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Influence of method of Storage on extending the longevity of coffee seeds (*Coffea arabica* L.)

Senthil Raj R and V Manonmani

Abstract
Coffee (*Coffea arabica* L.) seeds are classified into intermediate seeds which require high moisture content during the storage and possess a short shelf life. Coffee trees are propagated using seedlings derived from seeds. The present study evaluated the suitable storage method for prolonging the viability of the seed. The results showed that the shelf life of coffee seed was extended up to 80 days by storing in 300-gauge polythene bags at 15 °C. However, the seeds stored under environmental condition maintained the viability up to 20 days only. Also, the seeds stored along with moist media have not better way for extending the shelf life when compared with seed stored at 15 °C. The maximum seedling vigour and least electrical conductivity and free sugars were found in the seeds stored at 15 °C. The results showed that the coffee seeds can be stored up to 80 days in 300-gauge polythene bag at 15 °C.

Keywords: Coffee seeds, recalcitrant seed, storage temperature, storage medium, viability, desiccation sensitive

Introduction

Globally, coffee (*Coffea arabica* L.) is one of the most valuable commodities. The world's economy, which is second only to petroleum in international trade, is heavily dependent on the economy of Asia, Latin America, and Africa. Coffee occupies a place of pride in international trade next to petroleum, with a total industry turnover of nearly US $ 70 million. Coffee is one of the most popular commodities worldwide with world exports reaching around 10.5 billion kg of fruits in 2020, generating more than U$ 26 billion. India has emerged as a sixth largest quality coffee producer in the world, accounting for more than 40% of world coffee production and occupies the total production of 95,000 MT during 2019-20 from an area of 2.28 lakh ha contributing to a world share of 4.5 per cent. The bulk of production is done in southern states of India (Naidu *et al.*, 2018) [33]. In the states of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu, and North East, coffee was mainly cultivated commodity. In Tamil Nadu, production of coffee is about 13.7 MT in 27.8 hectares. (India statistics 2005). The majority of cultivated *Arabica* coffee is predominantly self-pollinating and cultivated by seeds. The self-pollination characteristic of Arabica coffee causes high genetic uniformity in the coffee plant population. The variations of *Arabica* coffee population are caused by the spontaneous mutation of genes that control the character of the fruits and seeds as well as the natural crossover that occurs between two types of Arabica coffee (Clifford & Willson, 1985, Gebreselassie *et al.*, 2010 [10, 17]. Planting material in the form of seeds in Arabica coffee is very popular (Rosa *et al.*, 2011, Nasiro *et al.*, 2017) [11, 34] because in addition to have genetic variation in a low population, it is also easier to distribute and produce large numbers of plants in one harvest period. The main obstacle to planting material of seeds on coffee plants is the short shelf life period. Coffee seeds are able to germinate shortly after harvest and seed viability can decline rapidly. Coffee seeds are classified as intermediate seeds (Ellis *et al.*, 1990; Hong & Ellis, 1996; Selmar *et al.*, 2006) [16, 20, 46] because they require high moisture content during the storage and the shelf life of coffee seeds is relatively short (Dussert *et al.*, 1997, Rahardjo, 2012) [13, 39]. The shelf life of coffee seeds will be longer if stored at low temperatures. Nasiro *et al.*, (2017) [34] revealed that coffee seeds stored at 15ºC were still able to maintain their germination of 71–78% while being stored at room temperature (± 28ºC) was only able to maintain seed germination of 43–55%. Coffee seeds still germinate after being stored for 10 months at 15 °C and the moisture content of 10-11%. (Ellis *et al.*, 1990) [16]. The storage using low moisture content is contrary to the provisions of the Regulation of Ministry of Agriculture No. 89 (2013) which stated that the moisture content of coffee seed stored is 30–40%. Nasiro *et al.*, (2017) [34] showed that coffee seeds stored at 15 °C were able to maintain seed viability more than 80% for

five months of storage while coffee seeds stored at room temperature only lasted for three months with a germination percentage of more than 80%. In contrast, Rosa *et al.*, (2011) [11] found that coffee seeds stored at 10-15 °C and 10–11% moisture content will produce poor quality seeds while if at 20 °C still produce good quality seeds. Apart from storage temperature, the germination rate is also influenced by the level of fruit maturity at harvest. Coffee seeds harvested from fully ripe fruit (physiologically ripe) have the most optimal germination compared to seeds harvested when still green or yellowish towards red (Rosa *et al.*, 2011, Baliza *et al.*, 2012) [11, 4]. The germination testing aims to determine the potential of germination in the seeds lots and can be used to compare the quality among the seeds lots. It is also used to predict the success of seedling in the nursery (Sudrajat *et al.*, 2017) [53]. The use of seeds which has high physiology potential is urgently needed for the success of production and it is also significantly contributing in the early process of seedling (Rahimi, 2013, Castan *et al.* 2018) [40, 6]. Therefore, the evaluations of germination potential in the seeds which have faster seed deterioration period and have been stored for a certain time are urgently needed. Seeds able to germinate when the root bulges or shoots appear. According to (Silva 2002) [12], the germination process of seeds starts when water was absorbed through an inhibition process and it lasts when the embryo axis begins to elongate, usually a potential root. Seeds can germinate when placed in appropriate condition of environment, adequate water supply and proper light so the testing in laboratory can be the basic to measure the viability potential of the seeds (Kader, 2005, Eira *et al.*, 2006, and Sudrajat *et al.*, 2017) [24, 14, 53].

Generally, storage is the major problem for recalcitrant seeds. So, any development in short-term storage will ease the problem of field collection and transportation to gene banks. The current successful short-term storage methods are limited to moist or imbibed storage, controlled atmospheric techniques and partial drying methods. These methods are successful for many of the recalcitrant seeds. In imbibed storage, the most common storage media are damp charcoal, saw dust, moist sand and other moisture conserving materials and chemicals. Also, the storage of seeds in controlled atmosphere with various gases or in sealed containers or waxing has had some success (Chin *et al.*, 1984) [8]. In addition, it is difficult to maintain quality of recalcitrant seeds during storage as the seeds themselves are variable in their size, moisture and viability. Also, there is a lack in understanding of the fundamental mechanisms and behaviour in recalcitrant group of seeds. So, the information on physiology of seed deterioration, seed longevity, storage behaviour and storage methods are inadequate. Hence, the present study was carried out to find the better storage method to prolong the viability of the coffee seeds and the changes caused during the desiccation of the seeds.

Materials and Methods

The freshly harvested coffee (*Coffea arabica* L cv. chandragiri) berries were collected from the Regional Coffee Research Station, Thandigudi was formed the base materials for the present study to identify suitable storage method for extending the shelf life of the seed. The freshly collected berries were used to extract the seeds and the uniform size seeds were obtained by manual grading. Then, the seeds were submitted to different storage treatments *viz.*, Ambient Storage (control) - SM_1, Seed in wood ash - SM_2, Seed in 5% moist charcoal - SM_3, Seed in 10% moist charcoal - SM_4, Seed in 5% moist sand - SM_5, Seed in 10% moist sand - SM_6, Seed stored @ 15 ℃ - SM_7, Seed stored @ 10℃ - SM_8, Seed stored @ 5 ℃ - SM_9. Then, the procedure described by Bhattacharyya and Basu (1992) [5] was followed for moist storage of coffee seeds. In case of moist sand incubation, medium grained building quality sand was used. The sand was thoroughly washed in running tap water, dried in the sun and again dried overnight in the hot air oven at 100 °C followed by cooling to room temperature before use. In charcoal incubation, fine quality materials were used. These materials were autoclaved at 15 lb pressure for 20 min followed by cooling to room temperature before use. Air dried incubation materials were mixed with carbendazim @ 2 g kg^{-1} and then moistened with respective solutions as per the following treatments. The seeds were uniformly covered with the pre-moistened incubation materials at the ratio of 1:3 and stored in loosely bound 300-gauge polythene bags under ambient room condition (25±2°C and 60% RH) for a period of 3 months. Seed samples were drawn at 10 days' interval and the following physiological and biochemical changes were estimated with four replicates.

Seed weight, and seed moisture content

The seeds kept for desiccation were weighed using an electronic balance and the mean values were expressed in gram. Moisture content of seed was determined by using hot air oven method. Empty weight of moisture bottle with lid (M_1) was noted. Then fresh seeds were weighed along with moisture bottle (M_2) and placed in hot air oven maintained at 105 °C for about 16 hour. Then the bottles were taken out and cooled in a desiccator containing calcium chloride ($CaCl_2$) for 20 minutes. The weight of bottle along with dried seeds (M_3) was recorded individually. The moisture content was calculated using the following formula and the mean was expressed as per cent.

$$\text{Moisture per cent} = \frac{M_2 - M_3}{M_2 - M} \times 100$$

Where,
M_1 = Weight of moisture bottle
M_2 = Weight of moisture bottle + sample before drying
M_3 = Weight of moisture bottle + sample after drying

Germination and vigour index

Germination test was conducted in sand medium using 25 seeds for each replication with ((ISTA, 1999) room temperature of 25±1°C and 95±2 per cent relative humidity. At the end of 45 days, the number of normal seedlings was counted and the germination per cent was calculated. Vigour index values were calculated adopting the following formula as suggested by Abdul-Baki and Anderson (1973) [1] and was expressed in whole number.

Vigour index = Germination (%) x Mean seedling length (cm)

Electrical conductivity of seed leachate and free sugars

Four replicates of 10 seeds were drawn and soaked in 20 ml of distilled water for 36 h and the electrical conductivity of seed leachate was measured using the digital electrical conductivity meter with an electrode possessing a cell

constant of one of Presley (1958) [38] using the electrical conductivity of seed leachate was expressed in μSm^{-1}. The leachates of coffee seeds after the electrical conductivity test were used for determining the amount of sugar following the method described Somogyi (1952) [50]. After measuring the electrical conductivity, the seed leachate was used to determine the free sugars. One ml of seed leachate was taken and one ml of copper reagent was added and boiled for 15 min. After cooling this mixture in running water, one ml of Nelson's arseno molybdate reagent was added for colour development and the final volume was made up to 10 ml with distilled water. The colour intensity was measured as optical density value in UV-VIS spectrophotometer at 620 nm. Then, the amount of sugar was calculated against a glucose standard and expressed in mg g^{-1}.

Experimental Results

Seed moisture, Seed weight germination and vigour index

Statistically significant differences were observed in seed moisture due to storage treatments, periods and their interactions. Maximum seed moisture (45.2%) was recorded in SM_7 and minimum in SM_1 (20.7%). Among the storage periods, seed moisture was maximum (59.6%) at initial period and minimum (10.3%) at 90 days after storage. Interaction between storage periods and treatments showed that SM_7 has recorded maximum seed moisture (21.3%) (Table. 1). Highly significant differences were recorded in seed weight due to storage treatments, periods and their interactions. Among the treatments, maximum seed weight (9.8 g) was recorded in SM_7 and minimum in SM_1 (4.6 g). In case of storage periods, seed weight was maximum (11.7 g) at initial period and minimum (4.3 g) at 90 days after storage. Interaction between treatments and storage periods showed that SM_7 has recorded maximum seed weight (7.9 g) at 90 days after storage (Table. 2). Highly significant differences were observed in germination due to storage treatments, periods and their interactions. Among the storage treatments, maximum germination (45%) was recorded in SM_7 and minimum (13%) at SM_1. In case of storage periods, the maximum germination (78%) was observed at initial period and minimum (3%) at 80 days after storage. Interaction between storage periods and treatments showed that SM_7 was recorded maximum germination (14%) at 80 days after storage (Table. 3). The variations in vigour index were noticed with significant differences due to the storage periods, treatments and their interactions. Among the treatments, maximum vigour index (106) was recorded in SM_7 and minimum in SM_1 (27). During period of storage, initial vigour (226) was reduced and recorded the lowest (3) at 80 days after storage. Interaction between storage periods and treatments showed that SM_7 has recorded the highest vigour index (17) at 80 days after storage (Table. 4).

Electrical conductivity of seed leachate, and free sugars

Electrical conductivity of seed leachate showed highly significant differences due storage treatments and periods. Among the treatments, minimum electrical conductivity (283 μSm^{-1}) was noticed in SM_7 and maximum (628 μSm^{-1}) in SM_1. During period of storage, the minimum electrical conductivity (109 μSm^{-1}) recorded at initial period was increased and recorded maximum (825 μSm^{-1}) on 90 days after storage (Table. 5). Highly significant differences were noticed for free sugars due to storage treatments, periods and their interactions. Among the storage treatments, minimum free sugars (3.09 mg g^{-1}) were recorded in SM_7 and maximum (6.76 mg g^{-1}) in SM_1. During period of storage, minimum (0.76 mg g^{-1}) free sugars were increased and reached maximum (14.07 mg g^{-1}) at 90 days after storage. Interaction between periods of storage and treatments showed that the seeds stored at 10°C (SM_7) have recorded minimum free sugars (7.55 mg g^{-1}) at 90 days after storage. (Table. 6).

Discussion

Many tropical recalcitrant species lose its viability even at short period for below 10 to 15 °C (Chin and Roberts, 1980) [7]. However, in this present study the results showed that the coffee seeds stored in sealed 300-gauge polythene bags at 15 °C maintained the maximum germination (14%) up to 80 days with the moisture content of 26.4 per cent (fig 2). Whereas, the seeds stored at ambient condition drastically reduced its viability due to heavy reduction in moisture content. Similar results of low temperature storage were recorded in cocoa in which, the seeds stored at 15 °C maintained better viability compared with 17 °C (Hor, 1984) [21]. Song *et al.* (1984) [51] also reported that *Hopea hainanensis* seeds stored at 15 to 20 °C maintained viability upto 6 months. Tompsett (1985) [57] found that the sudden loss of germination at 6°C was noticed in *Shorea robusta* seeds but the viability was maintained better at 11°C. The present findings also revealed similar trends that seeds stored at low temperature (5 °C) recorded sudden reduction in germination compared with seeds stored at 15 °C (fig 1). However, Barman (1990) found that tea seeds are viable when stored in sealed polythene bags at 4 °C for a period of one year. Contrastingly, Tompsett (1992) [58] found that moist *Dipterocarpus* seeds showed reduction in germination at low temperatures range from 0 to 16°C. The viability of clove, nutmeg, rubber seeds could successfully storage upto 45, 70 and 85 days at 10°C (Gunasekaran, 1997) [19]. Srimathi (1997) [52] found that jamun seeds stored at 2 per cent moist sand at 10°C prolong the viability up to three months. Similarly, low temperature storage was found in many crops *viz.*, jack at 10 °C (Shylla Merlin and Palanisamy, 2000) [47], *Calophyllum brasiliensis* at 15 °C (Vasquez, 2001), *Madhuca indica* at 15 °C (Varghese *et al*., 2002) [59], arecanut at 20 °C (Raja *et al*., 2005) [42] and *Embelia ribes* at 10°C (Sivalingam *et al*., 2011). Coffee seeds are classified as intermediate seeds (Ellis *et al.*, 1990; Hong & Ellis, 1996; Selmar *et al.*, 2006) [16, 20, 46] because they require high moisture content during the storage and the shelf life of coffee seeds is relatively short (Dussert *et al.*, 1997, Rahardjo, 2012) [13, 39]. The shelf life of coffee seeds will be longer if stored at low temperatures. Nasiro *et al.* (2017) [34] revealed that coffee seeds stored at 15ºC were still able to maintain their germination of 71-78% while being stored at room temperature (± 28 ºC) was only able to maintain seed germination of 43– 55%. Coffee seeds still germinate after being stored for 10 months at 15 °C and the moisture content of 10-11% (Ellis *et al.*, 1990) [16]. The storage using low moisture content is contrary to the provisions of the Regulation of Ministry of Agriculture No. 89 (2013) which stated that the moisture content of coffee seed stored is 30-40%. Nasiro *et al.* (2017) [34] showed that coffee seeds stored at 15 °C were able to maintain seed viability more than 80% for five months of storage while coffee seeds stored at room temperature only lasted for three months with a germination percentage of more than 80%. In contrast, Rosa *et al.* (2011)

[11] found that coffee seeds stored at 10–15 °C and 10-11% moisture content will produce poor quality seeds while if at 20°C still produce good quality seeds. Apart from storage temperature, the germination rate is also influenced by the level of fruit maturity at harvest. Coffee seeds harvested from fully ripe fruit (physiologically ripe) have the most optimal germination compared to seeds harvested when still green or yellowish towards red (Rosa *et al.*, 2011, Baliza *et al.*, 2012) [11, 4].

Among the storage methods, the seeds stored in water at 15 °C and at ambient conditions have recorded maximum electrical conductivity in seed leachate compared to the seeds stored at 15 °C (fig. 2). Similar results were observed in sub-ambient temperatures due to the changes in membrane thickness and permeability by Wolfe (1978). Ching (1972) reported the increased permeability of cellular membrane would result in the leaching of organic and inorganic solvent in imbibing medium. Tamari (1976) [56] also reported the increased electrical conductivity at low temperature storage in *Hopea* seeds. Similar findings were reported in cocoa (Hor, 1984) [21], *Hancornia speciosa* (Oliveria and Valio, 1992) [36], clove, nutmeg and rubber (Gunasekaran, 1997) [19], Jack (Shylla Merlin and Palanisamy, 2000) [47] and arecanut (Raja *et al*., 2005) [42]. In addition, free sugars of coffee seed leachate were increased with the advancement of storage period. The maximum free sugars were recorded in the coffee seeds stored in at 15 °C. However, the seeds stored at 15 °C recorded minimum values because of maintenance of better membrane integrity.

Conclusion

Obtaining commercially useful coffee seedlings is hindered by slow, uneven germination and low tolerance to desiccation as well as reduced coffee seed longevity. Coffee seeds have been considered recalcitrant, orthodox and even intermediate with varying results. However, after drying and storage, coffee seeds lose vigour, and seeds stored after drying cannot be used for producing seedlings. Coffee seedling producers usually sow seeds immediately or lightly dry them after harvest for a short storage period, if necessary. It is highly desirable that seeds are stored safely to optimize coffee seedling production at the appropriate time and season with ideal climatic conditions for planting in the field. Current recommendations suggest that coffee seeds can be safely stored between the seeds are viable for about 20 days only if stored under ambient condition. However, the seeds can be stored up to 80 days by storing in 300-gauge polythene bag at the temperature of 15 °C.

Statistical analysis

The analysis of variance was carried out and comparison was done by Duncan's Multiple Range Test (DMRT). The mean difference is significant at the P-values < 0.05. Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc., Chicago, USA). Data presented are means from four replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at P<0.05.

Conflict of interest

The authors have not claimed any conflict of interest.

Author's contributions

All the authors are contributed equally in the experiment. In which, first author R. Senthil Raj has carried out the experiment and interpreted the data. Next author, V. Manonmani has designed the experiment, guided and validated the manuscript.

Table 1: Effect of storage methods and period of storage on seed moisture (%) in coffee cv. Chandragiri

		Days after storage										
Storage methods		**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**Mean**
Ambient Storage (30±2°C)	SM_1	59.6±1.91^{a}	33.4±0.66^{d}	27.6±0.26^{e}	21.7±6.42^{f}	18.6±5.46^{e}	15.3±4.39^{f}	11.8±3.18^{g}	9.2±2.33^{f}	5.3±1.05^{f}	59.6±1.91^{a}	20.9±6.35^{e}
	SM_2	59.6±0.08^{a}	52.4±0.41^{abc}	45.1±0.88^{bcd}	42.6±1.09^{cd}	38.2±0.77^{b}	32.5±0.59^{c}	26.4±0.05^{8c}	21.2±0.29^{c}	12.7±0.20^{c}	59.6±0.08^{a}	34.4±0.36^{bc}
	SM_3	59.6±0.68^{a}	48.2±1.19^{abc}	39.5±0.26^{d}	34.2±0.83^{e}	27.6±0.41^{cd}	22.7±0.12^{de}	17.6±0.05^{8de}	11.4±0.08^{de}	7.2±0.02^{e}	59.6±0.68^{a}	27.6±0.68^{d}
	SM_4	59.6±1.45^{a}	49.8±0.05^{abc}	41.5±0.74^{cd}	36.4±0.88^{de}	31.8±0.296^{c}	24.9±0.41^{d}	19.5±0.41^{d}	13.4±0.17^{d}	8.6±0.20^{d}	59.6±1.45^{a}	29.5±0.20^{cd}
	SM_5	59.6±1.51^{a}	41.6±0.62^{c}	37.4±0.62^{d}	31.9±0.62^{e}	24.8±0.26^{d}	18.6±0.20^{e}	12.4±0.15^{f}	9.7±0.15^{e}	6.1±0.12^{e}	59.6±1.51^{a}	24.9±0.08^{d}
	SM_6	59.6±0.95^{a}	42.8±0.80^{bc}	38.4±0.29^{d}	33.7±0.53^{e}	25.4±0.29^{d}	20.8±0.47^{de}	14.5±0.12^{ef}	10.4±0.08^{e}	6.7±0.02^{e}	59.6±0.95^{a}	26.0±0.15^{d}
Refrigerated storage	SM_7	59.6±0.56^{a}	58.7±0.88^{a}	57.7±0.62^{a}	55.9±0.32^{a}	51.2±0.32^{a}	45.4±0.08^{a}	42.9±0.05^{a}	32.8±0.26^{a}	26.4±0.41^{a}	59.6±0.56^{a}	41.9±0.56^{a}
	SM_8	59.6±1.30^{a}	58.2±1.24^{a}	55.1±0.08^{ab}	53.4±0.36^{ab}	49.7±0.62^{a}	41.3±0.12^{ab}	38.6±0.36^{a}	28.4±0.36^{a}	17.6±0.15^{a}	59.6±1.30^{a}	40.6±0.15^{ab}
	SM_9	59.6±0.62^{a}	57.6±0.26^{b}	52.6±0.83^{abc}	51.1±1.12^{bc}	47.5±0.50^{b}	38.8±0.47^{bc}	37.3±0.26^{b}	27.6±0.17^{b}	16.8±0.36^{b}	59.6±0.62^{a}	39.7±0.74^{ab}
	Mean	59.6±0.57^{a}	49.2±0.27^{f}	43.9±0.74^{e}	40.1±1.75^{e}	35.0±0.48^{e}	28.9±0.12^{e}	24.6±0.79^{e}	18.2±0.46^{e}	11.9±1.47e	10.3±0.48^{e}	32.2±0.21^{e}

Ambient storage(30±2°C)		**Refrigerated storage**
SM_1 - Ambient Storage (control)	SM_4 - Seed in 10 % moist charcoal	SM_7 - Seed stored @ 15°C
SM_2 - Seed in wood ash	SM_5 - Seed in 5 % moist sand	SM_8 - Seed stored @ 10°C
SM_3 - Seed in 5 % moist charcoal	SM_6 - Seed in 10 % moist sand	SM_9 - Seed stored @ 5°C

Table 2: Effect of storage methods and period of storage on seed weight (g) in coffee cv. Chandragiri

		Days after storage										
Storage methods		**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**Mean**
Ambient Storage (30±2°C)	SM_1	11.7 ± 0.08 ^{a}	7.2 ± 0.12 ^{g}	6.3 ± 0.10 ^{g}	5.2 ± 0.08 ^{h}	3.8 ± 0.05 ^{g}	3.6 ± 0.02 ^{i}	2.7 ± 0.02 ^{h}	2.2 ± 0.01 ^{h}	1.7 ± 0.02 ^{h}	1.3 ± 0.02 ^{i}	4.6 ± 0.06 ^{h}
	SM_2	11.7 ± 0.23 ^{a}	9.3 ± 0.14 ^{c}	8.9 ± 0.21 ^{c}	8.2 ± 0.20 ^{d}	7.5 ± 0.06 ^{c}	6.2 ± 0.11 ^{d}	5.5 ± 0.04 ^{d}	4.8 ± 0.09 ^{d}	4.4 ± 0.02 ^{d}	3.6 ± 0.06 ^{e}	7.0 ± 0.08 ^{d}
	SM_3	11.7 ± 0.20 ^{a}	8.3 ± 0.13 ^{e}	7.3 ± 0.00 ^{e}	6.5 ± 0.03 ^{f}	5.8 ± 0.14 ^{e}	5.2 ± 0.05 ^{f}	4.6 ± 0.10 ^{e}	4.1 ± 0.04 ^{e}	3.8 ± 0.04 ^{e}	3.2 ± 0.02 ^{f}	6.1 ± 0.08 ^{e}
	SM_4	11.7 ± 0.18 ^{a}	9.1 ± 0.03 ^{c}	8.4 ± 0.08 ^{d}	7.3 ± 0.14 ^{e}	6.9 ± 0.03 ^{d}	5.6 ± 0.00 ^{e}	5.3 ± 0.10 ^{d}	4.6 ± 0.01 ^{d}	4.1 ± 0.05 ^{d}	3.8 ± 0.08 ^{d}	6.7 ± 0.11 ^{d}
	SM_5	11.7 ± 0.04 ^{a}	7.5 ± 0.15 ^{f}	6.9 ± 0.02 ^{f}	5.4 ± 0.04 ^{g}	5.1 ± 0.03 ^{f}	4.3 ± 0.02 ^{h}	3.8 ± 0.05 ^{g}	3.3 ± 0.03 ^{g}	3.1 ± 0.00 ^{g}	2.1 ± 0.02 ^{h}	5.3 ± 0.00 ^{g}
	SM_6	11.7 ± 0.08 ^{a}	7.8 ± 0.13 ^{d}	7.1 ± 0.09 ^{e}	6.2 ± 0.04 ^{g}	5.3 ± 0.10 ^{f}	4.9 ± 0.03 ^{g}	4.1 ± 0.03 ^{f}	3.8 ± 0.00 ^{f}	2.6 ± 0.05 ^{f}	2.4 ± 0.05 ^{g}	5.6 ± 0.11 ^{f}
Refrigerated storage	SM_7	11.7 ± 0.10 ^{a}	11.3 ± 0.04 ^{a}	10.9 ± 0.20 ^{a}	10.5 ± 0.13 ^{a}	10.1 ± 0.2 ^{a}	9.8 ± 0.02 ^{a}	8.9 ± 0.14 ^{a}	8.4 ± 0.16 ^{a}	8.1 ± 0.06 ^{a}	7.9 ± 0.11 ^{a}	9.8 ±0.12 ^{a}
	SM_8	11.7 ± 0.20 ^{a}	11.1 ± 0.03 ^{a}	10.7 ± 0.08 ^{b}	10.3 ± 0.16 ^{b}	9.8 ± 0.07 ^{b}	9.5 ± 0.12 ^{b}	8.4 ± 0.06 ^{b}	8.1 ± 0.14 ^{b}	7.7 ± 0.11 ^{b}	7.3 ± 0.06 ^{b}	9.5 ± 0.16 ^{b}

	SM_9	11.7 ± 0.27 ^{a}	10.9 ± 0.24 ^{a}	10.5 ± 0.11 ^{c}	10.1 ± 0.04 ^{c}	9.6 ± 0.03 ^{c}	9.1 ± 0.15 ^{c}	8.0 ± 0.04 ^{c}	7.6 ± 0.09 ^{c}	7.2 ± 0.09 ^{c}	6.8± 0.07 ^{c}	9.2 ± 0.14 ^{c}
	Mean	11.7 ±0.54 ^{e}	9.2 ±0.47 ^{e}	8.6 ±0.76 ^{e}	7.7 ±0.73 ^{e}	7.1 ±0.04 ^{e}	6.5 ±0.7 ^{e}	5.7 ±0.6 ^{e}	5.2 ±0.27 ^{e}	4.7 ±0.04 ^{e}	4.3 ±0.47 ^{e}	7.1 ±0.18 ^{e}

Ambient storage(30±2°C)		Refrigerated storage
SM_1 - Ambient Storage (control)	SM_4 - Seed in 10 % moist charcoal	SM_7 - Seed stored @ 15°C
SM_2 - Seed in wood ash	SM_5 - Seed in 5 % moist sand	SM_8 - Seed stored @ 10°C
SM_3 - Seed in 5 % moist charcoal	SM_6 - Seed in 10 % moist sand	SM_9 - Seed stored @ 5°C

Table 3: Effect of storage methods and period of storage on germination (%) in coffee cv. Chandragiri

		Days after storage										
Storage methods		**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**Mean**
Ambient Storage (30±2°C)	SM_1	78±0.83^{a}	31±1.31^{bc}	18±1.06^{c}	0	0	0	0	0	0	0	13±21.25^{a}
	SM_2	78±0.47^{a}	64±1.54^{ab}	53±1.21^{abc}	43±0.15^{b}	35±0.12^{ab}	22±0.05^{ab}	12±0.20^{ab}	8±0.17^{ab}	0	0	32±0.17^{a}
	SM_3	78±0.98^{a}	47±1.19^{bc}	43±0.68^{bc}	22±0.20^{b}	19±1.41^{b}	11±0.05^{a}	7±0.12^{ab}	3±0.18^{ab}	0	0	23±0.47^{a}
	SM_4	78±1.09^{a}	51±0.26^{bc}	48±0.38^{bc}	31±0.74^{b}	25±0.47^{ab}	14±1.12^{ae}	9±0.05^{ab}	5±0.14^{ab}	0	0	26±0.56^{a}
	SM_5	78±0.77^{a}	42±0.98^{c}	36±0.62^{c}	15±0.53^{b}	12±0.12^{b}	6±0.32^{ab}	3±0.47^{abc}	0	0	0	19±0.05^{a}
	SM_6	78±1.66^{a}	45±1.19^{bc}	41±0.77^{c}	20±0.41^{b}	17±0.15^{ab}	8±0.058^{ab}	4±0.76 ^{ab}	0	0	0	21±0.53^{a}
Refrigerated storage	SM_7	78±1.51^{a}	73±1.66^{a}	71±0.36^{a}	67±1.30^{a}	49±0.47^{a}	41±0.47^{a}	33±0.26^{a}	21±0.29^{a}	14 ±0.14^{a}	0	45±0.68^{a}
	SM_8	78±0.53^{a}	71±0.88^{a}	69±0.77^{ab}	53±0.62^{b}	37±0.26^{ab}	29±0.10^{ab}	21±0.15^{ab}	16±0.12^{a}	12±0.26^{ab}	0	39±0.74^{a}
	SM_9	78±0.74^{a}	70±0.95^{a}	62±1.04^{b}	48±0.50^{b}	31±0.56^{ab}	24±0.20^{ab}	15±0.08^{ab}	10±0.29^{a}	0	0	34±0.36^{a}
	Mean	78±0.77 ^{e}	55±0.71 ^{e}	49±0.14 ^{e}	33±0.96 ^{e}	25±0.87 ^{e}	17±0.46 ^{e}	12±0.73 ^{e}	7±0.33 ^{e}	3±0.77 ^{e}	0	28±0.46 ^{e}

Ambient storage(30±2°C)		Refrigerated storage
SM_1 - Ambient Storage (control)	SM_4 - Seed in 10 % moist charcoal	SM_7 - Seed stored @ 15°C
SM_2 - Seed in wood ash	SM_5 - Seed in 5 % moist sand	SM_8 - Seed stored @ 10°C
SM_3 - Seed in 5 % moist charcoal	SM_6 - Seed in 10 % moist sand	SM_9 - Seed stored @ 5°C

Table 4: Effect of storage methods and period of storage on vigour index in coffee cv. Chandragiri

		Days after storage										
Storage methods		**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**Mean**
Ambient Storage (30±2°C)	SM_1	226±75.09^{ac}	38±1.54^{b}	7±0.38^{c}	0	0	0	0	0	0	0	27±0.28^{b}
	SM_2	226±5.29^{a}	147±1.87^{b}	101±0.65^{c}	74±0.83^{cd}	50±0.47^{cd}	22±0.31^{cd}	12±0.47^{cd}	7±0.76^{d}	0	0	64±0.95^{b}
	SM_3	226±4.21^{a}	86±0.08^{b}	65±0.83^{cde}	32±0.17^{cde}	20±0.15^{d}	8±0.03^{e}	6±0.08^{de}	3±0.41^{d}	0	0	45±0.62^{b}
	SM_4	226±5.62^{a}	103±1.60^{b}	82±1.30^{cd}	46±0.59^{cde}	32±0.36^{d}	13±0.03^{de}	8±0.08^{de}	4±0.47^{d}	0	0	51±0.20^{b}
	SM_5	226±1.40^{a}	55±1.30^{bc}	44±0.26^{ef}	14±0.29^{de}	9±0.05^{d}	5±0.04^{e}	3±0.14^{e}	0	0	0	36±0.03^{b}
	SM_6	226±5.29^{ab}	73±0.68^{bc}	54±0.26^{def}	22±0.29^{de}	14±0.08^{d}	6±0.01^{e}	3±0.04^{e}	0	0	0	40±0.77^{b}
Refrigerated storage	SM_7	226±1.41^{a}	197±3.24^{a}	184±0.24^{a}	154±1.09^{a}	104±0.05^{a}	83±0.47^{a}	60±0.53^{a}	33±0.71^{a}	17±0.17^{a}	0	106±1.81^{a}
	SM_8	226±2.46^{a}	185±3.69^{a}	165±0.47^{b}	117±1.69^{b}	71±0.17^{b}	45±0.24^{b}	29±0.15^{b}	18±0.05^{b}	10±0.36^{ab}	0	87±0.12^{a}
	SM_9	226±0.05^{d}	175±0.45^{c}	130±0.45^{f}	87±0.78^{e}	54±0.46^{d}	31±0.24^{e}	17±0.76^{de}	9±0.49^{d}	0	0	73±0.43^{c}
	Mean	226±0.04^{e}	118±0.42 ^{e}	92±0.24 ^{e}	61±0.76 ^{e}	39±0.73 ^{e}	24±0.43 ^{e}	15±0.11 ^{e}	8±0.77 ^{e}	3±0.76 ^{e}	0	59±0.72 ^{e}

Ambient storage(30±2°C)		Refrigerated storage
SM_1 - Ambient Storage (control)	SM_4 - Seed in 10 % moist charcoal	SM_7 - Seed stored @ 15°C
SM_2 - Seed in wood ash	SM_5 - Seed in 5 % moist sand	SM_8 - Seed stored @ 10°C
SM_3 - Seed in 5 % moist charcoal	SM_6 - Seed in 10 % moist sand	SM_9 - Seed stored @ 5°C

Table 5: Effect of storage methods and period of storage on electrical conductivity (dS m^{-1}) in coffee cv. Chandragiri

		Days after storage										
Storage methods		**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**Mean**
Ambient Storage (30±2°C)	SM_1	109±35.17^{a}	190±62.62^{ab}	347±114.68^{abc}	541±1.85^{a}	625±3.84^{a}	688±2.68^{a}	763±49.03^{a}	939±109.65^{ab}	1022±1.62^{ab}	1056±1.41^{ab}	628±9.72^{a}
	SM_2	109±0.17^{a}	138±1.71^{ab}	152±0.13^{bc}	284±1.15^{g}	329±7.88^{g}	395±4.07^{e}	441±4.11^{d}	538±10.65^{de}	611±1.86^{de}	753±1.13^{bc}	375±4.17^{f}
	SM_3	109±0.89^{a}	157±3.33^{ab}	215±0.89^{abc}	354±2.98^{d}	416±6.78^{d}	547±7.14^{c}	614±11.55^{b}	715±17.46^{bc}	798±16.597^{bc}	887±2.26^{ab}	481±10.79^{d}
	SM_4	109±0.77^{a}	145±3.03^{a}b^{a}	188±2.46^{abc}	317±4.94^{f}	358±8.33^{f}	429±0.48^{d}	553±5.48^{c}	628±4.98^{cd}	699±8.73^{cd}	832±1.08^{abc}	426±4.12^{e}
	SM_5	109±2.48^{a}	185±1.33^{ab}	283±6.63^{a}	485±0.77^{b}	549±11.73^{ab}	642±2.62^{ab}	723±0.61^{a}	851±19.28^{a}	963±2.05^{a}	1011±1.53^{e}	580±6.61^{b}
	SM_6	109±0.64^{a}	172±4.31^{b}	257±2.54^{ab}	415±1.91^{c}	567±7.96^{cd}	628±2.93^{b}	698±1.44^{a}	759±9.79^{ab}	849±1.67^{ab}	971±17.11^{e}	543±0.89^{c}
Refrigerated storage	SM_7	109±0.08^{a}	112±2.36^{g}	121±1.83^{c}	186±0.61^{d}	217±1.29^{g}	293±0.12^{g}	341± 1.01^{f}	417±5.86^{ab}	510±7.16^{e}	526±8.49^{a}	283±2.74^{i}
	SM_8	109±0.74^{a}	115±0.13^{g}	129±1.19^{c}	212±2.42^{h}	268±1.51^{b}	324±4.32^{g}	379±2.53^{ef}	435±10.48^{e}	524±5.91^{e}	678±2.86^{d}	317±7.75^{8h}
	SM_9	109±2.65^{a}	121±0.12^{f}	148±0.05^{bc}	234±1.36^{h}	297±4.35^{g}	357±5.54^{f}	428±7.13^{de}	489±9.64^{e}	568±0.26^{de}	713±11.12^{c}	346±3.83^{g}
	Mean	109±2.14^{a}	148±0.24^{d}	204±0.47^{abc}	336±0.17^{e}	403±0.23^{d}	478±0.46^{d}	549±0.11^{e}	641±2.71^{e}	727±1.67^{e}	825±0.18^{e}	442±0.17^{e}

Ambient storage(30±2°C)		Refrigerated storage
SM_1 - Ambient Storage (control)	SM_4 - Seed in 10 % moist charcoal	SM_7 - Seed stored @ 15°C
SM_2 - Seed in wood ash	SM_5 - Seed in 5 % moist sand	SM_8 - Seed stored @ 10°C
SM_3 - Seed in 5 % moist charcoal	SM_6 - Seed in 10 % moist sand	SM_9 - Seed stored @ 5°C

Table. 6: Effect of storage methods and period of storage on free sugars (µg g^{-1}) in coffee cv. Chandragiri

		Days after storage										
Ambient Storage (30±2°C)	SM_1	0.76±0.08^{a}	1.86±0.12^{a}	2.04±0.05^{a}	2.56±0.12^{a}	2.98±0.273^{a}	4.51±0.76^{ab}	7.98±2.001^{abc}	10.8±2.85^{abc}	14.8±4.21^{abc}	19.3±5.74^{abc}	6.76±1.3^{de}
	SM_2	0.76±0.07^{a}	1.38±0.03^{e}	1.45±0.11^{d}	1.58±0.15^{de}	1.97±0.47^{bc}	2.68±0.03^{cde}	5.01±0^{cd}	6.18±0,15^{bc}	9.32±0.17^{abc}	14.4±0.26^{ab}	4.47±0.31^{bc}
	SM_3	0.76±0.01^{a}	1.55±0.03^{cd}	1.68±0.14^{c}	1.82±0.07^{c}	2.48±0.058^{a}	3.36±0.08^{bc}	6.58±0.17^{abc}	7.41±0.12^{abc}	11.8±0.29^{abc}	16.4±0.20^{a}	5.38±0.07^{bc}
	SM_4	0.76±0.04^{a}	1.42±0.06^{de}	1.54±0.03^{d}	1.69±0.08^{cd}	2.04±0.13^{b}	3.11±0.05^{bcd}	5.28±0.14^{bcd}	7.03±0.03^{abc}	10.1±0.15^{abc}	15.7±0.26^{a}	4.87±0.02^{a}
	SM_5	0.76±0.06^{a}	1.74±0.04^{b}	1.84±0.06^{b}	2.37±0.24^{a}	2.62±0.08^{a}	4.29±0.09^{a}	7.75±0.12^{a}	9.83±0,14^{a}	13.5±0.32^{a}	18.1±0.36^{a}	6.28±0.07^{a}
	SM_6	0.76±0.02^{a}	1.67±0.03^{be}	1.71±0.05^{c}	2.08±0.05^{b}	2.56±0.04^{a}	3.72±0.03^{ab}	7.28±0.24^{ab}	8.54±0.07^{ab}	12.4±0.20^{ab}	17.2±0.41^{a}	5.79±0.14^{b}
Refrigerated storage	±SM_7	0.76±0.04^{a}	0.93±0.24^{g}	1.15±0.23^{e}	1.34±0.03^{f}	1.57±0.23^{d}	1.89±0.05^{e}	3.28±0.033^{d}	5.08±0.08^{c}	7.34±0.14^{c}	7.55±0.12^{c}	3.09±0.06^{d}
	SM_8	0.76±0.08^{a}	1.04±0.03^{g}	1.26±0.13^{e}	1.37±0.23^{f}	1.69±0.08^{cd}	2.04±0,13^{e}	4.57±0.23^{cd}	5.48±0.07^{bc}	8.07±0.17^{bc}	8.97±0.08^{bc}	3.53±0.07^{d}
	SM_9	0.76±0.03^{a}	1.21±0.46^{f}	1.38±0.24^{d}	1.46±0.21^{f}	1.86±0,33^{bcd}	2.31±0.47^{de}	4.73±0.43^{cd}	5.81±0.08^{bc}	8.58±0.20^{bc}	9.04±0.06^{bc}	3.71±0.21^{e}
	Mean	0.76±0.07^{a}	1.42±0.21^{de}	1.56±0.21^{d}	1.81±0.41^{e}	2.20±0.26^{b}	3.10±0.11^{bcd}	5.83±0.28^{cd}	7.35±0.27^{abc}	10.66±0.24^{abc}	14.07±0.11^{ab}	4.88±0.7^{bc}

Ambient storage(30±2°C)		Refrigerated storage
SM_1 - Ambient Storage (control)	SM_4 - Seed in 10 % moist charcoal	SM_7 - Seed stored @ 15°C
SM_2 - Seed in wood ash	SM_5 - Seed in 5 % moist sand	SM_8 - Seed stored @ 10°C
SM_3 - Seed in 5 % moist charcoal	SM_6 - Seed in 10 % moist sand	SM_9 - Seed stored @ 5°C

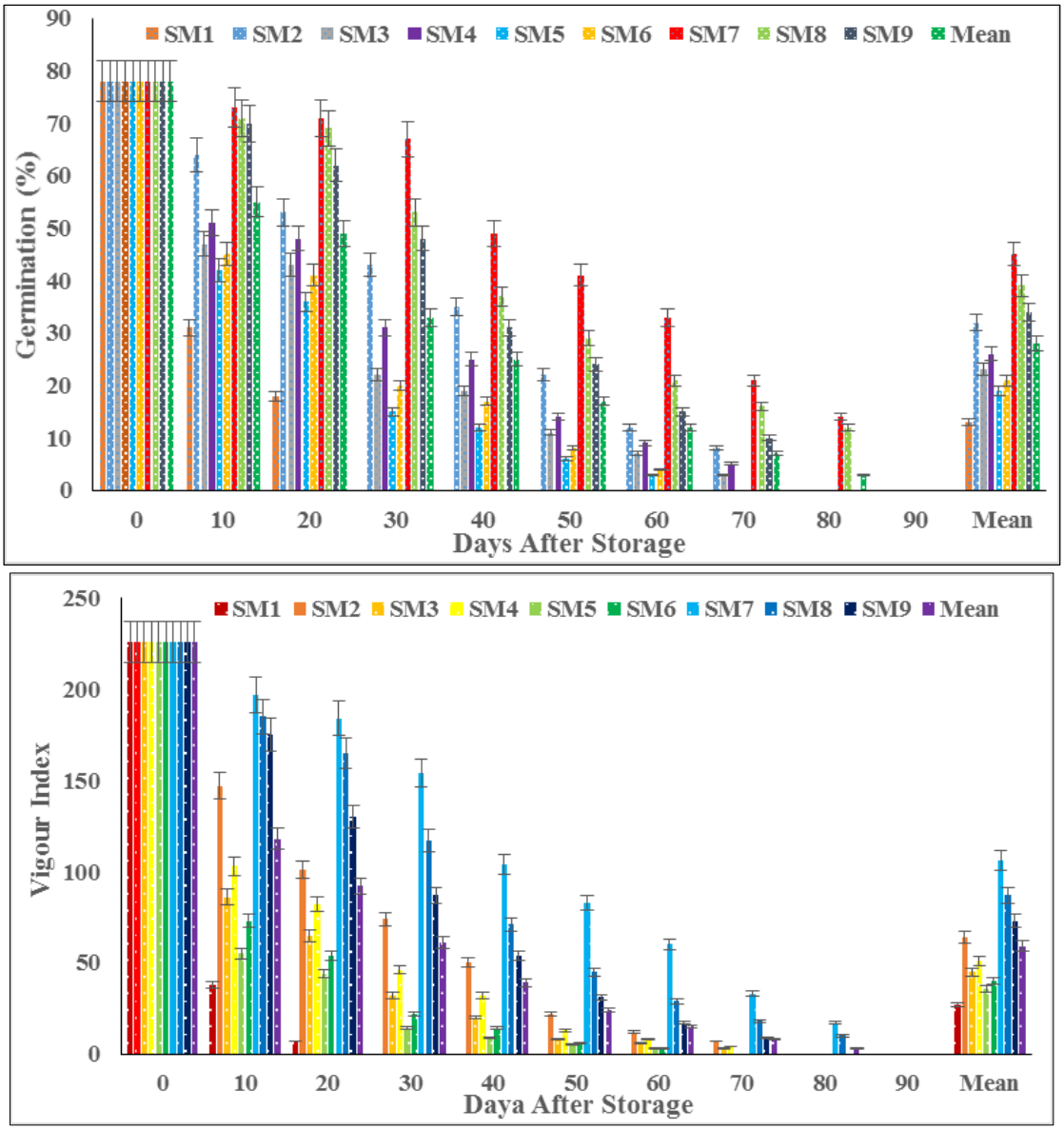

Fig 1: Effect of storage methods and period of storage on germination (%) and Vigour index in coffee cv. Chandragir

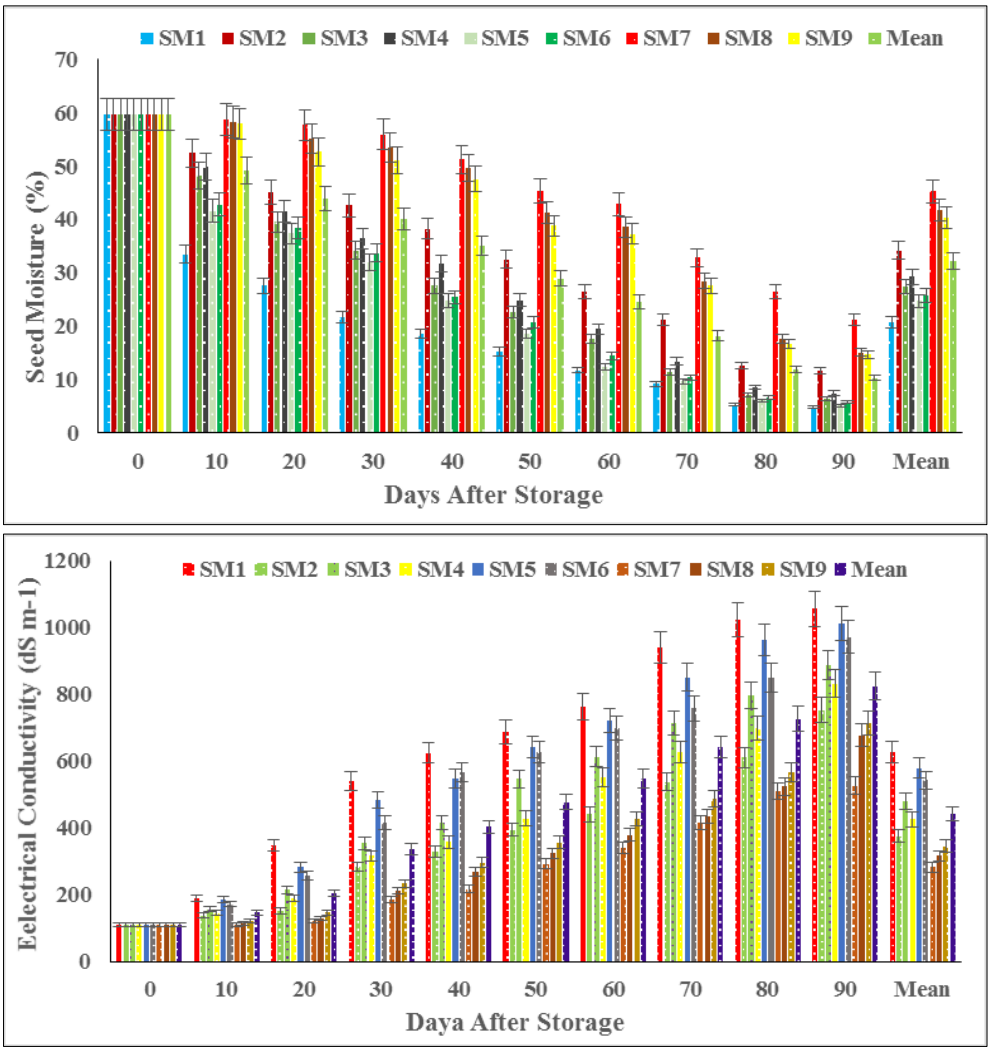

Fig 2: Effect of storage methods and period of storage on seed moisture (%) and Electrical conductivity (dS m^{-1}) in coffee cv. chandragir

References

1. Abdul-Baki A, Anderson JD. Vigour determination in soybean seed by multiple criteria 1. Crop Science. 1973;13(6):630-633.
2. Abdul-Baki AA. Biochemical aspects of seed vigour. Horticultural Science. 1980;15(2):765-771.
3. Ali-Khan ST, Youngs CG. Variation in protein content of field peas. Canadian Journal of Plant Science. 1973;53(1):37-41.
4. Baliza DP, Caixeta F, Pinho ÉVDRV, Cunha RLD, Martins DC, Rosa SDVFD. Physiological quality of coffee seeds produced under different levels of solar radiation and maturation stages. Revista Brasileira de Sementes. 2012;34:416-423.
5. Bhattacharyya AK, Basu RN. Retention of vigor and viability of jackfruit (*Artocarpus heterophyllus* Lam.) seed." Indian Agriculturist. 1992;36:65-74.
6. Castan DOC, Gomes-Junior FG, Marcos-Filho J. Vigor-S, a new system for evaluating the physiological potential of maize seeds. Scientia Agricola. 2018;75:167-172.
7. Chin HF, Roberts EH. Recalcitrant crop seeds. Recalcitrant crop seeds. 1980.
8. Chin HF, Hor YL, Lassim M. Identification of recalcitrant seeds. Seed Science and Technology. 1984;12(2):429-436.
9. Clegg JS. Metabolism and the intracellular environment: The vicinal-water network model. Cell Associated Water. 1979;2(3):363-413.
10. Clifford MN, Willson KC. Coffee botany, biochemistry and production of beans and beverage. The Avi Publishing Company Inc. Connecticut, United States of America. 1985.
11. Da Rosa SDVF, Carvalho AM, McDonald MB, Von Pinho ERV, Silva AP, Veiga AD. The effect of storage conditions on coffee seed and seedling quality. Seed Science and Technology. 2011;39(1):151-164.
12. Da Silva EA, Toorop PE, Van Aelst AC, Hilhorst HW. Anatomy and morphology of the coffee (*Coffea arabica* cv. Rubi) seed and fruit during germination. Germination: mechanism and regulation, 2002, 7pp.
13. Dussert S, Chabrillange N, Engelmann F, Anthony F, Hamon S. Cryopreservation of coffee (*Coffea arabica* L) seeds: importance of the precooling temperature. Cryo-Letters. 1997;18(5):269-276.
14. Eira MT, Silva EA, De Castro RD, Dussert S, Walters C, Bewley JD, *et al*. Coffee seed physiology. Brazilian Journal of Plant Physiology. 2006;18:149-163.
15. Ellis RH, Roberts EH. Towards a rational basis for seed testing seed quality. (Ed. P. Hebblethwaitei). Seed Production. Butterworths, London. 1980.
16. Ellis RH, Hong TD, Roberts EH. An intermediate category of seed storage behaviour? I. Coffee. Journal of Experimental Botany. 1990;41(9):1167-1174.
17. Gebreselassie W, Ali M, Anteneh N. Pre-sowing

treatment of coffee (*Coffea arabica* L.) seeds to enhance emergence and subsequent growth of seedlings. Research Journal of Seed Science. 2010;3(4):218-226.
18. Girija T. Physiological investigation on recalcitrancy behaviour of mango (Mangifera indica L.) and rattan (Calamus spp.) seeds. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore, 1998.
19. Gunasekaran M. Seed technological studies in clove (*Syzygium aromaticum* L.), nutmeg (Myristica fragrans Houtt) and rubber (Hevea braseliensis Muell-Avg)." M.sc. (Ag.) Thesis, Tamil Nadu Agricutural University, Coimbatore. 1997.
20. Hong TD, Ellis RH. A protocol to determine seed storage behaviour (No. 1). Bioversity International, 1996.
21. Hor YL. Storage of cocoa (*Theobroma cacao* L.) seeds and changes associated with their deterioration. Ph.D. Thesis, Universiti Pertanian Malaysia, Malaysia. 1984.
22. ISTA. International rules for seed testing. Bassersdorf: International Seed Testing Association, 2013.
23. ISTA. International rules for seed testing. International Seed Testing Association, Bassersdorf, Switzerland, 2015.
24. Kader MA. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceeding of the Royal Society of New South Wales. 2005;138:65-75.
25. King MW, Roberts EH. The storage of recalcitrant seeds: achievements and possible approaches: a report on a literature review carried out for the International Board for Plant Genetic Resources. The storage of recalcitrant seeds: achievements and possible approaches: a report on a literature review carried out for the International Board for Plant Genetic Resources, Rome, 1979.
26. Kittock DL, Law AG. Relationship of seedling vigor to respiration and tetrazolium chloride reduction by germinating wheat seeds. Agronomy Journal. 1968;60(3):286-288.
27. Kundu M, Kachari J. Desiccation sensitivity and recalcitrant behavior of seeds of Aquilaria agallocha Roxb. Seed Science and Technology. 2000;28(3):755-760.
28. Loomis WD, Battaile J. Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry. 1966;5(3):423-438.
29. Maguire JD. Speed of Germination Aid in Selection and Evaluation for Seedling Emergence and Vigour 1. Crop Science. 1962;2(2):176-177.
30. Malik CP, Singh MB. Plant enzymology and histo-enzymology." Kalyani publishers, New Delhi, 1980, 53pp.
31. Malik CP, Singh MB. Plant enzymology and histo-enzymology. Kalyani publishers, New Delhi, 1980, 53pp.
32. Malik CP, Singh MB. Plant enzymology and histo-enzymology. Kalyani Publishers, New Delhi, 1980, 53pp.
33. Naidu S, Posner EA, Weyl G. Antitrust remedies for labour market power. Harvard law review. 2018;132(2):536-601.
34. Nasiro K, Shimber T, Mohammed A. Germination and seedling growth rate of coffee (*Coffea arabica* L.) seeds as influenced by initial seed moisture content, storage time and storage condition. International Journal of Agriculture Biosciences. 2017;6(6):304-310.
35. Nautiyal AR, Purohit AN. Seed viability in sal. III. Membrane disruption in ageing seeds of Shorea robusta. Seed Science and Technology. 1985;13:69-76.
36. Oliveira LM, Valio IFM. Effects of moisture content on germination of seeds of Hancornia speciosa Gom (Apocynaceae). Annals of Botany. 1992;69(1):1-5.
37. Paul AK, Mukherji S, Sircar SM. Metabolic changes in rice seeds during storage. Indian Journal of Agricultural Science. 1970;40(12):1031-1036.
38. Presley JT. Relation of protoplast permeability to cotton seed viability and predisposition to seedling disease. Plant Disease Reporter. 1958;42(7):852-854.
39. Rahardjo P. Batas pengamatan perkecambahan benih kopi Arabika. Warta Balai Penelitian Perkebunan Jember. 1988;7:1-3.
40. Rahimi A. Seed priming improves the germination performance of cumin (*Cuminum syminum* L.) under temperature and water stress. Industrial Crops and Products. 2013;42:454-460.
41. Raja K, Palanisamy V, Selvaraju P, Shanmugasundaram KA. Desiccation sensitivity of avocado (Persea americana Mill) seeds. The Project on Handling and Storage of Recalcitrant and Intermediate Tropical Forest seeds, Newsletter, 2001, 22-24.
42. Raja K, Palanisamy V, Selvaraju P. Deteriorative changes associated with the loss of viability in desiccation–sensitive Arecanut (*Areca catechu* L.) seed. Seed Science and Technology. 2005;33(1):177-184.
43. Rajeswari Dayal BR, Kaveriappa KM. Effect of desiccation and temperature on germination and vigour of the seeds of Hopea parviflora Beddome and H. ponga (Dennst.) Mabb. Seed Science and Technology. 2000;28(2):497-506.
44. Roberts EH. Quantifying seed deterioration. In: Physiology of seed deterioration (Eds. M.B. Mc Donals Jr. and C.J. Nelson). Crop Sci. Soc., America Special Publication Number 11, CSSA, Madison, WI, 1986, 101-123pp.
45. Roberts EH. Storage environment and the control of viability." In Viability of seeds, 14-58. Springer. 1972.
46. Selmar D, Bytof G, Knopp SE, Breitenstein B. Germination of coffee seeds and its significance for coffee quality. Plant Biology. 2006;8(02):260-264.
47. Shylla Merlin J, Palanisamy V. Seed viability and storability of jack fruit (*Artocarpus heterophyllus* L.). Seed Research. 2000;28(2):166-170.
48. Simon EW, Ann Minchin, Mary M, McMenamin, Smith JM. The low temperature limit for seed germination." New Phytologist. 1976;77(2):301-311.
49. Sivalingam R, Patric Raja D, Irudayaraj V, Latha KV. Standardization of storage conditions to prolong viability of seeds of Embelia ribes Burm a medicinal climbing shrub. International Journal Biological Technology. 2011;2(2):106-108.
50. Somogyi M. Notes on sugar determination. Journal of Biological Chemistry. 1952;195:19-23.
51. Song X, Chen Q, Wang D, Yang J. A study on the principal storage conditions of Hopea hainanensis seeds. Scientia Silvae Sinicae. 1984;20:225-236.
52. Srimathi P. Research focus on seed collection, processing and storage of amla (Emblica officianilis Gaetn.), jamun (Syzygium cuminii Skeels) and ber (Zizyphus mauritiana Lamk.). Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore. 1997.

53. Sudrajat DJ, Nurhasybi BY, Bramasto Y. Standar pengujian dan mutu benih tanaman hutan. Forda Press. 2015.
54. Szezotka Z. Amylolytic activity acorns of Quercus borealis mich X. during storage under controlled conditions. Arboretrum Hornickve. 1974;20:129-134.
55. Szezotka Z. Changes in the viability of protein synthesis in the embryo axes of Northern Red oak (Quercus borealis Mich X) and English oak (Q. *robur* L.) during storage under controlled conditions. Astroretum Hornickve. 1975;19:129-134.
56. Tamari C. Phenology and seed storage trials of dipterocarps. Research Pamphlet, Forest Research Institute, Peninsular Malaysia. 1976;9:59-62.
57. Tompsett PB. The influence of moisture content and storage temperature on the viability of Shorea almon, Shorea robusta, and Shorea roxburghii seed. Canadian Journal of Forest Research. 1985;15(6):1074-1079.
58. Tompsett PB. A review of the literature on storage of dipterocarp seeds. Seed Science and Technology. 1992;20:251-267.
59. Varghese B, Naithani R, Dullo ME, Naithani SC. Seed storage behaviour in Madhuca indica JF Gmel. Seed Science and Technology. 2002;30(1):107-118.
60. Yoshida S, Douglas A, James H. Laboratory manual for physiological studies of rice. Laboratory manual for physiological studies of rice. Phlippines, IRRI Publication. 1971.