EFFECTS OF MATURITY STAGE, DESICCATION AND STORAGE PERIOD ON SEED QUALITY OF CLEOME (*Cleome gynandra* L.)

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ABSTRACT

Cleome gynandra (L.) is a weed that grows throughout the tropics and sub-tropics. In Kenya it is gradually being domesticated and grown as a vegetable that is used in many culinary systems for its remarkable nutritional and medicinal properties. However, the seed planted by farmers has a germination that is erratic and occurring over an extended period. The maturity stage and postharvest handling of Cleome seeds could be contributing to the poor quality of seeds planted by farmers. The aim of this study was to investigate the effects of seed maturity stage, desiccation and storage period on the quality of Cleome seed. To achieve the above objectives, Cleome was harvested at three developmental stages characterized by pod colour: yellow; yellow-green; green. The seeds were then dried above silica gel to 20%, 10%, 5% and 2% moisture contents. For each maturity stage and moisture content level, initial viability and vigour tests were conducted prior to storage. Viability and vigour tests were then conducted after three and six months of storage. Data was subjected to analysis of variance (ANOVA) using statistical package for social scientists (SPSS). The findings showed that Cleome seeds harvested from yellow pods maturity stage tolerated desiccation to 5% moisture content and recorded the highest seed quality after six months of storage.

Key words: *Cleome gynanadra*, maturity stage, desiccation, storage, seed quality

1.0 INTRODUCTION

Cleome gynandra (L.) (Plate 1) is a weed that grows throughout the tropics and sub-tropics[1]. It is an erect herb that grows up to 1.5 m tall[2]. 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[2]. In Kenya it is grown as a leafy vegetable and is also used to treat many diseases. It is used in many culinary systems for its remarkable nutritional and anti-oxidant properties[1]. The tender leaves, young shoots and occasionally flowers are eaten boiled as potherb, relish, stew or side dish. The leaves are utilized in fresh form or dried as powder. Since the leaves are bitter they are sometimes cooked with milk and/or with other leafy vegetables such as cowpea leaves,

amaranth and nightshades (*Solanum nigrum*). In some communities in Kenya, boiled Cleome leaves are traditionally given to lactating mothers[1]. The leaves are made into a concoction that treats diarrhea and anemia. An infusion of the roots is used as a medicine for chest pain[1]. The glands on the stems and leaves have insect repellent properties; cabbage and related crops intercropped with Cleome suffer less attack from diamond back moth larvae. Similarly, in French bean intercropped with Cleome, the beans are less infested with flower thrips and are therefore of better quality for export[1]. The seeds are used to feed birds. The seed contains edible polyunsaturated oil, which is extracted by simple pressing and does not need refining. The seed cake can be used as animal feed[1]. Existing evidence suggest that *Cleome gynandra* is endowed with higher level 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[3].
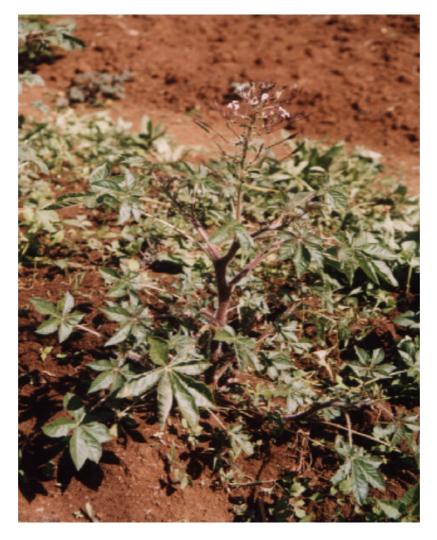

Plate 1: *Cleome gynandra* **plant photographed from one of the experimental plots at Moi University, Kenya.**

1.1 Maturity Stage

High quality seed lots may improve crop yield in two ways: first, because seedling emergence from the seedbed is rapid and uniform, leading to the production of vigorous plants, and second because percentage seedling emergence is high[4], so optimum plant population density could be achieved under a wide range of environmental condition[5]. These are the main reasons for farmers' interest in buying and cultivating high quality seed[4]. Seed maturation is one of the main components of seed quality and a prerequisite for successful germination and emergence. Therefore, a seed crop should be harvested when quality traits of seed are maximal[6]. Generally the seed quality parameters in any crop are associated with stage at which the seed crop is harvested[4]. If the seed crop is harvested in an early seed development stage, the seed quality parameters will be poor due to more number of immature and undeveloped seeds. While in delayed harvesting seed yield and quality are affected by the vagaries of weather in the field. Hence harvesting of the seed crop at physiological maturity is better as seeds will be having maximum dry weight, higher viability and vigor, besides higher seed yield[7]. During germplasm collection missions, collectors often encounter crops at different stages of maturity because of either genotypic differences in crop duration or differences in planting time[8]. From the storage point of view, although it is desirable to collect fully mature seeds, often immature seeds are also collected during collecting missions. Therefore, knowledge on seed maturity in relation to seed quality is important to gene bank managers, germplasm collectors and famers. However, little information of this type is available for *Cleome gynandra* (L).

Seed and pod morphology changes during maturation and development have attracted attention in a number of studies. For example, physiological changes can occur after seed maturity leading to development of hard seeds of certain crops[9].In *Millettia leucantha* (Vatke), seeds from immature stage are leaf green while mature seed is milk coffee in colour[10]. Rosecoco and Mwezi Moja bean cultivars change from green at immature stages to red purple in mature seeds at physiological maturity[11]. Surprisingly, few studies on seed morphology changes have been related precisely to seed maturity parameters during development, yet visual morphology features are indispensable field diagnostic indicators that would guide farmers and seed collectors particularly when linked to seed physiological age. In this study, pod colour changes of *Cleome gynandra* was used as indicator of seed maturity stages.

1.2 Desiccation

Desiccation tolerance is defined as the ability of the seed to withstand rapid desiccation after harvest and germinate upon subsequent rehydration (provided the seeds are not dormant) [12]. Three sequential phases of seed development are recognized[13, 14, 15]. Firstly, following fertilization, rapid cell division and differentiation of the embryo occurs. This can be referred as the histo-differentiation phase. This is followed by a phase of cell expansion marked by an initial increase in fresh weight, dry weight and decrease in moisture content and reserve materials (proteins, lipids and carbohydrates) accumulate. Cell division is fully arrested during this phase. The end of maturation phase is signaled by the cessation of dry weight increase at the point referred to as mass maturity[16] or physiological maturity[17]. This point coincides with the formation of an abscission layer between the parent plant and the vascular connection of the seed[18]. Consequently, the final stage is often referred to as the post abscission phase during which the seeds of many species undergo maturation drying, as moisture is rapidly lost to the atmosphere.

Seed moisture content is one of the most important factors affecting seed quality from the time seed mature in the field until they are planted[4]. Moisture determines how long mature seed will maintain high quality in storage. Seeds have high moisture content at early stages of development. During maturation

seed water content tend to decrease. The initial phase of dehydration is slow, and is accelerated from the time the seeds reach maximum dry weight; at that time, seeds possess 35% to 55% moisture content for orthodox monocot and dicot seeds, respectively[4]. This decrease in moisture content proceeds until hygroscopic equilibrium is attained[4]. From that point on, moisture content changes are associated with variations in relative humidity. However, seeds produced in fleshy fruits have a lower decrease in moisture content than seeds produced in dry fruits. In this study, Cleome seeds were dried to four moisture content levels and evaluation of viability and vigour was conducted for seeds from each moisture level.

1.3 Storage

Storage of seeds as *ex situ* germplasm is an essential step for the long-term conservation of plant genetic resources. Maintaining seed viability for longer period is very essential to preserve the genetic integrity in stored samples. Several factors, namely, temperature, nature of the seeds, seed moisture content, relative humidity, and initial seed quality influence the seed longevity during storage[19, 20]. There is a close relationship between the loss of seed viability during storage and the accumulation of genetic damage in the surviving seeds[21]. Seed moisture content, temperature, and storage periods are among the main factors affecting above relationship[22]. Depending on the duration and method adopted, drying and long-term storage may lead to considerable reduction in germination or to eventual death of the seeds. Before storage, if the seeds are not properly dried, the high moisture content may reduce the seed viability by promoting fungal growth. Such deterioration could further result in decline of seed germination capacity[23]. Proper storage conditions, however, may effectively retain substantial viability in seeds over a considerable storage period[24, 20].

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[25]. 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[26]. The best alternative to avoid the risks associated with storage is to avoid storing the seeds. However, there are times when seed growers and dealers carry over seed lots from one year to the next due to a weak market, to insure an adequate supply the following year, or for other reasons. Under such circumstances the question is how to manage the seeds to maintain their quality (viability and vigor) throughout the storage period[27]. In general, seeds maintain their quality under favorable storage conditions for longer periods of time than if stored under poor conditions. A question that is frequently asked is whether good storage conditions enhance the quality of the seed? The answer is no. However, the quality of seeds can be maintained and the rate of seed deterioration can be slowed down by good storage environment. Once seeds deteriorate, their physiological quality can not be restored because seed deterioration is inexorable and irreversible process, just like aging[25]. In this study, Cleome seeds dried at four moisture levels were stored at minus 20°C for three and six months and seeds evaluated for viability and vigour.

2.0 MATERIALS AND METHODS
2.1 Maturity Stage

Seeds of *Cleome gynandra* (L.) were grown on a randomized complete block design (RCBD) at Moi University farm, Kenya. To ensure that seeds were harvested at different maturity stages even on a single plant, individual flowers with anthers exposed were tagged using strings of different colours for each date of

tagging, which corresponded to the date of fertilization. Seeds were harvested at three developmental stages: yellow pods - 55 days after tagging (DAT); yellow-green pods - 45 DAT and green pods -15 DAT.

2.2 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°C for 17 hours[27]. The rest of the bulk pods and seed lots not used for desiccation were left overnight at room temperature until moisture contents results were calculated after 17 hours. After removing the seeds from the oven, seeds were allowed to cool for about 30 - 45 minutes inside a desiccator before their weights were taken and seed moisture content expressed on a fresh weight basis as:

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

2.3 Desiccation Experiment.

The protocol developed by DFSC and IPGRI in 1999 was followed with certain modifications to determine the seed desiccation tolerance at each of the three-development stages[28]. Seeds were dried in silica gel in a ratio of 1:5 and enclosed in 6 cm x 8 cm perforated nets to allow the easy separation of the small seeds from the silica during re-weighing. For each maturity stage, randomly selected seed samples were dried to four target moisture levels namely 20%, 10%, 5%, and 2%, using the method described in the DFSC/IPGRI protocol (1999):

$$\text{Weight of seed (g) at TMC} = \frac{(100\text{-IMC})}{(100\text{-TMC})} \times \text{initial seed weight (g)}$$

Where, TMC is the target moisture content and

IMC is the initial moisture content.

Periodically, seeds were removed from silica gel for re-weighing to monitor water loss and time taken to attain the various target moisture contents noted. To ensure consistent rapid drying of seeds, hydrated silica gel was re-activated once daily in all the containers at the same time. There was also periodic thorough mixing of seeds particularly during re-weighing and changing of silica gel. After achieving the four-desiccation levels (20%, 10%, 5%, and 2% moisture content), germination, electrical conductivity and actual moisture content tests were carried out[27].

2.4 Storage Period

Seeds dried above silica gel to four target moisture levels: 20%, 10%, 5% and 2% moisture content (from the desiccation experiment above) were sealed in aluminum foil and stored at minus 20°C for three and six months. For each treatment, 400 seeds were used for germination, 100 seeds for moisture content determination and 100 seeds for electrical conductivity test. After each storage period, seed samples were removed from the storage conditions and viability and vigour tests carried out[27].

2.5 Germination

Germination tests were carried out according to recommendations by International Seed Testing Association[27]. At the beginning of the study and subsequently after each sampling interval four replicates each of 100 seeds were used[27] for germination test. These seed replicates were allowed to imbibe on 1% agar-water at 25°C ($\pm$ 0.5) in a germination cabinet (LMS cooled incubators, Jencons-PLS,) with a 12-hour photoperiod daily. Sterilin petridishes (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.

2.6 Mean germination time

The speed at which seeds germinate is often evaluated as a measure of variations associated with differences in seed vigour[27]. Generally, seeds with high vigour germinate more rapidly compared to those with low vigour. In the current study, germination speed (vigour) has been evaluated on the basis of germination at first count and on daily basis for ten days. The test was held on the premise that, seeds high in vigour will produce more normal seedlings within the first four days of evaluating germination. This is because it is well established that low vigour seeds usually have slow and widely distributed germination rates compared to high vigour seeds[30, 27].

2.7 Electrical Conductivity

After each storage period, samples were drawn for germination and electrical conductivity test. 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, at ambient temperatures (25-30°C). A control bottle containing distilled water only was set up with each test run. All bottles were maintained in a room at ambient temperatures for 24 hours. 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 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 International Seed Testing Association[27].

1.8 Statistical Analysis

In this study, data sets were analysed using Scientific Package for Social Scientists (SPSS), where data was subjected to analysis of variance (ANOVA), and descriptive analysis. Levels of significance, means and standard deviations were obtained for various data sets. Separation of means was done by least significant differences.

RESULTS AND DISCUSSION

3.1. Maturity Stage

The results of this study showed that there was an increase in germination percentage as well as in vigour, as seeds developed to full maturity. Seeds from green pods had the least germination percentage, while seeds from yellow pods had the highest germination percentage. Seeds from yellow-green pods were intermediate (Table 1). Highest seed quality is attained at physiological maturity[31, 32, 12], which in this study could be the yellow pod maturity stage. Following fertilization, there is the histo-differentiation phase, followed by cell expansion phase and finally physiological maturity[14, 15, 16, 33]. From the findings of this study the green maturity stage was probably at the histo-differentiation stage, the yellow-green stage at cell expansion phase, while the yellow pod maturity stage was close to physiological maturity of Cleome seeds and hence gave higher seed quality than green and yellow-green pod maturity stages.

3.2 Desiccation

Percentage germination improved with decrease in moisture content for seeds from yellow-green and yellow pod maturity stages (Table 2). Green pods maturity stage recorded zero germination at 5% and 2% moisture contents. This implies that seeds at green pod maturity stage were immature and could not tolerate desiccation. Desiccation had no significant effects on mean germination time and electrical conductivity on seeds from yellow pod maturity stage. Yellow pod maturity stage took the least time to dry to various target moisture contents. This was due to the fact that the initial moisture content was low. In this study the initial germination results obtained were very low and could be attributed to primary dormancy factors. Freshly harvested seeds of Cleome exhibit dormancy[34]. Thus, given that Cleome seeds used in this study were freshly harvested and immediately processed for storage, there was a high possibility of primary dormancy being expressed, at least in the initial germination tests.

Orthodox seeds do not tolerate desiccation at all stages of their development[35].The development ability to tolerate desiccation to low moisture contents may occur at different developmental stages in different species[36]. When immature desiccation-intolerant embryos of bean (*Phaseolus vulgaris* L.) were desiccated, there was a general collapse of the membranes, which was not apparent when more mature, desiccation-tolerant embryos were dried under the same conditions[37]. In this study, zero germination was observed when the seeds from green pods were dried to 5% and 2% moisture contents, indicating desiccation tolerance had not been attained or young *Cleome gynandra* seeds could not survive rapid drying. This was in contrast to mustard seeds, which were partially germinable prior to the attainment of desiccation tolerance[38]. Tolerance of rapid desiccation usually seems to be delayed until most of the reserve materials have been laid down, close to maximum dry weight or physiological maturity[39]. The present study is in agreement with the above observation as seeds from yellow-green and yellow pods were tolerant to desiccation and viability increased with reduction in moisture content but seed from green pods was intolerant to desiccation as indicated by the zero germination.

Reduction in moisture content resulted in reduction in vigour. This could be attributed to rate at which drying was carried out (1:5; seed: silica gel). It was demonstrated that while gradual rates of water loss result in the germination of castor bean seeds as young as 25 days after pollination (DAP), rapid drying over silica gel is fatal to seeds younger than 55 DAP[40]. Imbibition injury can occur in seeds that have been over dried[41].

This study agrees with this observation especially for *Cleome gynandra* seed dried to 2% moisture content where low percent germination was recorded across all the maturity stages.

3.3 Storage Period

Seed quality was higher for seeds stored for six months as compared to those stored for three months (Table 3 and Table 4). Initial germination was low but increased after three and six months of storage (Table 5). The low initial germination could be attributed to relative dormancy exhibited by freshly harvested Cleome seeds, which is then released in storage. After-ripening dormancy loss in stored seed has been observed in *Amaranthus retroflexus*[42] and *Festuca idahoensis*[43]. Viability increased in storage possibly due to loss of dormancy.

Although viability increased in storage, there was gradual seed deterioration as indicated by mean germination time and electrical conductivity (Table 5). The germination percentage is an indicator of the ability of the seed to emerge from the soil to produce a plant in the field under normal conditions. Seed vigor is the capacity of seed to emerge from the soil and survive under potentially stressful field conditions and to grow rapidly under favorable conditions. The loss of a seed's ability to germinate is the last step (not the first step) in a long process of deterioration (gradual loss of viability)[4]. Decrease in seed vigor and other physiological changes happen before loss of germination. Therefore seed with acceptable germination can be low in vigor[4]. A viability test is limited in detecting quality differences among high germinating seed lots[44]. The results of seed storage are unlikely to adequately reflect the degree of seed deterioration that has taken place[32]. This has been reflected in this study by the high germination of 95%, yet *Cleome gynandra* seeds have deteriorated in storage as indicated by the electrical conductivity measurements (Table 5). Deteriorated seed lots have high electrolyte leakage and are classified as low vigour, while those with low leakage are considered high vigour[27].

Maturity Stage

Table 1: Effect of maturity stage on percent germination, mean germination time and electrical conductivity of *Cleome gynandra* seeds.

Maturity stage	Percent Germination	Mean germination time (days)	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)
Green pod	0.00a	0.00a	629.05a
Yellow-green pod	12.25b	2.06b	27.10b
Yellow pod	14.50c	2.04c	25.94c
LSD (0.05)	1.45	0.01	0.93

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.

The results shown in Table 1 indicate that there were significant ($P<0.05$) effects of maturity stages on percent germination, mean germination time and electrical conductivity of Cleome seeds.

Desiccation of Cleome seeds

TABLE 2. Effect of desiccation on percent germination, mean germination time and electrical conductivity of *Cleome gynandra* seeds harvested at different maturity stages.

Maturity stage(pod colour)	IMC	TMC	AMC.	Desiccation tolerance	Germination (%)	Mean germination time	Electrical conductivity
Green	**70.2**				1.50a	4.18a	420.77a
		20	12.80	10.00	1.25b	4.32b	536.65b
		10	10.00	10.75	0.75c	4.33c	543.21c
		5	4.70	30.25	---	---	629.05d
		2	2.30	33.42	---	---	658.48e
Mean					0.70	2.57	557.63
LSD $_{0.05}$					0.120*	0.001*	4.31*
Y/green	**41.3**				5.00a	2.02a	22.61a
		20	12.13	2.08	6.75b	2.04b	24.61b
		10	10.80	2.75	9.00c	2.05c	25.75c
		5	4.40	26.42	12.00d	2.06d	27.18d
		2	2.10	29.42	11.75d	2.14e	32.18e
Mean					8.90	2.062	26.261
LSD $_{0.05}$					1.25*	0.002*	0.83*

					Germination	Mean germination time	Electrical conductivity
Yellow	27.1				6.75a	2.00a	20.51a
		20	11.60	1.25	9.25b	2.01a	20.89a
		10	9.80	2.33	11.50c	2.01a	21.17a
		5	4.30	25.58	14.50d	2.02a	22.94a
		2	2.40	28.75	14.00e	2.04a	24.47a
Mean					11.20	2.016	21.99
LSD $_{0.05}$					0.35*	0.05Ns	4.12Ns

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. --- = seeds did not germinate, Ns = no significance according to LSD test.

Key: TMC = target moisture content, IMC = initial moisture content, AMC = actual moisture content

Percentage germination improved with decrease in moisture content for seeds from yellow-green and yellow pod maturity stages (Table 2). Green pods maturity stage recorded zero germination at 5% and 2% moisture contents.

Storage of Cleome seeds: Three months storage of desiccated Cleome seeds

TABLE 3. Effect of seed storage moisture content on percent germination, mean germination time and electrical conductivity of yellow pod *Cleome gynandra* seeds stored at minus 20°C for 3 months.

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

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.

After three months of storage, results of Table 3 indicate that reduction in seed moisture content had significant ($P \leq 0.05$) effects on percentage germination and vigour. In general, viability and vigour improved with decrease in moisture content up to 5%, but further seed moisture content reduction to 2% resulted to decrease in seed quality.

Six months storage of desiccated Cleome seeds

TABLE 4. Effect of moisture content on percent germination, mean germination time and electrical conductivity of yellow pod *Cleome gynandra* seeds stored for six months at minus 20°C.

Moisture content	Germination (%)	Mean germination time (days)	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)
20	76.5a	2.26a	33.41a
10	78.8a	2.23b	31.99b
5	94.5b	2.21c	29.27c
2	55.5c	2.38d	35.67d
LSD $_{(0.05)}$	11.7*	0.01*	1.33*

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.

After six months of storage, reduction in moisture content had a significant ($P \leq 0.05$) effect on percent germination, mean germination time and electrical conductivity of Cleome seeds. Results of Table 4 show a general trend of seed quality improvement with moisture content reduction up to 5%.

Storage Period

Table 5. Effect of storage period on percent germination, mean germination time and electrical conductivity of yellow pod *Cleome gynandra* seeds dried to 5% moisture content.

Storage period	Germination (%)	Mean germination time (days)	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)
0 months	14.5a	2.04a	25.94a
3 months	77.5b	2.10b	27.36b
6 months	94.5c	2.21c	35.25c
Significance	*	*	*
LSD $_{0.05}$	7.3	0.04	1.12

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.

Storage period caused positive significant effects ($P \leq 0.05$) on viability of Cleome seeds. Initial germination was as low but increased after three and six months of storage (Table 5). There were negative significant effects ($P \leq 0.05$) caused by storage period on vigour of Cleome seeds as indicated by high values of mean germination time and electrical conductivity.

4.0 CONCLUSIONS

The experiments carried out in this study have depicted Cleome seed as orthodox since viability increased with decrease in moisture content. On the basis of this study Cleome seeds should be dried to moisture content between 5% and 2%. The decrease in percent germination from 5% moisture content to 2% moisture content indicated that the critical moisture content for Cleome seeds could be between 5% and 3%.

Based on the results of this study, it may be concluded that, to achieve high seed quality, Cleome seed should be harvested at yellow pod maturity stage, dried to 5% moisture content and stored for six months.

5.0 ACKNOWLEDGEMENTS

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