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Longevity of Bean (*Phaseolus vulgaris*) Seeds Stored at Locations Varying in Temperature and Relative Humidity

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Seed is an important input in agricultural production and its quality is essential in determining maximum potential crop yield. Conditions under which the seed is stored is often a major cause of poor seed quality. The combined effect of high moisture content and storage temperature are critical factors that affect storage potential and eventual quality of seed at planting time. The aim of this study was to determine effects of temperature and relative humidity on the viability of bean seeds stored under stockists store conditions. Mwitemania- GLP 92 bean seeds were stored by stockists at different locations varying in temperature and relative humidity. The same seeds were also stored under controlled temperature of -20˚C and relative humidity of 50% at the Seed Science laboratory, Chepkoilel Campus. Mean temperature and relative humidity of the three stockists stores in each town were recorded daily and their means calculated. The bean seeds were packed in clear polythene bags and stored by stockists in Bungoma, Nyeri, Nairobi and Mombasa. Viability and vigour tests were performed at zero days of storage and after every 30 days for 12 months of storage. Data was subjected to Analysis of Variance (ANOVA) and means separated by Least Significant Difference (LSD) at $P<0.05$. Results showed that the seeds stored under controlled conditions at Chepkoilel Campus maintained their quality for 12 months while the seeds stored by stockists in Mombasa with recorded mean maximum temperature and relative humidity of 30.8˚C and 80.1 % respectively showed a rapid decrease in viability which went below the accepted levels after one month of storage. In Nyeri, Bungoma and Nairobi, seeds remained viable above the accepted levels for 6 months. It was concluded that longevity of seeds depends on the ambient temperature and relative humidity at the stockists stores.

Key words: Longevity, seed storage, temperature, relative humidity, Mwitemania –GLP 92.

Introduction

Seed quality deterioration often occurs due to conditions under which the seed is stored. Variation in temperature and relative humidity during storage may affect the seed vigour and viability during germination. In the humid tropical countries, the high relative humidity of the storage environment leads to an increase in moisture content which in combination with high ambient temperatures; result in rapid deterioration leading to a decline in seed vigor and ultimate loss of viability. Poor storage conditions have been reported to cause 10% loss in seed quality in the tropics (Genchev, 1997). Low seed moisture content and storage temperature slows down the rate of aging (Coolbear, 1995). Seed aging processes lead to deterioration in seed quality and aged seed show decreased vigour leading to weak seedlings which cannot withstand the rigours of weather when introduced to field conditions (Rokich *et al.,* 2000). Ellis and Roberts (1987) reported that the activities of storage fungi are influenced by the relative humidity of interseed atmosphere than by the moisture of the seeds themselves.

In Kenya, the Seed and Plant Varieties Act, Cap 326, stipulates the validity period of horticultural seeds to be six months and maize and beans to be one year. However, this does not take into account the diverse conditions under which the seeds are stored.

Improved seed is required to go through a series of seed handling processes before reaching the growers, including seed processing, distribution, and marketing. These processes require a certain level of seed standards. However, loss of viability may occur during transportation and storage due to the combined effects of temperature and relative humidity (McCormack, 2004). Seed deterioration is viewed both as biochemical and bio-physical process that accumulate over time, eventually resulting in the loss of germination (Priestly, 1986) and Smith and Berjak (1995) suggested that deterioration may result from changes in chromosome aberrations and damage to DNA, changes in RNA and protein synthesis, changes in enzyme and reserve substances, loss in respiratory activity and ATP production, membrane changes and eventual cell destruction.

Once seed standards are specified, seed quality must conform to these standards. Usually, under the harsh conditions in SSA, expensive hybrid seed should be stored in air-conditioned facilities (Cromwell *et al.*, 1993). However, costs are prohibitive to store large volumes of seed in air conditioned structures. For storing certified and lower grade seed, simpler principles should be followed. Seeds should be packaged and stored in well-ventilated, low humidity facilities. These parameters, if properly met, are adequate for short-term storage. Currently, very few countries of SSA have adequate storage facilities (FAO, 1994).

Bean seeds are categorized as orthodox seed, implying that the seeds can be dried to low moisture content ranging from 2-5% and over a wide range of storage environments without loss in viability. The viability of orthodox seed conforms to some general rules, that for each 1% decrease in seed moisture content the storage life of the seed is doubled and for each 5.6˚C decrease in seed storage temperature the storage life of a seed is doubled (Black *et al*., 1987). A simple method for calculating the combined effects of temperature and relative humidity on longevity of seed was found. The method is as follows: the sum of storage temperature in degrees Fahrenheit (F), plus the relative humidity (in percent) should not exceed 100. Since seed moisture is the most important concern, the rule stipulates that not more than half the sum should be contributed by the temperature (Harrington, 1972). Roberts (1973) further stated that viability of seeds in storage increases with decrease in seed storage moisture and temperature. When the moisture content is high (>30%), non-dormant seeds may germinate, and from 18-30% moisture content rapid deterioration by micro-organisms can occur (Ellis *et al.*, 1990).

Seeds stored at moisture content > 18-20% will respire, and in poor ventilation the generated heat will kill them. Below 8-9% moisture content there is little or no insect activity, and below 4-5% moisture content seeds are immune from insect attack and storage fungi (Black *et al.,* 1987).

Fluctuating storage conditions affect the seeds differently depending on the type of seed (Priestly, 1986). Onion seeds stored under conditions of alternating high and low relative humidity lose viability proportionately to the length of time the seeds are subjected to high relative humidity. Thus, fluctuating storage conditions may be harmful to the bean seeds and may determine the quality of seeds during the storage period. Wein and Kueneman (1981) reported that the viability of some lines of soybean seeds stored under ambient conditions of 27˚C to 32˚C and 80% relative humidity was reduced by almost 50% in 8 months. Bosco and Silveira (1980) studied the suitability of ambient conditions of cowpea seed and found that 27.2˚C and 61.7% relative humidity and 23.9˚C and 81.2% relative humidity were favorable conditions for open storage for a period up to 8 months. After 12 months, germination and vigor dropped rapidly. At 26.7˚C and 83.35% relative humidity, germination and vigor were significantly lower after only 4 months in storage. AOSA (1981) gave general rules about storage conditions of maize that, for every 5˚C increase in the storage temperature over the range of 0˚C to 50˚C, reduces the life of the seed by half.

Materials and Methods

The study was carried out in five towns within Kenya. The towns (Bungoma, Nyeri, Nairobi, and Mombasa) were selected because of their variations in temperature and relative humidity.

The seeds were stored by three stockist in each of the towns. The stockists according to the seed companies who provided the seeds met the minimum requirements by Kenya Plant Health Inspectorate Service (KEPHIS) to stock the seed. The seeds were packaged in half-kilogram clear polythene bags. Each stockist in Bungoma, Nyeri, Nairobi and Mombasa received twelve samples of seed for storage. One sample was collected from the stockists every month and carried to the seed science laboratory at Chepkoilel Campus for analysis. A cold box with a temperature and relative humidity of minus 5ºC to 5ºC and 50-55%, which minimizes deterioration, was used to ferry the samples. The samples were placed immediately into the chest freezers maintained at minus 20ºC and 50% relative humidity to await viability and vigour tests. The tests were carried out as per ISTA (2003) rules. Seeds were equilibrated for 24 hours at room temperature (20-27 ºC) before the tests were conducted to avoid imbibition injury. Seeds in controlled conditions were stored in a chest freezer maintained at -20ºC and 50% relative humidity at the Seed, Crop and Horticultural Science laboratory in Chepkoilel campus.

Storage sites

(a) Bungoma

Bungoma lies on latitude 0º 25.3'N and longitude 35º 04’E of Greenwich Meridian. The average annual temperatures vary between 21ºC to 25ºC (G.O.K, 1997).

The three major stockists in the town who stored the seeds were; Nomadic Vet. Services Bungoma Chemist and Henrose Farmers Center. All the stockists had their stores built with concrete walls, cemented floors and fitted with ceiling material that screens heat from sun. The experimental seeds samples were subjected to open/shelf storage conditions like other commercial seed sold by the stockist. Daily temperature and relative humidity readings were recorded from which mean maximum temperature and relative humidity was calculated for the twelve months of storage (Table 1 and2).

Table1: Mean maximum temperature (˚C) of seed stockists stores at Bungoma

	Storage period in months												
Stock	1	2	3	4	5	6	7	8	9	10	11	12	**Mean**
Henrose	27.6	27.7	27.6	27.3	27.1	25.1	27.2	28.1	27.6	28	27.0	27.2	**27.3**
Bungma Chemst	27.2	27.8	26.8	26.5	27.1	25	26.9	27.5	27.2	28.1	27.4	26.9	**27.1**
Nomadic	27.7	27.5	27.1	27.2	26.8	26	27.1	27.8	26.5	27.8	27.3	27.0	**27.2**

Table 2: Mean maximum relative humidity (%) of seed stockists stores at Bungoma

	Storage period in months												
Stockist	1	2	3	4	5	6	7	8	9	10	11	12	**Mean**
Henrose	65.4	66.5	59.4	69.1	71.2	69.1	67.2	66.5	66.6	68.5	66.0	66.5	**66.8**
Bungoma Chemist	66.0	66.5	60.1	68.8	70.3	70.2	70.0	69.0	66.5	65.9	67.5	66.8	**67.2**
Nomadic	67.3	65.8	63.4	66.7	70.0	71.4	68.7	67.7	66.4	66.2	64.8	66.7	**67.1**

(b) Nyeri

Nyeri lies between the equator and latitude 0º38S and longitude 30º and 38ºE. The area has an average temperature of 11-23ºC (G.O.K, 1997).

Three stockists namely Sagada Agrovet, Kahigaini stores and Rupshi Meghji who are major seed sellers in town according to sales representative, Kenya Seed Company, stored the seeds. These stockists meet the minimum requirements by KEPHIS that the stores should have concrete walls and cemented floors beside a water proof roof. The storage of experimental seed was similar to that of commercial seeds meant for sale. The seeds were placed on wooden racks and on open shelves.

Temperature and relative humidity readings of the stockist stores were recorded daily by the shop keepers and mean temperature and relative humidity calculated (Table 3 and 4).

Table3: Mean maximum temperature (˚C) of seed stockists stores at Nyeri

	Storage period in months												
Stockist	1	2	3	4	5	6	7	8	9	10	11	12	**Mean**
Rupshi Meghji	20.1	21.3	20.0	21.4	21.6	19.2	18.2	17.3	18.1	16.7	19.3	21.6	**19.6**
Kahigaini	19.4	20.1	20.2	19.4	20.3	19.7	18.4	17.9	19.2	17.6	19.2	20.5	**19.2**
Sagada	20.0	19.3	20.1	18.7	22.1	17.5	19.1	16.6	17.6	16.8	20.1	19.4	**18.9**

Table 4: Mean maximum relative humidity (%) of seed stockists stores at Nyeri

	Storage period in months												
Stockist	1	2	3	4	5	6	7	8	9	10	11	12	**Mean**
Rupshi meghji	66.1	68.3	70.1	67.0	67.0	65.4	66.4	67.5	68.7	65.6	66.7	65.5	**67.1**
Kahigaini	67.0	67.7	71.0	70.7	66.4	65.7	69.0	67.3	70.0	68.9	66.5	64.9	**67.9**
Sagada	69.0	68.2	69.4	67.9	67.0	64.8	69.3	68.0	70.1	67.8	65.9	65.3	**67.8**

(c) Nairobi

Nairobi lies between an altitude of 2º38'S and longitude 30ºE. The average daily temperature range between 13- 27ºC (G.O.K, 1997).

Jumbo Agrovet, DIP Chem on Haile Sillasie avenue and Kiambu Fertilizer at the Wakulima market stored the seeds. These stockists have stores build with conrete walls, cemented floors and are provided with ventilation fans on the walls just below the ceiling. Experimental seed samples were placed on the shelves and others on wooden racks together with other seeds sold by the stockist. Temperature and relative humidity readings of the storage area were recorded on daily basis by shop attendants and calculations for mean temperature and relative humidity made (Table 5 and6).

Table 5: Mean maximum temperature (˚C) of seed stockists stores at Nairobi

	Storage period in months												
Stockist	1	2	3	4	5	6	7	8	9	10	11	12	**Mean**
DipChem	23.7	24	24.2	22.4	21.3	21.2	21.0	21.1	21.7	23.4	23.0	23.2	**22.5**
Kiambu fert.	22.8	23.7	23.8	22.7	21.0	21.4	21.7	21.1	20.7	22.7	22.7	23.0	**22.3**
Jumbo agrovet	24.0	23.2	22.9	21.8	20.5	20.7	21.5	22.1	21.2	23.5	23.2	22.8	**22.3**

Table 6: Mean maximum relative humidity (%) of seed stockists stores at Nairobi

	Storage period in months												
Stockist	1	2	3	4	5	6	7	8	9	10	11	12	**Mean**
Dipchem	77.6	71.7	72.0	68.7	69.1	73.3	69.9	70.1	70.0	70.1	72.4	69.8	**71.2**
Kiambu fert	80.1	79.8	68.5	69.3	70.4	71.4	72.1	67.9	70.5	70.4	68.8	72.3	**71.8**
Jumbo agrovet	71.5	72.4	70.4	67.6	71.1	68.6	69.8	70.9	70.7	72.0	72.9	74.2	**71.0**

(d) Mombasa

Mombasa lies between latitude 3º80' and 4º10'S of the equator and longitude 39º60' and 39º80'E of the Greenwich meridian. The average temperature range is 22.7ºC and 33.1ºC (G.O.K, 1997).

Kenya Farmers Association at the Mwembe Tayari market, Antipest Kenya Ltd at Jela Barridi in Mvita and Farm Plus at the Kongowea market stored the seeds in the town. Mwembe Tayari and Jela Baridi are within the city centre and the two stockists are about one kilometer apart. Kongowea market is around three kilometers from the city centre and Farm Plus chose this area strategically to benefit from farmers coming to sell their produce. The stores of the three stockists were built with permanent materials (concrete walls, cemented floors, iron sheet roofing lined on the inside with ceiling material). They were also fitted with ventilation fans that were switched on to run throughout the storage period. Experimental seed samples were subjected to open shelf storage conditions by placing them on the wooden racks and on the shelves similar to other seeds meant for sale by the stockists.

Temperature and relative humidity readings of the stockists stores were recorded daily by the shop attendants using dry and wet bulb thermometers. From these readings, mean maximum temperature and relative humidity were calculated (Table 7 and 8).

Table7: Mean maximum temperature (˚C) of seed stockists stores at Mombasa

	Storage period in months												
Stockist	1	2	3	4	5	6	7	8	9	10	11	12	**Mean**
Antipest	30.9	32.3	33.2	31.5	32.5	30.0	29.0	28.7	28.6	28.6	29.3	29.5	**30.3**
KFA	29.3	30.5	32.7	30.6	29.8	30.1	28.9	29.5	28.4	28.4	29.4	29.6	**29.8**
Farmplus	30.3	31.7	33.4	31.7	32.4	31.6	29.0	30.0	29.1	29.7	30.6	30.2	**30.8**

Table 8: Mean maximum relative humidity (%) of seed stockists stores at Mombasa

	Storage period in months												
Stockist	1	2	3	4	5	6	7	8	9	10	11	12	**Mean**
Antipest	78.5	79.2	81.4	80.2	80.5	82.1	80.6	79.4	78.7	80.5	80.4	79.9	**80.1**
KFA	80.1	80.6	81.3	78.6	79.6	81.5	80.8	81.4	76.4	81.1	78.6	80.2	**80.0**
Farmplus	79.6	77.8	76.9	81.2	80.2	81.6	79.2	81.1	80.6	78.6	78.6	79.5	**79.6**

Germination test

Seed germination test was carried out according to ISTA (2003) rules. Seeds were first equilibrated at room temperature (22-25 ºC) for 24 hours, and then each package opened and four (400) seeds were counted and divided into replicates of 100 seeds each. The 100 seeds could not be adequately spaced in the germination trays; therefore the seeds were further sub-divided into 50 seed, so that two trays make up one replicate, and then germinated on moist sand substrate after covering with a thin layer of sand. They were then incubated in a growth chamber at 23ºC and 70% relative humidity for seven days when evaluation was carried out (ISTA, 2003). Each seedling was evaluated in accordance with ISTA rules for germination. Normal, abnormal and dead seedlings count was made. The number of normal seedlings was assessed and used to calculate the percentage germination of the sample.

Electrical conductivity test

Seeds were equilibrated from cold storage for 24 hours at room temperature. Four replicates of twenty (20) seeds each sample were placed in plastic jars with 125ml-distilled water then covered with aluminum foil. They were then incubated for 24 hours at room temperature (20-27 ºC). After stirring for 15-20 seconds electrical conductivity of the leachates was measured using an electrical conductivity meter type Fielblab- Lf and LF 513T-electrode dip-type cell (Schott Gerate Glass Company, Mainz, Germany). Results were expressed in µS/cm (ISTA, 1995). A control container with distilled water was set up for each replicate and the readings from this as used as a baseline in calculating the electrical measurements of the soaked seeds. Between measurements, the dip cell was rinsed in distilled water and dried using tissue paper. The mean readings in µS/cm were obtained after subtracting measurements of the control. High values indicate low vigour and vice versa.

Results

Viability as tested by germination percentage and electrical conductivity tests

Quality of bean seeds declined gradually in the stockists stores in Bungoma, Nyeri and Nairobi compared to the rapid decline at the stockists stores in Mombasa. Seed quality decline in controlled conditions, did not drop below the minimum accepted level of 80% germination and the electrical conductivity was within the critical level of 6.0 μS $cm^{-1}g^{-1}$ for the twelve months of storage (Fig.1). Germination percentage of seeds in Mombasa declined below the accepted level of 80% after storage for two months and to zero after nine months while the electrical conductivity shot above the critical level after two months (Fig1). Deterioration in quality of bean seeds stored by stockists in all the sites was significantly higher compared to deterioration in controlled conditions. When a comparison of seed quality was made between seeds stored by stockists in each town, the results showed that there was no significant difference in viability loss between the seeds stored in Mombasa, however their viability differed significantly from the seeds in controlled conditions (Table 9). In Bungoma, seeds stored at Nomadic had a mean germination percentage of 75% and Bungoma Chemist stores had 75% and there was no significant difference in seed quality loss between these two stockists. However seeds at Henrose had a lower mean germination of 73%, which differed significantly from the other two stockists (Table 9). Seeds stored by Rupshi Meghji and Kahigaini stores in Nyeri had a lower mean germination percentage of 75% and 76% respectively compared with Sagada (78%) (Table 9). Mean germination percentage of seeds stored at Dip chem. (75%) and Jumbo Agrovet stores (75%) in Nairobi had their viability not significantly different as tested by LSD at (P<0.05), however the quality of seeds stored by Kiambu fertilizer (77%) were significantly higher from the seeds stored by the two stockists (P<0.05) for the twelve months of storage.

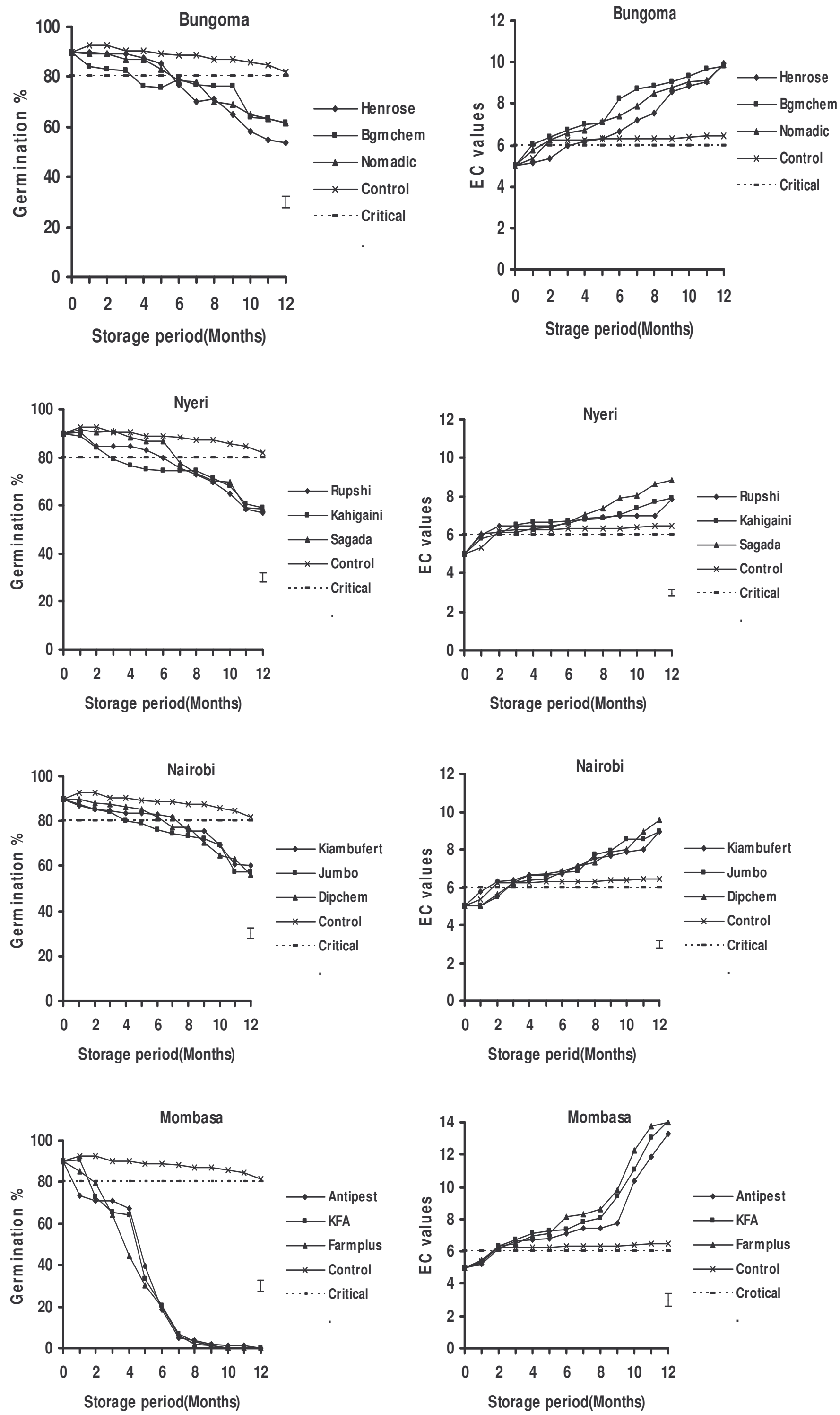

Fig.1: Percentage germination and electrical conductivity of seed stored over time (Jan, 2004-Dec, 2004) by three stockists in Bungoma, Nyeri, Nairobi and Mombasa towns. The bars represent LSD P<0.05 for comparison of stockist × time of storage.

Table 9: Mean percent germination and electrical conductivity of seed stored for twelve months by stockists in Bungoma, Nyeri, Nairobi and Mombasa towns. Values followed by the same letter in a column do not differ significantly as measured by LSD at *P*<0.05.

Bungoma			Nyeri			Nairobi			Mombasa		
Stock	Germ %	EC value	Stock	Germ %	EC value	Stock	Germ %	EC value	Stock	Germ %	EC value
Henr	73a	6.25a	Rupshi	75a	6.25a	Dipchem	75a	6.25a	Farmplus	27a	6.25a
Bgm chem	75b	7.22b	Kahigaini	76a	6.75b	Jumbo	75a	7.08b	Antipest	28a	8.07b
Nomadic	75b	7.75c	sagada	78b	6.84b	Kiabufert	77b	7.13b	KFA	29a	8.63c
Control	88c	8.08d	Control	88c	7.24c	Control	88c	7.16b	Control	88b	8.94d
LSD	2	0.25	LSD	1	0.37	LSD	2	0.37	LSD	6	0.21

Discussions

Germination percentage and vigour of common bean seeds significantly differed in stockist stores over time (Fig.1 and Table 9). Seeds stored at the stockist stores in Mombasa recorded the highest percent germination loss compared to the seeds in controlled conditions which recorded high viability throughout the storage period (Fig.1). Seed deterioration has been associated with a sequence of chromosome aberrations and damage to DNA, changes in RNA and protein synthesis, changes in enzyme and reserve substances, loss in respiratory activity and ATP production, membrane changes and eventually cell destruction which is displayed by failure of the seed to germinate (Smith and Berjak, 1995). These changes during deterioration are accelerated by high temperature and relative humidity and this explains why the seeds in controlled conditions had low viability loss compared to the seeds in the stockists stores. The ambient/stockist temperature and relative humidity fluctuation in storage affected the quality of seed during the storage period in Mombasa, Nyeri, Bungoma and Nairobi. Similar observation was made by Wein and Kueneman (1981) while studying viability of soybean seed under open storage. They reported that seed stored under ambient conditions of 27˚C to 32˚C and 80% relative humidity had its viability reduced by 50% in eight months. Electrical conductivity results indicated that there was significant increase in the amount of leachetes from the seed with increase in time of storage, temperature and relative humidity (Fig1) which led to seed ageing. Disruption of membranes because of ageing could lead to diverse metabolic changes, all of which contribute to seed deterioration and loss of viability. Loss in integrity of the plasma membrane leads to leakage of cytoplasmic components to the external medium. This implies that bean seeds are subjected to physiological changes that lead to deterioration. This deterioration is attributed to imbibition leakage which is not completely understood, but is likely that the selective permeable membranes of the plasmalemma that normally retains solutes within cells lose their integrity upon drying of mature seed (Halmer and Bewley, 1984). Mathews and Bradnock (1986) and Perry, (1981) also observed that loss in the integrity of plasma membrane has been demonstrated in deteriorated seed by the extent of leakage of cytoplasmic components to the external medium. These observations partly explains why bean seeds stored by stockists in Mombasa had low viability and vigour (Fig.1)

Seeds stored by stockists within the sites did not show any significant difference in viability and vigour loss. This was attributed to the small differences in mean maximum temperature and relative humidity variation between the stockists (Table 1, 2, 3, 4, 5, 6 and 7). This can be explained by the fact that if the seeds packaged in bags allow air movement, seed moisture is determined by the relative humidity of the air (Cabrera and Lansakara, 1995). Regardless of storage conditions, the moisture content of seed eventually comes into equilibrium with the moisture in the surrounding. Bean seeds were packaged in polythene bags which could not allow free movement of air into and/or out of the seed, hence moisture change could not result in significant difference in the loss of viability and vigour between the stockist stores within the sites hence temperature could have led to the differences in seed viability loss.

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