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Assessment of Storage Dependent Physiological Parameters of *Sesamum indicum* seeds

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A B S T R A C T

In this study experiments was conducted to evaluate the effect of augmented storage of seed. Sesame (*Sesamum indicum* L.) is one of the world's most imperative oil seed crops, storage of which is one of the foremost concerns. The objective of this work was to evaluate the effects of moisture content and electrical conductivity (EC) as well as the germination index in the four storage patterns of Sesame seed. Based on the obtained results, it can be concluded that the electrical conductivity test has not shown to be a good indicative of the deterioration process of seeds stored at low temperatures and moisture content was correlated with EC. Maximum germination percentage was achieved immediately after harvesting and gradually decreases with storage time. Seed aging is the main problem of seed storage. Application of accelerated storage study could be used to assess seed vigor and quality. Physiological changes that occur in seeds during storage are manifested as reduction in seedling vigor index, number of germination, percentage germination, speed of germination and rate of germination among others. The study thus, was aimed at prolonging the longevity of sesame seed through different characteristic premeditated during of the storage.

Introduction

An important oil seed: Sesame (*Sesamum indicum* L.) is usually grown in tropical zones as well as in temperate zones amongst the latitudes 40^{o}N and 40^{o}S. It has been cultivated for centuries, especially in Asia and Africa. In last decade, the world production of sesame seed was 3,976,968 tons and the major production area were Asia (2,489,518 tons) and Africa (1,316,690 tons), constituting about 62.6% and 33.1%

of the total world production. Besides being the major source of edible oil, its industrial applications include preparation of sweets, confectionary and bakery products (Ekasit, 2012).

Being such an important seed its preservation and storage should be highly specified, opposite to this seed processing and storage problems are common in tropical countries like India, reason for which could be the predominant hot and humid tropical and subtropical conditions with great variation in relative humidifies and temperatures throughout the year (as per the season: summer, winter, and rainy), including biennial variation. Frequent fluctuation in temperature, moisture content and storage time make the processing and storage of seeds difficult (Suma et al., 2013). To minimize the loss of viability and change in genetic integrity of the seed and thereby conservation of genetic resources further studies are the need of the hour.

Research on the seed storage has corroborated that germination potential, electrical conductivity, high temperature and relative humidity conditions accelerate the deterioration of the seed and thereby cause ageing. Therefore, exposure of seed to high temperature and high moisture conditions for different storage time will cause ageing and has been commonly used for accelerated ageing of seeds in the laboratory as well as for farmers in the fields. Such study of seed storage pattern prior to germination may yield useful information concerning the loss of seed viability, vigour, and longevity of viability and may be used as a tool for predicting the relative seed-longevity. Thus deterioration would occur relatively slowly at low moisture and temperature (Suma et al., 2013). The type of storage component in the seed also influences the equilibration of seed moisture content. Because of the differences in anatomical structure and storage components of seeds, the equilibrium moisture content differs between similar species. Therefore, there is a need to investigate the effect of different storage time, electrical conductivity, germination percentage, seed vigorous properties and Moisture content to accelerate the seed desirable efficiency for long storage.

The most important factor in crop production is high quality seed. Seed vigor is also the key components of seed quality. Seed aging is recognized by some parameters like delay in germination and emergence, slow growth and increasing of susceptibility to environmental stresses in various duration of storage. Seed quality (viability and vigor) decreases under long storage conditions due to aging. It is the reason of declining in germination characteristics. Aging is manifested as reduction in germination percentage and those seeds that do germinate produce weak seedling. The aim of seed vigor and germination tests is to distinct low and high seed with each other, also these tests provide ways to examine the ability of field performance of different seeds in laboratory conditions.

Seeds deteriorate and lose their germinability during periods of prolonged storage (Ghahfarokhi et al., 2014).

The electrical conductivity (EC) test is one of those used to evaluate seed vigor.The relation between water content, organizational level of seed cellular membranes and quantity of leachates in the soaking solution is the theoretic base of the EC test. Thus, higher the speed of the restoring of cell membrane integrity which occurs at the onset of the germination process, lower the amount of leachates released to the soaking solution, indicating high seed vigor. Several factors affect EC test results, such as seed size, temperature, imbibition period, initial seed water content and storage temperature. A positive correlation have been found between EC and accelerated aging results, that is, seeds with low germination after accelerated aging showed low EC results, or, in other words, the results of one test contradicted the results of the other (Adriana et al., 2012).

Seed deterioration starts immediately after a crop has attained the physiological maturity stage. Thus, in order to prevent the quantitative and qualitative losses due to several biotic and abiotic factors during storage, several methods are being adopted such as seed treatment with suitable chemicals or plant products, as well as seed storage in safe containers.

Oyekale et al. (2012) reported that seed deterioration during storage was due to the damage in cell membrane and other chemical changes in the seed system such as the protein and nucleic acid accumulation. Such degenerative changes result in complete disorganization of membranes and cell organelles and ultimately causing death of the seed and loss of viability. The most common and consistent ultra-structural changes in all the cell organelles were the loss in integrity of membranes, which invariably leads to increased seed deterioration especially during storage. Oyekale et al. (2012) reported that for every one percent moisture decline and for each 5^0C decrease in temperature the storage period will be doubled. In open and pervious containers, seed moisture content is determined by relative humidity of the surrounding atmosphere and temperature of the storage environment. Adriana et al.(2012) stated that seeds stored in ambient conditions lose their viability and vigour very fast due to changes in storage conditions of temperature and relative humidity. The storability of seeds is also influenced by the type of packaging material. Seeds stored in moisture-proved sealed containers provide suitable environment for storage, offer protection against contamination and also acts as a barrier against the escape of seed treatment chemicals than in moisture pervious containers higher viability and vigour was retained for longer period in moisture-proof containers followed by moisture resistant and impervious containers respectively (Oyekale et al., 2012).

Thus the study was undertaken to relate the moisture content, germination index and electrical conductivity with the storage period of the seed.

Materials and Methods

Sampling and Storage Condition

The seeds were procured and present investigation was carried out at Biochemistry Laboratory of Project Coordination Unit, All India Coordination Project (Sesame and Niger), Jawaharlal Nehru Krishivishwavidyalaya, Jabalpur (M.P.), India. The *S. indicum*seed materials used in the present study include four different seed coat colors viz white, black, dark brown and light yellow and three different storage conditions i.e. Short term (including the Year 2014, 2013 and 2012) mid-term (including the Year 2011, 2010 and 2009) and long term (including the Year 2008, 2007 and 2006 cold storage -20^0C) against the control (freshly harvested year 2015 seeds in four different seed coat colors) respectively. Short term and mid-term storage seed samples were stored in the Laboratory at 28±2°C.

Morphological Characterization

The raw sesame seed was first winnowed to remove the dust and chaff present, and then washed manually. The cleaned sesame seeds were grouped into samples for further studies. Mature seeds of all four seed coat colors and storage pattern of *S. indicum* were studied by using Electronic Digital Vernier Caliper and Caliper Gauge for the dimensions of seeds, length, width and Seed weight was measured with the help of a single pan electric balance (Afcoset, ER 180 A, Japan). The mean values of seed dimension parameters were obtained from a set of n=10 and for weight of seeds n= 100 seeds in triplicates of each colors (IPGRI, 2004).

Germination and Seedling Vigor Index Tests

Germination and Seedling vigor index tests were conducted using 100 seeds in three replications. Seeds were plated in petri plates with wet filter paper and kept in germinator maintaining adequate humidity and temperature of 20^0C. Germination count and length of seedling was taken every day up to the seventh day. The formula used to evaluate germination percentage, seedling length, and seedling vigor index (Abdul-Bakiand Anderson, 1973).

Moisture Determination

The most common method of determination of seed moisture is oven drying method. The principle underlying this method is the elimination of water from the seed by drying precisely for prescribed duration and temperature. The moisture content is expressed as the percentage of the seed dry weight. For determining the seed moisture content, 100 seed sample weight in gm. each was drawn randomly in three replications of each seed coat colors. Seeds were dried at $103\pm2^{\circ}$C in order to attain constant weight (Suma et al., 2013). The moisture contents were calculated using the formula

Moisture percentage (%) = Initial weight – final weight /Initial weight x 100

Electrical Conductivity (EC) Test

The electrical conductivity (EC) was investigated randomly in three replications of each seed coat colors according to the method of (Hampton and TeKrony, 1995). Fifty seeds of each color were weighed and placed in 250 ml of distilled water containing beaker. Further, beakers sealed by aluminum foil and kept at 20^0C for 24 h. The electrical conductivity of the seed leachates was measured by using an EC meter and results were expressed per gram of seed weight as µS cm^{-1} g^{-1} for each sample.

Results and Discussion

The stored seeds revealed a decline on their physiological quality during the storage times (Short term, mid-term and long term) detected by the morphological characteristics, germination, Moisture and electrical conductivity at all the storage period studied. During the measurement of length and width entire storage seeds variations were almost observed negligible. But 100 seed weight of the entire storage seed, variations were observed in mostly in mid-term storage seed as comparisons to control and other type of storage patterns. Standard error for seed width and 100 seed weight were almost negligible. From the analysis of presented data (Fig.1) it is obvious that analyzed seed quality traits varied amongst tested. Agronomic crops as well as within different storage time with statistically highly significant differences.

Therefore, the storage life of seeds stored under moisture controlled environment is usually longer than seeds stored under ambient storage conditions; and seed deterioration is often faster in the later. Storage pattern of black color sesame seeds through short, mid and long term storage showed variation in various morphological characteristics (Fig. 3).

Short term storage showed value near to control (Fresh seeds). With length 3.02 mm and width 1.81 mm. whereas less variation was observed in mid-term and long term storage with 2.89mm and 2.96mm length; 1.72mm and 1.78mm width respectively for mid-term and long term storage whereas weight of seeds showed slight deviation in mid-term and long term storage with 0.29 gm/100 seeds whereas weight of control and long term storage were comparable with 0.31 gm/100seeds.Storage pattern of white color sesame seeds through short, mid and long term storage showed variation in various morphological characteristics. Mean width was similar with short term and long term storage with 1.81 mm whereas mean length was almost similar in various storage periods. Light yellow and dark brown colored seeds also showed similar patterns in morphological characteristics with similar length, width and weight. Overall texture of various colored seed showed similarity with soft appearance accepts the mid-term storage which has slight rough appearance.

Seed processing and storage is greatly influenced by moisture content and germination index but type of storage periods and condition majorly effect moisture content. This study of germination index and moisture content is indeed a vital parameter for seed quality assessment correlation between these parameters depicts the pattern that revealed that black color sesame seed has highest germination and moisture percent 43.56 and 4.66 respectively at short term storage (Fig. 2). Similar result were observed after analyzing the aforementioned parameters with different color seeds with highest value at short term storage simultaneously followed by long term and midterm storage.

Electrical conductivity (EC) and vigor index were analyzed for all storage periods including all the four color of sesame seeds. Electrical conductivity as vigor test could be used to detect seed deterioration at various storage periods (Fig. 4). Analysis of the present evaluated data revealed that electrical conductivity was maximum in dark brown sesame seed with 15.81μ cm^{-1} g^{-1} followed by long term storage of white seed with 15.28 μ cm^{-1} g^{-1}.These results were also analyzed statistically using box plot analysis which showed variance in EC and median of all the four color in the same area with a little difference in white and dark brown seed. Two way correlation cluster analysis also confirmed the above results with close correlation between short and long term storage of sesame seeds (Fig. 3 and 4).

These results were corroborated some studies in the literature, which indicated that the electrical conductivity results might be influenced by the storage temperature. Vigor index showed highest value in short term storage with 98.41 % in white sesame seed and 75.3 % in black. Similar observation was assessed in statically analysis where box plot showed strong auto correlation between white and black seeds.

Tabatabaei and Naghibalghora (2014) scrutinized that the Sesame (*Sesamum indicum* L.) cultivated in arid and semi-arid regions, is an annual oil seed crop cultivated for centuries, particularly in the developing countries of Asia and Africa, for its high content of both excellent quality edible oil (42-54%) and protein (22- 25%). Seed germination is an essential process in plant development to obtain optimal seedling numbers that results in higher seed yield. Germination and seedling growth declined with many abiotic factors such as salt and drought stress that are perhaps two of the most important grounded abiotic stress that limit number of seedling and seedling growth

Adriana et al.(2012) found a positive correlation between EC and accelerated aging results, that is, seeds with low germination after accelerated aging showed low EC results, or, in other words, the results of one test contradicted the results of the other. These result was in accordance to our finding which showed that lower germination index of short term white color seasame seed (Fig. 4a).

Among all the storage pattern scrutinized short term is way better of storage followed by long term and midterm for assessing the seed potential for better regermination and viability these conclusion of our was in harmony with Oyekale et al. (2012) reported that seed deterioration during storage was due to the damage in cell membrane and other chemical changes in the seed system such as the protein and nucleic acid accumulation. Such degenerative changes result in complete disorganization of membranes and cell organelles and ultimately causing death of the seed and loss of viability. The study illustrated the role of seed moisture content while processing and storing seeds of *Seasamem indicum*. Moisture content and germination content is slightly affected by the storage pattern. In the given data (Fig. 3) analyzed by the values obtained by performing these experiments related with the trend observed by various researchers that was true for vigor index also since vigor index is a consequence of germination percentage and seedling vigor. Hence storage of seeds with high moisture content (4-6%) caused faster deterioration in all accessions and was least at 2%, without having any adverse effect on physiological parameters. On analyzing the variability in seed storage behavior, it was observed that germination percentage was recorded maximum in short term storage 68-75%followed by mid-term and minimum in long term seeds of *Seasamem indicum*

Hence, it is convincingly proved that moisture and germination content plays an important role in maintaining the seed storage and thus directly affecting seed viability. The type of storage component in the seed also influences the equilibration of seed moisture content (Fig. 2). Because of the differences in anatomical structure, genotypic characters and storage components of seeds, the equilibrium moisture content differs between different colored seed. Therefore, there is a need to investigate the effect of temperature and relative humidity for equilibration of seed moisture content to desirable levels.

These results were in accordance with the results obtained by Suma et al. (2013) studied the Role of relative humidity in processing and storage of seeds and assessment of variability in Storage Behavior in *Brassica* spp. and *Eruca sativa* and reported the same trends in results.

Fessel et al. (2006) recounted that the electrical conductivity (EC) and the accelerated aging (AA) tests are valid to assess seed vigor (Association of Official Seed Analysts, 2002). EC test results (Fig. 4) have demonstrated a desirable efficiency to indicate seed performance to establish the stand under a wide range of field conditions as well as an important vigor test.

Table.1 Morphological Variation in Different Types of Seeds

	Black color seeds					White Color Seeds					Light Yellow Color Seeds					Dark Brown Color Seeds				
Storage Patterns	Mean Length (mm) μ ± S.D n= 10	Mean Width (mm) μ ± S.D n= 10	Mean Texture μ ± S.D n= 10	Seed shape	Mean of 100 seed weight (g)	Mean Length (mm) μ ± S.D n= 10	Mean Width (mm) μ ± S.D n= 10	Mean Texture μ ± S.D n= 10	Seed shape	Mean of 100 seed weight (g)	Mean Length (mm) μ ± S.D n= 10	Mean Width (mm) μ ± S.D n= 10	Mean Texture μ ± S.D n= 10	Seed shape	Mean of 100 seed weight (g)	Mean Length (mm) μ ± S.D n= 10	Mean Width (mm) μ ± S.D n= 10	Mean Texture μ ± S.D n= 10	Seed shape	Mean of 100 seed weight (g)
Control (2015)	3.04 ± 0.023	1.86 ± 0.086	Soft	Oval with convex side	0.31 ± 0.001	3.03 ± 0.010	1.87 ± 0.075	Soft	Oval with convex side	0.30 ± 0.004	3.02 ± 0.028	1.82 ± 0.027	Soft	Oval with convex side	0.30 ± 0.004	3.03 ± 0.022	1.89 ± 0.030	Soft	Oval with convex side	0.31 ± 0.004
Short Term	3.02 ± 0.032	1.81 ± 0.05	Soft	Oval with convex side	0.29 ± 0.003	3.05 ± 0.056	1.86 ± 0.04	Soft	Oval with convex side	0.30 ± 0.006	3.06 ± 0.03	1.89 ± 0.02	Soft	Oval with convex side	0.30 ± 0.001	3.02 ± 0.036	1.83 ± 0.036	Soft	Oval with convex side	0.30 ± 0.006
Mid Term	2.89 ± 0.035	1.72 ± 0.008	Soft +Rough	Oval with convex side	0.29 ± 0.002	2..98 ± 0.02	1.81 ± 0.033	Soft + Rough	Oval with convex side	0.28 ± 0.008	3.04 ± 0.014	1.85 ± 0.04	Soft + Rough	Oval with convex side	0.27 ± 0.005	2.92 ± 0.03	1.83 ± 0.020	Soft + Rough	Oval with convex side	0.28 ± 0.003
Long Term	2.96 ± 0.058	1.78 ± 0.035	Soft	Oval with convex side	0.30 ± 0.003	3.01 ± 0.056	1.81 ± 0.070	Soft	Oval with convex side	0.28 ± 0.096	3.02 ± 0.054	1.90 ± 0.047	Soft	Oval with convex side	0.30 ± 0.003	3.02 ± 0.079	1.85 ± 0.027	Soft	Oval with convex side	0.29 ± 0.003

Figure.1 Different Seed Coat Color of Sesame Seed

Figure.2a Interactive Effect of Moisture on Germination of Black Sesame Seeds

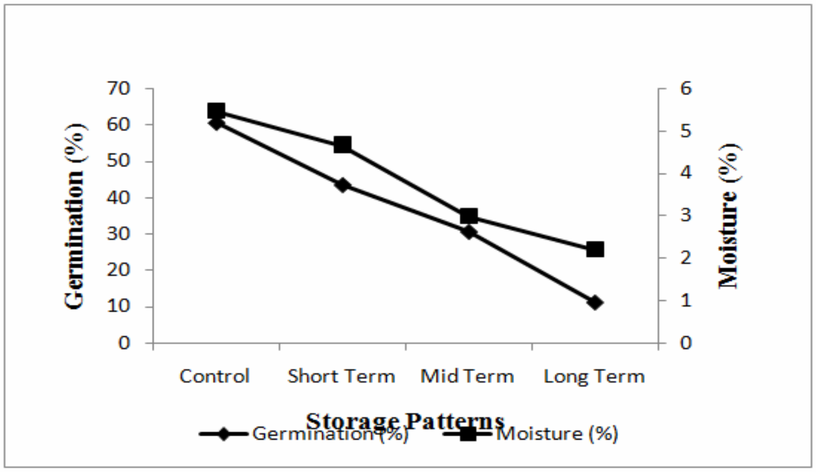


Figure 2b Interactive Effect of Moisture on Germination of White Sesame Seeds

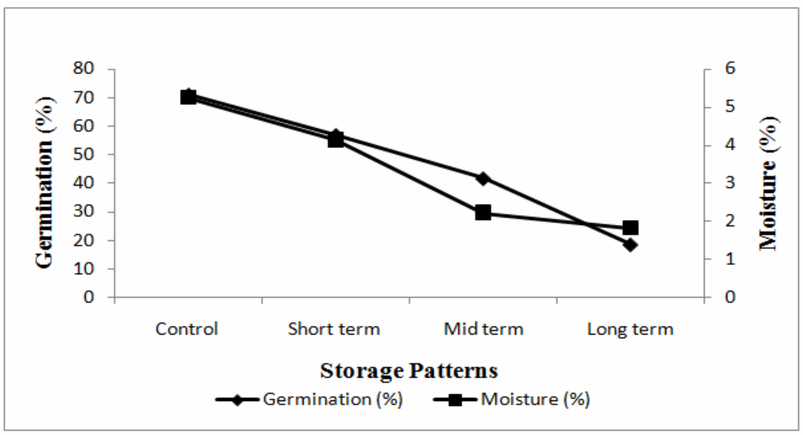


Figure.2c Interactive Effect of Moisture on Germination of Dark Brown Sesame Seeds

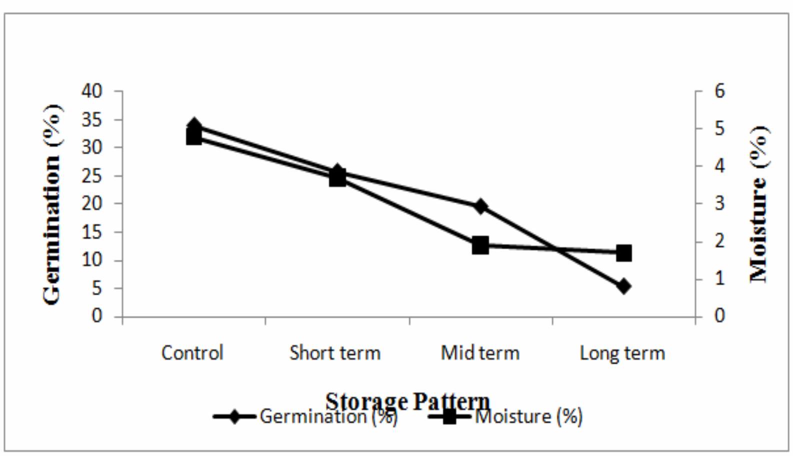


Figure.2d Interactive Effect of Moisture on Germination of Light Yellow Sesame Seeds

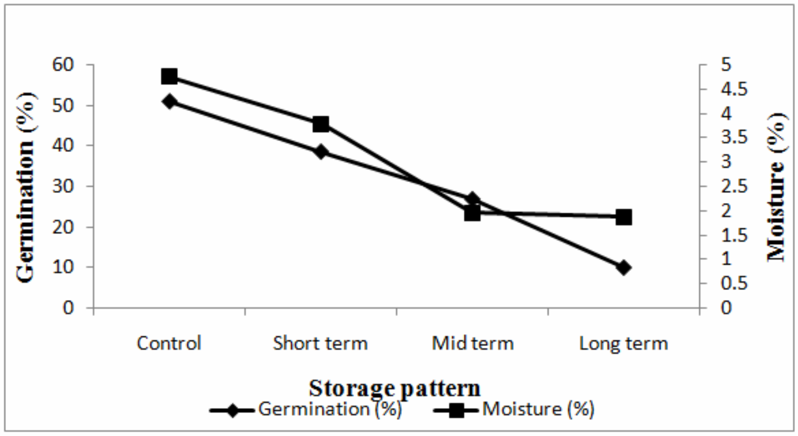


Figure.3a Interactive Effect of *S. indicum*Vigour Index Percentage (%) of Seeds on Different Storage Pattern

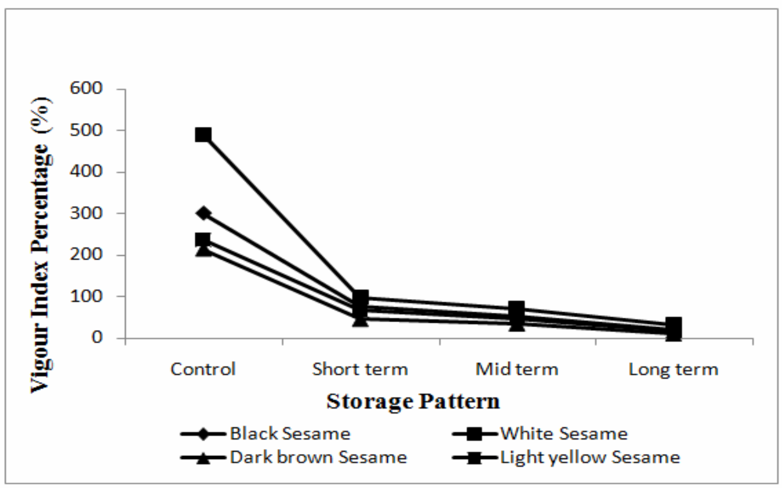


Figure.3b Cluster Analysis

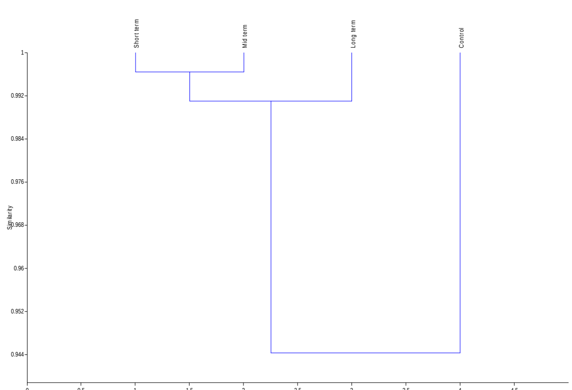


Figure.3c Box Plot Analysis

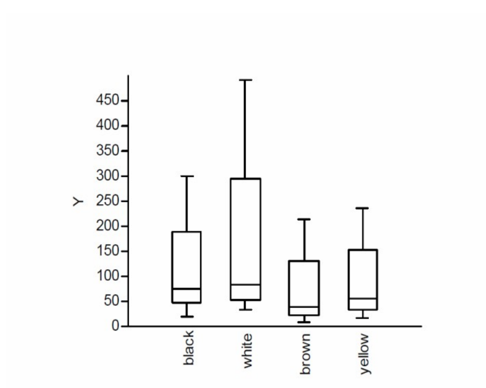


Figure.4a Interactive Effect of *S. indicum* Electrical Conductivity of Seeds on Different Storage Pattern and its Statistical Analysis

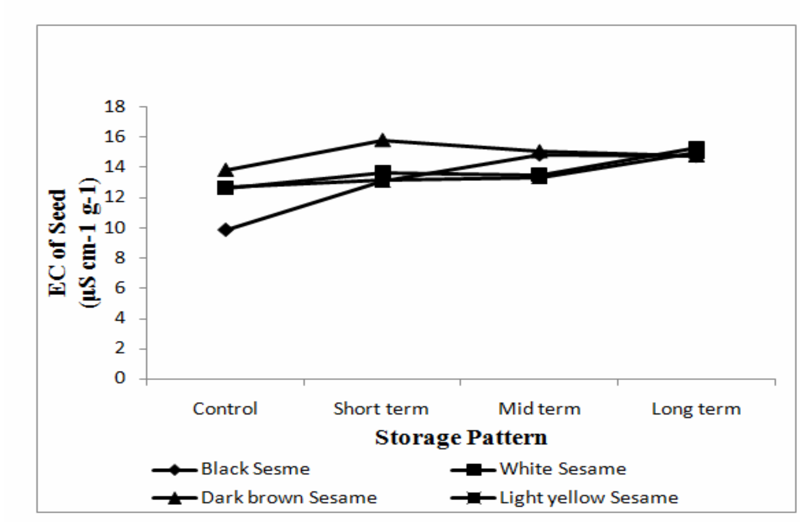


Figure.4b Cluster Analysis

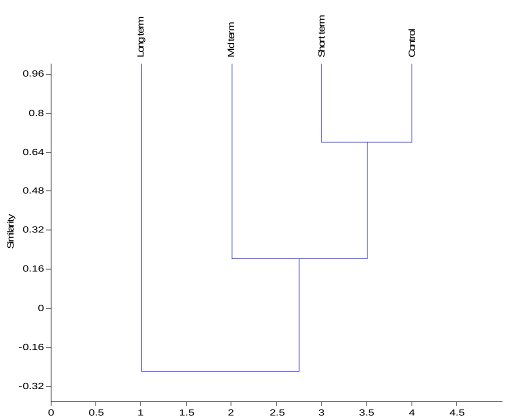
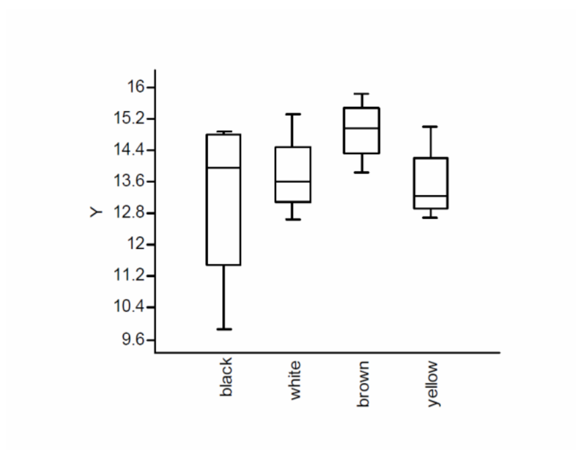


Figure.4c Box Plot Analysis



Similar was our finding that electrical conductivity was maximum in dark brown sesame seed with 15.81µS cm^{-1} g^{-1} with short term storage. These findings were important because not many studies on electrical conductivity to estimate changes in the physiological quality of seeds during storage have been developed.

The main causes for the seed ageing process are not yet fully elucidated, given the high number of cytological and metabolic alterations involved. Several researchers have considered cell membrane disintegration, with consequent increased permeability as one of the first detectable signs of deterioration, even if the mechanisms involved in such process are not yet fully clear. Thus, reviewing the deterioration process of seeds. On the other hand, Panobianco et al.(2007) found that alterations in carbohydrates during storage could affect the cell membrane permeability, thus contributing to the reduction in the physiological quality and germination of seeds. This could be the reason of our finding that short term. The damage caused to membrane through deterioration that provides lower selectivity and hence increase in the leakage of solutes to the environment has been one of the main causes of the decline in the physiological quality of seeds. As result, the electrical conductivity test is considered as an important tool to evaluate the seed vigour, since it indirectly assesses the cell membrane degradation degree by determining the amount of electrolytes released in the seed soaking solution. The electrical conductivity results may be affected by several factors. Some research has revealed that the storage temperature may influence the electrical conductivity test, especially lower ones. There are results showing that soybean seeds stored at 10ºC, when evaluated by the germination and accelerated ageing tests, present reduction in physiological quality; however, this fact is not detected by the conductivity test. Thus, the deterioration of seeds at low storage temperatures does not seem to be directly related to the loss of membrane integrity.

During storage, seed quality can remain at the initial level or decline to a level that may make the seed unacceptable for planting purpose what is related to many determinants: environments conditions during seed production, pests, diseases, seed oil content, seed moisture content, mechanical damages of seed in processing, storage longevity, package, pesticides, air temperature and relative air humidity in storage, biochemical injury of seed tissue and similarly Storage longevity may varies from six months (usually for maize, soybean and sunflower), up to 20 months or longer if the seeds are to be carried over. Longevity of seed in storage is influenced by the stored seed quality as well as stored conditions. Irrespective of initial seed quality, unfavorable storage conditions, particularly air temperature and air relative humidity, contribute to accelerating seed deterioration in storage.

We, through our results observed that as the duration of storage time increases electrical conductivity decreases (Fig.4) these finding was similar to the verdicts of Adriana et al.(2012) who also reported the inversely proportional relation of electrical conductivity with the aging and storage of oil seed.

Seed deterioration starts immediately after a crop has attained the physiological maturity stage. In a crop seed like sesame (*Sesamum indicum L.*) deterioration is even faster immediately after harvest from the field; this is owing to its high oil content and fast cellular respiration occurring in the seed. Several methods are being adopted therefore to encourage crop/seed shelf (storage) life while preserving it; and these may include: seed treatment with suitable chemicals or plant products, as well as seed storage in safe containers. Roberts reported that seed deterioration during storage was due to the damage in cell membrane and other chemical changes in the seed system such as the protein and nucleic acid accumulation. Such degenerative changes result in complete disorganization of membranes and cell organelles and ultimately causing death of the seed and loss of viability. The most common and consistent ultra-structural changes in all the cell organelles were the loss in integrity of membranes, which invariably leads to increased seed deterioration especially during storage. Seeds stored in moisture-proved sealed containers provide suitable environment for storage, offer protection against contamination and also acts as a barrier against the escape of seed treatment chemicals than in moisture pervious containers. Dried seeds of onion, carrot, cabbage, cucumber and tomato in desiccators and stored in moisture vapor-proof resistant and impervious containers. Higher viability and vigour was retained for longer periods in moisture-proof containers followed by moisture resistant and impervious containers, respectively.

The most important factor, and probably the easiest to control, is seed moisture content. Correct drying and packaging can make the difference between seeds surviving for a few months at best to being a high quality resource available for use for many years, possibly decades, into the future. The preservation of orthodox seeds by cold storage helps in sustaining seed viability; but the problem of high cost of electricity hampers this process because it raises the cost of seed preservation. Harrington suggested that within the normal range of moisture and temperature for stored seed, each 1% reduction in seed moisture content or each 5ºC reduction in temperature doubles storage life of the stored seed. Since sesame is a small flat seed, it is difficult to move much air through it in a storage bin. Therefore, the seeds need to be harvested as dry as possible and stored at 6% moisture or less. If the seed is too moist, it can quickly heat up and become rancid. Environmental conditions which are safe for seed storage from harvest to planting are not necessarily safe for longer periods. Controlled seed storage is thus very essential especially in tropical and subtropical conditions in order to maintain seed viability and seedling vigour for longer periods (Oyekale et al., 2014).

Results of our study were in accordance with Oyekale et al. (2012) reported that with increase in moisture content the storage ability of seed decreases i.e.: both the factors are inertial proportional whereas temperature plays an important role in storage so it could be concluded that low temperature could be the criteria to improve condition for storage of seed. Therefore long term storage included in the experiments had provided suitable environment for stowage of seed.

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