field parameters, to assess the efficacy of different seed vigour tests as a predictor of field potential and to determine the speed of germination. All the seed lots were subjected to various viability, vigour tests with three replications, and observations were recorded on standard germination, seedling length, seedling fresh weight, seedling dry weight, vigour index-I, vigour index-II, seed weight, electrical conductivity, dehydrogenase activity, tetrazolium test,pH exudates test, accelerated ageing test, field emergence and field emergence index.

It was observed that freshly harvested seed lot showed highest mean value for all the parameters except electrical conductivity. Among the genotypes HM-247, HM-292 and HM-548 were observed to be superior for seedling length, seedling dry weight, seedling fresh weight, vigour index-I, vigour index-II, dehydrogenase activity and tetrazolium tests. The genotypes which could bear larger stress reduced more formazan and vice versa. Simple correlation studies indicated that all the viability and vigour parameters (except test weight and seedling length) were positively associated among themselves. Higher the values of above said parameters, better was the seed quality and vice-versa. Electrical conductivity test showed negative association with seed quality indicating the status of membrane integrity. Genotypes HM-548, HM-292, HM-247 show better performance under laboratory as well as field conditions than other genotypes. . The genotype UM-305 was found to be inferior for test weight, standard germination, dry weight, vigour index-I, vigour index-II, dehydrogenase activity test, tetrazolium test.

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