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EFFECTS OF PACKAGING AND STORAGE CONDITIONS ON QUALITY OF SPIDER PLANT (*Cleome gynandra* L.) SEED

Kamotho GN[1]*, Mathenge PW[1], Muasya RM[2] and ME Dulloo[3]

Grace Kamotho

*Corresponding author email: gracekamotho@yahoo.com

[1]School of Agriculture and Biotechnology, Karatina University, P.O. Box 1957-10101,Karatina, Kenya

[2]South Eastern Kenya University, P.O. Box, 170-90200, Kitui, Kenya

[3]Bioversity International,ViadeiTreDenari 472/a 00057, Maccarese (Fiumicino), Rome, Italy.

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ABSTRACT

In Kenya, spider plant (*Cleome gynandra* L.) has gained popularity among consumers due to its nutritional and medicinal values. In the local markets, bundles of leafy shoots as well as uprooted young plants are offered at fairly high prices in many parts of Kenya. Existing evidence suggests that spider plant is endowed with higher level of nutrients than its exotic counterparts. The leaves contain over and above the normal recommended adult daily allowance of vitamins A and C, calcium and iron. However, quality of spider plant seed is affected by one or more factors that cause negative response during seed handling and storage. The purpose of this research was to increase insight into how the seed quality of spider plant is affected by different packaging containers, seed moisture content and storage temperatures, with a view to finding out the optimal method of packaging and storing of these seeds. This study was carried out using seeds dried above silica gel to four target moisture levels: 20%, 10%, 5% and 2% moisture content. Dried seeds were sealed in aluminum foil packets and polyethylene packets and stored at three storage temperatures: ambient (22^oC to 30^oC), 5^oC and minus 20^oC for three and six months. After each storage period, seed samples were drawn and viability and vigour tests carried out. Data sets were factorially combined and subjected to Analysis of Variance (ANOVA)and descriptive analysis. Means separation was by Least Significance Difference (LSD).Levels of significance, means and standard deviations were obtained for various data sets. Seed stored for six months at 5% moisture content and minus 20^oC recorded the highest seed quality. There were no significant differences between seeds packaged in aluminum foil packets and polyethylene packets. In this study, a germination of 85% was recorded for seed dried to 5% moisture content and stored at room temperature. Therefore, on the basis of these findings, farmers can dry their seeds at about 5% moisture content, package them in polyethylene (since readily available) and store at room temperatures for six months.

Key words: Containers, moisture content, storage temperatures

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INTRODUCTION

Spider plant (Plate.1) is an erect herb that grows up to 1.5 m tall. Its petals are white, pink or lilac, while the capsules (silique) are green, turn yellow when ripe, and dehisce easily when dry to release seeds. Although the species is widely distributed in Africa and Asia, its range of genetic diversity has hardly been studied. In Kenya, spider plant has gained popularity among farmers due to its nutritional and medicinal values [1]. In the local markets, bundles of leafy shoots as well as uprooted young plants are sold at high prices in many parts of Kenya [1].

Plate.1: Photograph of spider plant (*Cleome gynandra* L.) taken from one of the experimental plots

It is popular in cultural diets and existing evidence suggests that spider plant is endowed with higher levels of nutrients than its exotic counterparts [2]. The leaves

contain over and above the normal recommended adult daily allowance of vitamins A and C, calcium and iron. The amino-acid composition of spider plant leaf-protein has a high chemical score, comparable to that of exotic vegetables such as spinach. High levels of nutritionally critical amino acids, like lysine, arginine, aspartic acid, glutamic acid, tyrosine and histidine have been reported [2].The leaves and seeds of spider plant are used in indigenous medicine in many countries [3].Indigenous knowledge possessed by rural women in Kenya indicates that spider plant has several medicinal uses [3]. Its leaves are crushed to make a concoction that treats scurvy and marasmus. Sap from young leaves treats epilepsy and recurrent malaria. Seeds and roots are ingested for the expulsion of roundworms and bruised leaves are applied on boils to prevent formation of pus.

Though not actually threatened with extinction, spider plant is facing the danger of genetic erosion. With increasing pressure on agricultural land, its ecological niches are fast disappearing. Genetic erosion, hence, is bound to be rapid. In general, little is known about the cultivation techniques, the extent and structure of genetic variation, and the seed physiology of spider plant [2]. This is primarily because of the low priority and status accorded to this crop nationally and internationally. This is regrettable considering the significant contribution this local vegetable has played in the nutritional well being of many rural populations, especially in Africa. Furthermore, beyond Africa this vegetable has a significant role to play in widening the world's currently narrow food base [4].

The primary objective of storing seeds for plant genetic resource conservation is to maintain the genetic integrity of preserved accessions for as long a period as possible. Commercial seed companies and farmers are also faced with the task of prolonging the lifespan of certain seed lots, for future seed production and planting seasons, respectively [5]. This is a challenging task due to inevitable deterioration of seeds in storage, which leads to low vigour, reduced number of viable seeds and genetic drift [5]. Seeds may be stored by farmers in such a way that their levels of germination and vigour are least affected at the time of sowing in the forthcoming or subsequent season(s). For this reason, agricultural seeds need to be stored for one or two cropping seasons or years. Secondly, some quantity of seed may be stored for two to three years (or more) for utilization in commercial seed production, as "carry over stock". The third and the much more difficult task in seed storage is for the conservation of plant genetic resources in gene banks for utilization in crop improvement programmes by present and future generations. In this, seeds of a wide range of species and cultivars are stored for prolonged periods of time, normally extending to hundreds of years [5].

Deterioration in seeds may be defined as an increased probability of death of an individual seed per unit time as age increases, failure to germinate being indicative of seed death [6]. Seed ageing, however, cannot be considered solely as a function of time, since the environmental factors during storage are important. It has generally long been known that, the greater the moisture content and storage temperature of orthodox seeds, either singly or in combination, the shorter is the period of seed survival [6]. This qualitative statement, however, is only of limited use in designing and managing seed

storage systems unless the relationship between longevity (period until seed death) and the environment is quantitatively described [6].

Changes in some quantifiable traits such as viability occur when seeds deteriorate, some of which have been used to estimate and quantify deterioration. Perhaps the most widely accepted and useful index of seed deterioration is the reduction in viability [7]. A viable seed will germinate and develop into plant, given favourable conditions and provided any dormancy that may be present is removed [8]. Viability of a seed or seed lot may be defined thus, as the degree to which a seed is alive, metabolically active and possesses enzymes capable of catalyzing metabolic reactions needed for germination and growth [9]. Thus, a seed that is dormant but capable of germination when the dormancy – imposing mechanism is relieved is considered viable [10].

To maintain seed viability during storage, it is vital that seeds are of maximum quality when they are placed in storage [11]. The seeds should also be stored under conditions which will optimize longevity [12]. The most important factor that influences the potential longevity of seeds is moisture content [13]. In the first instance, it is the response of the seed to a reduction in moisture content (that is the response to drying) that will determine whether or not it can be stored successfully using conventional seed storage methods [14]. All seeds lose viability during storage with a loss of vigour preceding the loss in germination [15]. Seed vigour is "the sum of all attributes which favour stand establishment under unfavourable conditions" [16].

Following a series of other definitions, it was evident that vigour was a concept that described several characteristics, which in turn were associated with various aspects of performance of germinating seed or subsequent seedling [16]. A broadly based definition was thus adopted by the ISTA congress in 1977 as: "the sum total of those properties of seed which determine the level of activity and performance of the seed or seedling emergence. Seeds which perform well are termed as high vigour seeds and those which perform poorly are termed as low vigour seeds" [16]. The definition also specifies those aspects of performance reported to show variations associated with differences in vigour as: biochemical processes and reactions during germination, such as enzyme reactions and respiratory activities; rate and uniformity of seed germination and seedling growth; rate and uniformity of seedling emergence and growth in the field; and emergence ability of seeds under unfavourable conditions [16].

Seed vigour loss precedes viability decline, so that, although seed lots may have similar high germination values, they may differ in their physiological age and so differ in vigour and ability to perform [15]. Viability test alone may as such be of limited ability in detecting physiological quality differences among seed lots [8]. It is on the basis of such circumstances, therefore, that a more sensitive differentiation of potential seed performance is necessary [16]. Moreover, seed vigour testing could supplement a viability test with more information about the physiological quality of a seed lot. Furthermore, although a seed may be viable, it may still fail to germinate under stressful

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conditions. Results of vigour tests could help identify when such stand failures are likely [17].

It has been reported that one of the major problems in the cultivation of spider plant is the unavailability of high quality seeds [2]. A survey carried out found that the majority of farmers use tins (plastic and metallic) and polyethylene bags as storage packaging materials because they are readily available [1]. The purpose of this research was to study the effect of packaging and storage conditions on seed quality of spider plant with a view to finding out the optimal method of handling and storing these seeds.

MATERIALS AND METHODS

Storage experiments were set using seeds dried above silica gel to four target moisture levels: 20%, 10% 5% and 2%. Dried seeds were sealed in aluminium foil packets and polyethylene packets and stored at three storage temperatures: ambient (22^oC to 30^oC), 5^oC and minus 20^oC for three and six months. For each treatment, 400 seeds were used for germination, 250 for moisture content determination and 100 seeds were used for electrical conductivity test. After each storage period, seeds were removed from the storage conditions and germination and vigour tests carried out according to the International Seed Testing Association (ISTA) [9].

Germination test

Germination test was done as described in the ISTA procedure [9]. At the beginning of the study and subsequently after each sampling interval, four replicates each of 100 seeds were used. These seed replicates were allowed to imbibe on 1% agar-water at 25^oC ($\pm$ 0.5) in a germination cabinet (LMS cooled incubators, Jencons-PLS,) with a 12-hour photoperiod daily. Sterilinpetridishes (of 9 cm) from Bibby Sterilin Limited, Stone, U.K. were used. Prior to placing seeds on water-agar, seeds were sterilized in 1% sodium hypochlorite, (Rackitt Colman, Nairobi) for 10 minutes to reduce fungal growth.

Moisture content determination

Initial moisture content expressed on fresh weight basis was determined gravimetrically in five replicates, each of 50 seeds in a well-ventilated oven at 103^oC for 17 hours [9]. The rest of the bulk pods and seed lots not used for desiccation were left overnight at room temperature until moisture content results were calculated after 17 hours. After removing the seeds from the oven, seeds in the dishes were allowed to cool for about 30 - 45 minutes inside desiccators before their weights were taken, and seed moisture content expressed on a fresh weight basis as:

Percent seed moisture content

$$= \frac{\text{Initial seed weight (g) - seed weight after drying (g)}}{\text{Initial seed weight (g)}} \times 100$$

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Electrical conductivity test

After each storage period, samples were drawn for germination and electrical conductivity tests. Four replicates of 25 seeds from each storage treatment combination were weighed to three decimal places before being soaked into 100 ml distilled water in plastic bottles. A control bottle containing distilled water only was set up with each test run. All bottles were maintained at ambient temperatures for 24 hours in the seed science laboratory of Moi University, Chepkoilel campus. After the soak period, the solution and seeds in each bottle were gently swirled for 10 to 15 seconds, and conductivity (μScm^{-1}) of the soak water measured using a Fieldlab-Lf conductivity meter and LF 513T-electrode dip-type cell (Schott Gerate Glass Company, Mainz, Germany). Several measurements were taken until a stable result was obtained. Between measurements, the dip cell was rinsed twice in distilled water and dried using clean dry paper towels. After subtracting the control bottle measurements (the mean of the readings), conductivity was expressed per gram of seed ($\mu Scm^{-1}g^{-1}$). The conductivity measurement was conducted according to recommendations by the International Seed Testing Association [9].

RESULTS

Three Months Storage

Effect of storage temperatures on viability and vigour of spider plant seeds

After three months of storage, seed viability and vigour were significantly ($P<0.05$) affected by storage temperatures. Seed viability as represented by percent germination was highest (78%) in seeds stored at minus 20^{o}C. Seeds stored at room temperatures recorded the least percent germination of 67.5% while those stored at 5^{o}C were intermediate with a percent germination of 72%. Seed vigour as indicated by mean germination time of 1584 minutes or 2.10 days and electrical conductivity of 26.36$\mu Scm^{-1}g^{-1}$ was also highest at minus 20^{o}C. Seeds stored at 5^{o}C were intermediate in vigour with 1612 minutes, 48 seconds or 2.12 days mean germination time and an electrical conductivity of 28.81$\mu Scm^{-1}g^{-1}$. The lowest seed vigour of 1828 minutes, 48 seconds or 2.27days mean germination time, and electrical conductivity of 31.67$\mu Scm^{-1}g^{-1}$ was obtained for seeds stored at room temperatures (Table 1).

Effect of storage moisture content on viability and vigour of spider plant seeds

Reduction in storage moisture content had significant ($P<0.05$) effects on viability and vigour of spider plant seeds dried to 2%, 5%, 10% and 20% moisture content levels. Though there were no significant differences between 20% and 10% moisture content levels, in general, viability and vigour improved with decrease in moisture content up to 5%, but further reduction to 2% moisture content resulted in decrease in seed quality. Highest viability as represented by a percent germination of 77.5% and highest vigour as represented by a mean germination time of 1584 minutes or 2.10 days and electrical conductivity of 26.37$\mu Scm^{-1}g^{-1}$ was obtained for spider plant seeds dried to 5% moisture content. Lowest viability of 46.5% percent germination and

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lowest vigour of 1857 minutes, 36 seconds or 2.29 days mean germination time and 31.83µScm^{-1}g^{-1} electrical conductivity was recorded for seed dried to 2% moisture content (Table 2).

Six Months Storage

Effect of temperature and moisture content on viability and vigour of spider plant seed

The three temperature and four moisture content regimes used in this study were significantly different ($P<0.05$) in their effects on percent germination, mean germination time and electrical conductivity of spider plant seeds stored for six months. Results indicated in figures 1, 2 and 3 show a general trend of seed quality improvement (high percent germination, lower mean germination time and lower electrical conductivity) with moisture content reduction from 20%to 5%. However, further drying to 2% moisture content resulted in a decline in seed quality. Seeds dried to 5% moisture content gave better seed quality across all the storage temperature regimes compared to seed dried to 2%, 10% and 20% moisture contents. Seeds dried to 5% moisture content and stored at minus 20^{o}C recorded the highest viability as represented by a percent germination of 95% and highest vigour as indicated by a mean germination time of 1598 minutes, 24 seconds or 2.11 days and electrical conductivity of 26.36 µScm^{-1}g^{-1}, while seeds dried to 2% moisture content and stored at room temperature had the least seed quality (percent germination of 55.5%, mean germination time of 1944 minutes or 2.35 days and electrical conductivity of 35.13µScm^{-1}g^{-1} .

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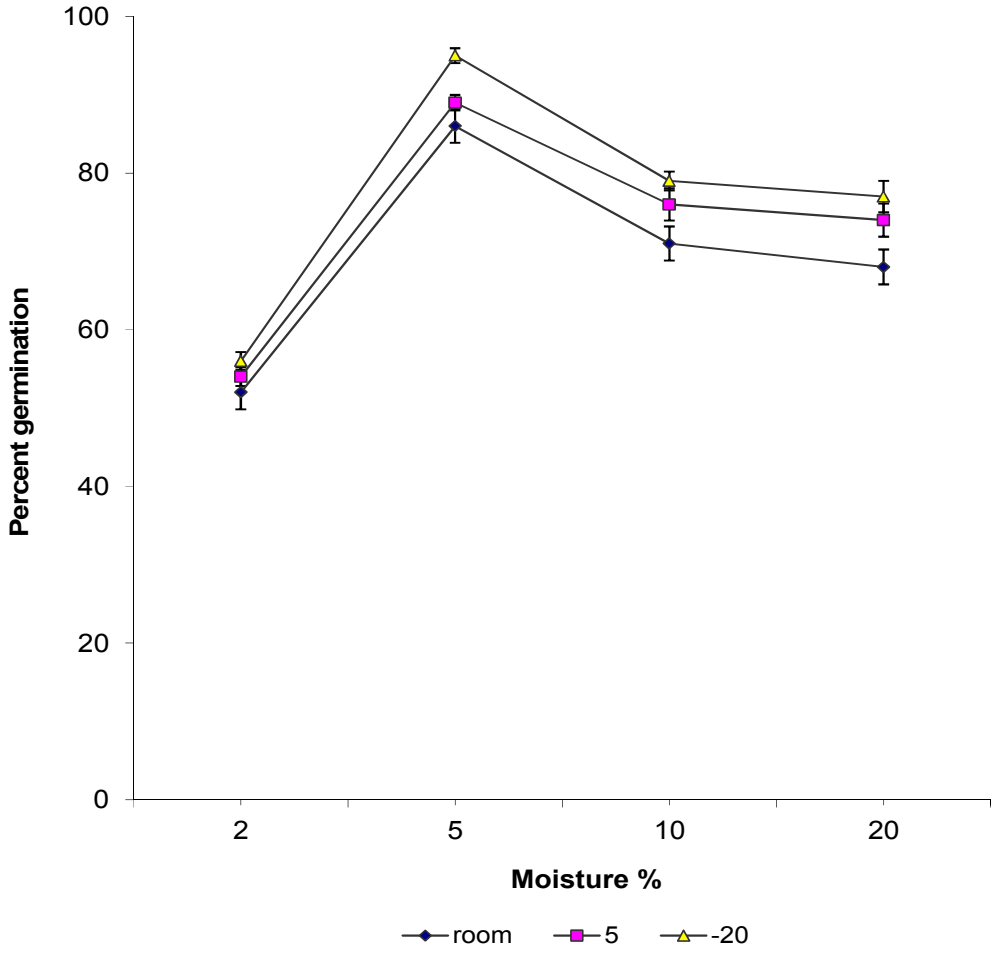

Figure 1: Effect of moisture and temperature on percent germination of spider plant seeds stored for six months

Cleome seed stored at minus 20^{o}C had higher percent germination across the four moisture content levels. Seeds dried to 5% moisture content and stored at minus 20^{o}C recorded the highest percent germination of 95%. In general, seeds stored at room temperature recorded the least percent germination in all the four moisture content levels, while storage temperature of 5^{o}C was intermediate (Fig.1).

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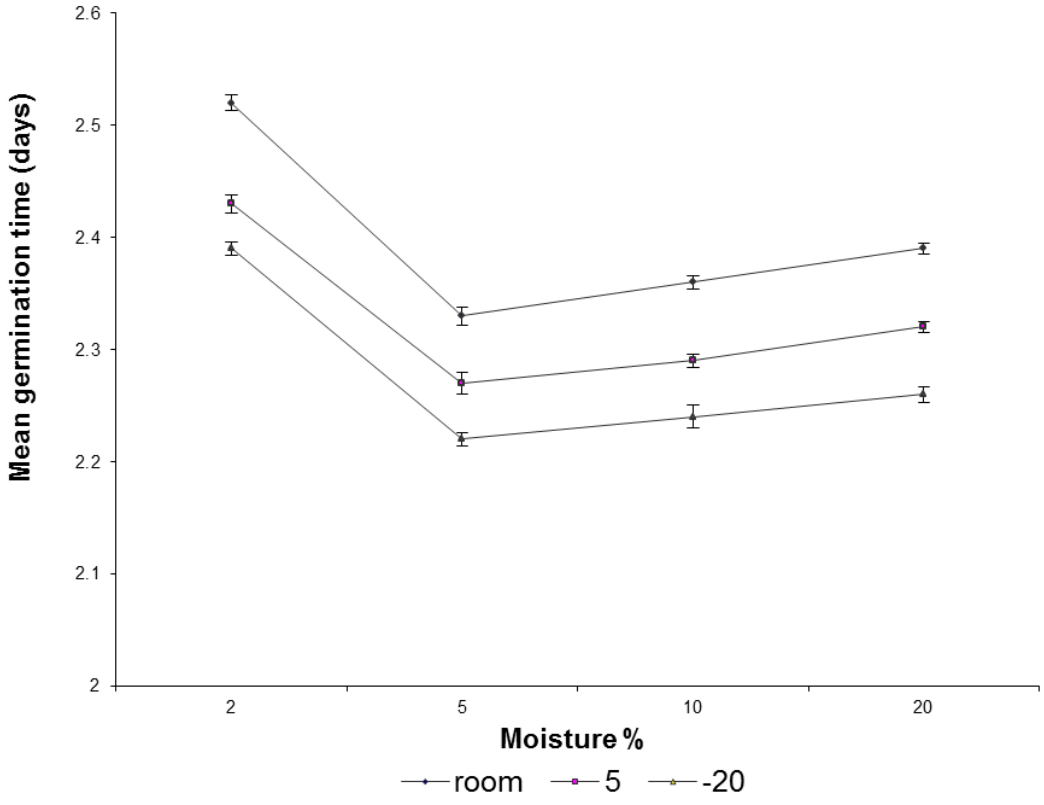

Figure 2: Effect of moisture and temperature on mean germination time of spider plant seeds stored for six months…put this at the bottom of the figure

Figure 2 shows a high mean germination time (low vigour) for seed dried to 2% moisture content across the three storage temperature regimes. Least mean germination time (high vigour) was recorded for seed dried to 5% moisture content and stored at minus 20^{o}C. Across the four moisture content levels, seed stored at room temperature recorded the highest mean germination time (low vigour).

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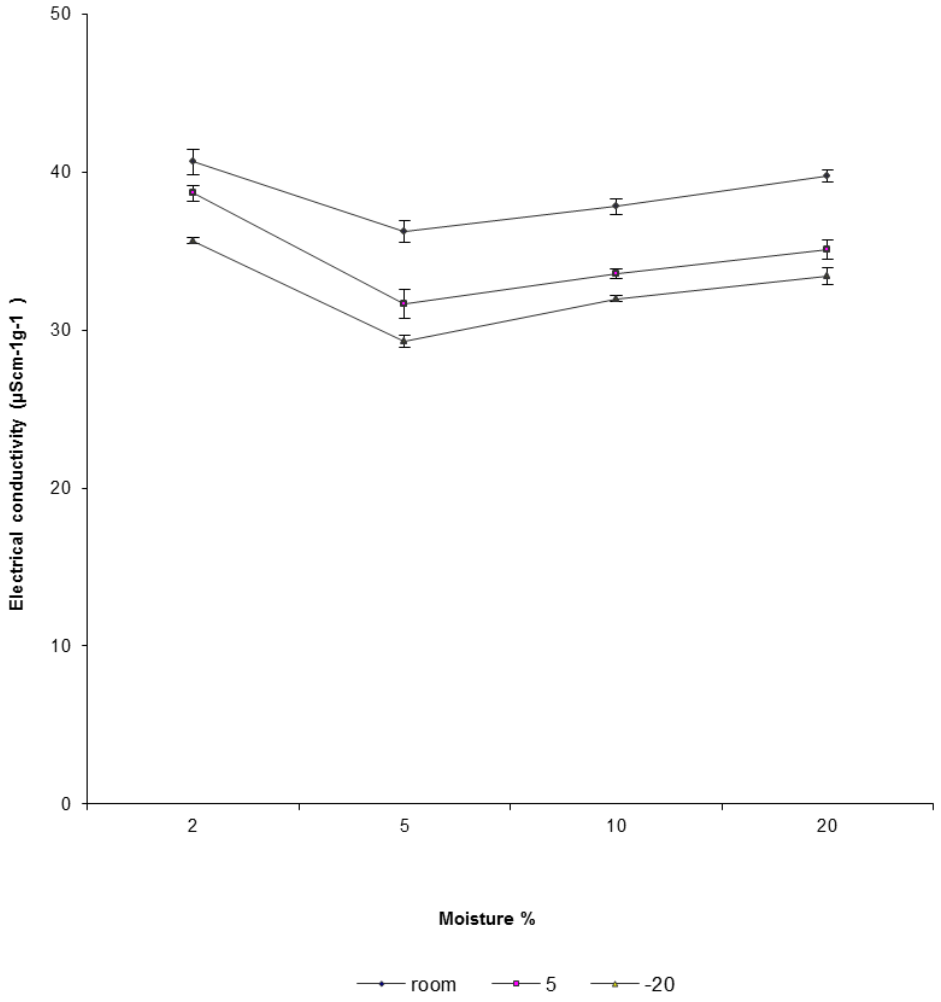

Figure 3: Effect of moisture and temperature on electrical conductivity of spider plant seeds stored for six months

Figure 3 depicts a general trend of seed electrical conductivity reduction with a decrease in moisture content from 20%, 10% to 5%. However, further drying to 2% moisture content resulted in an increase in seed electrical conductivity. Seeds dried to 5% moisture content gave least seed electrical conductivity (high vigour) across all the storage temperature regimes. Storage temperature of minus 20^{o}C recorded the least electrical conductivity (high vigour) across the four moisture content levels. High seed electrical conductivity (low vigour) was obtained for seed stored at room temperature.

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Effect of containers on viability and vigour of spider plant seeds stored for six months

In this study, aluminium foil packets and polyethylene packets did not differ significantly in their effects on seed quality of spider plant seeds stored for six months. Percent germination of seeds stored in aluminium foil packets and polyethylene packets was 94.5% and 93.5%, respectively. Mean germination time and electrical conductivity for seeds stored in aluminium foil packets were 1742 minutes, 24 seconds (2.21 days) and 29.27$\mu Scm^{-1}g^{-1,}$ respectively, while for seed stored in polyethylene packets, the mean germination time was 1756 minutes, 48 seconds (2.22 days) and electrical conductivity was 29.28$\mu Scm^{-1}g^{-1}$ (Table 3).

Effect of storage period on seed viability and vigour

Storage period caused positive significant effect (P<0.05) on viability of spider plant seeds. Initially, germination was as low as 14.5%, but increased to 78% and 95% after three and six months, respectively (Table 4). There were negative significant effects (P<0.05) caused by storage period on vigour of spider plant seeds. Seed vigour as indicated by mean germination time and electrical conductivity decreased in storage. The initial mean germination time and electrical conductivity was 1497 minutes, 36 seconds (2.04 days) and 23.94$\mu Scm^{-1}g^{-1,}$respectively. However, after three and six months of storage, mean germination time increased to 1584minutes (2.10 days) and 1742 minutes, 24 seconds (2.21 days), respectively, while electrical conductivity also increased to 26.36$\mu Scm^{-1}g^{-}$ and 29.27$\mu Scm^{-1}g^{-1,}$respectively (Table 4).

DISCUSSION

Storage temperatures of minus 20^{o}C gave seeds of highest quality, implying that spider plant seeds are cold tolerant and probably chilling has a dormancy breaking effect. Other studies have demonstrated that high seed vigour and viability levels can be maintained in storage by lengthening the pre-chilling period [18]. Orthodox seeds can be dried to low moisture content (5 % or less) without damage and, over a wide range of environments, their longevity increases with decrease in storage moisture content and temperature in a quantifiable and predictable way [19, 20]. The findings of this study concurred with the above observation as spider plant seeds dried to 5% moisture content and stored at minus 20^{o}C had the highest quality.

Removing every last bit of water from seeds is detrimental [13]. Thus, reports of low germination in seeds stored under extremely dry conditions were not unexpected [21]. Reports using numerous species have demonstrated that drying to extremely low water contents may shorten seed longevity and for many seeds there is an optimum moisture level for storage at which longevity is maximized and below which seeds are damaged. This is the critical water content [13]. In the present study, there was an increasing trend in germination percent with moisture reduction up to 5% but the trend declined at 2% moisture content. In the two storage periods (three and six

months), seed dried to 2% moisture content recorded the least germination percent and the least vigour. Therefore, the findings of this study are in agreement with the aforementioned observation that drying seeds to extremely low water contents could be detrimental [13].

Seeds should be packaged in water proof containers and hermetically sealed without delay following removal from the drying room or cabinet [22]. Different types of containers are available for packaging seeds. The choice depends on storage conditions and species. Laminated aluminium foil packets are the most commonly used containers in gene banks as they occupy little space and can be resealed [22]. Studies have indicated that although polyethylene is not suitable for long-term storage of orthodox seeds, it can be used for short-term or medium-term storage [23]. In this study, seeds were packaged in aluminium foil packets and polyethylene packets. Polyethylene packets are cheap and readily available to farmers [1]. The two containers did not differ significantly in their effect on seed quality at least in the test period (six months), which could be referred to as short- term.

Although viability increased in storage, there was gradual seed deterioration as indicated by vigour (mean germination time and electrical conductivity). Seeds dried to 20% moisture content and stored at room temperatures recorded low vigour as compared to seed dried to 5% moisture content and stored at minus 20^{o}C. This agrees with other studies that deterioration of orthodox seeds in storage increases with increase in seed moisture and temperature [19, 20]. It has generally long been known that, the greater the moisture content and storage temperature of orthodox seeds, either singly or in combination, the shorter is the period of seed survival [19, 20]. Therefore, the high percent germination and high vigour (low mean germination time and low electrical conductivity) exhibited by spider plant seeds stored at 5% moisture content and minus 20^{o}C is in agreement with the above observation.

Percent germination of freshly harvested spider plant seeds was as low as 14.5% but increased to 95% after six months storage. This could be attributed to relative dormancy exhibited by freshly harvested spider plant seeds, which is then released in storage. After-ripening dormancy loss in stored seed has been observed in *Amaranthusretroflexus* and *Festucaidahoensis* [24, 25]. A viability test is limited in detecting quality differences among high germinating seed lots [8]. It was further observed that the results of seed storage are unlikely to adequately reflect the degree of seed deterioration that has taken place [12]. This has been reflected in this study by the high percent germination of 95%, after six months of storage, yet the seeds have deteriorated as indicated by the electrical conductivity measurements.

Electrical conductivity measurement of soak water in which seeds have been soaked identifies such deteriorated seed lots. Seed lots with high electrolyte leakage (high electrical conductivity) are classified as low vigour, while those with low leakage (low electrical conductivity) are considered high vigour [9]. In the present study, results of the electrical conductivity measurements revealed significant changes in

amount of electrolyte leaked from seeds stored at different storage periods. This could be attributed to deterioration of spider plant seeds in storage due to ageing.

CONCLUSION

This study has established the beneficial effect of drying seeds to low moisture contents and storing at low temperatures. Based on the results of this study**,** it may be concluded that, to achieve high seed quality**,** spider plant seed should be dried to 5% moisture content and stored at sub zero temperatures (preferably at minus 20^{o}C) for a period of six months. However, these conditions can only be available in such places as the gene banks and other institutes that conserve seed for long-term storage. In this study, a germination of 85% was recorded for seed dried to 5% moisture content and stored at room temperatures.

The study showed that aluminium foil packets and polyethylene packets are equally good as packaging materials for spider plant seed (at least for short-term storage). From the findings of this study, it can be concluded that at the farm level, spider plant seeds can be dried to 5% moisture content, packaged in polythene packets and stored for six months at room temperatures. For gene bank storage, spider plant seed can be dried to 5% moisture content, packaged in aluminium foil packets and stored for six months at minus 20^{0}C.

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Three Months Storage

Table 1: Effect of storage temperatures on percent germination, mean germination time and electrical conductivity of spider plant seeds stored for three months at 5% moisture content

Storage temperature	Germination (%)	Mean germination time (days)	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)
Room temperatures (22-30^{o}C)	67.50a	2.27a	31.67a
5^{o}C	72.00b	2.12b	28.81b
- 20^{o}C	78.00c	2.10c	26.36c
LSD $_{0.05}$	3.1	0.01	0.82

Any two means having different letters within a column are significantly different at 5% level of significance according to the least significant difference (LSD) test

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Table 2: Effect of seed storage moisture content on percent germination, mean germination time and electrical conductivity of spider plant seeds stored at minus 20^{o}C for 3 months

Moisture content	Germination (%)	Mean germination time (days)	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)
20	71.5a	2.18a	28.27a
10	73.5a	2.17a	27.99a
5	77.5b	2.10b	26.37b
2	46.5c	2.29c	31.83c
LSD $_{0.05}$	2.5	0.02	0.32

Any two means having a common letter within a column are not significantly different at 5% level of significance according to the least significant difference (LSD) test

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Table 3: Effect of container on percent germination, mean germination time and electrical conductivity of spider plant seeds stored for six months at minus 20^{o}C and 5% moisture content

Storage container	Germination (%)	Mean germination time (days)	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)
Foil	94.5a	2.21a	29.27a
Polythene	93.5a	2.22a	29.28a
$LSD_{0.05}$	2.8	0.03	1.15

Any two means having a common letter within a column are not significantly different at 5% level of significance according to the least significant difference (LSD) test

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Table 4: Effect of storage period on percent germination, mean germination time and electrical conductivity of spider plant seeds dried to 5% moisture content and stored at minus 20^{o}C

Storage period	Germination (%)	Mean germination time (days)	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)
0 months	14.5a	2.04a	23.94a
3 months	77.5b	2.10b	26.36b
6 months	94.5c	2.21c	29.27c
$LSD_{0.05}$	7.3	0.04	1.12

Any two means having a common letter within a column are not significantly different at 5% level of significance according to the least significant difference (LSD) test

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