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EFFECT OF COLD AND DRY STORAGE ON SEED VIABILITY AMONG THREE PROVENANCES OF *ACACIA TORTILIS* SUBSPECIES *RADDIANA* AND SUBSPECIES *SPIROCARPA*

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ABSTRACT
The aim of the study was to evaluate the inter-specific variation and effect of time on seed viability and moisture content among three provenances of *Acacia tortilis* and two subspecies (raddiana and spirocarpa) under cold and dry storage conditions. Seeds of the two subspecies, which were collected from three provenances were subjected to cold and dry storage conditions for 18 months. Seed viability and moisture content were tested every 6 month interval. For germination the seeds were treated with electric burner (hot wire) and compared to control untreated seed. Results revealed high significant differences between treatment and subspecies, whereas no significant differences were exist between the provenances. The differences was increased with increasing storage time. Seeds across provenances and subspecies retained high viability under both storage conditions. Dry storage increased seed germinability with increasing storage time in both subspecies of the three provenances. The results revealed that cold and dry storage conditions are suitable for *Acacia tortilis* seeds preservation in the three provenances. But the cold store excelled the dry storage since the seed did not break dormancy with increasing time.

KEYWORD: *Acacia tortilis*, subspecies, raddiana, spirocarpa, cold storage, dry storage, viability, longevity.

INTRODUCTION

Seed storage is very important to secure good quality seeds for planting programs whenever needed. Seed longevity (the period seeds will remain viable in store) depend on genetic and physiological factors as well as storage conditions (Schmidt, 2000). The most important factors that influence storage are temperature, moisture, seed characteristics, micro-organism geographical location and storage structure (Govender, *et al*., 2007). It is necessary to improve methods that increase potential seed longevity in storage (Coronado, *et al*., 2007). Temperature and moisture content are particularly most important factors influencing the longevity of seeds and affecting the rate of deterioration (Spano *et al*., 2007). In particular, high temperature and moisture content reduced seed longevity, and therefore the ageing of seeds is closely tied to storage conditions The ageing process is characterised by many physiological and biochemical changes and natural ageing in seeds is accompanied by the loss of the ability to germinate and by a reduction in viability (Ellis, 1991; Spano *et al*., 2007).

Seed viability can be extended by cold or dry storage at seed moisture content below 5% (Huang *et al*., 2003). Dormancy plays a majors role in regulating germination in dry forest species. Dry environment is evidently characterised by the large variation but unpredictable seasonal changes in moisture and temperatures (Teketay, *et al*., 1997). An increase in situ storage resulted in reduces seed germination of both *Pachira equatica* and *Annona glabra* seeds because of viability loss or appearance of dormancy due to the toughness of seed coat (Baskin and Baskin, 1998; Mata and Casasola, 2005). For orthodox seed, in general, the combination of 3-7% moisture content and storage temperature below 0 $^{\circ}$C would permit long term seed preservation (Ruize *et al*., 1999; Vertucci, *et al*., 1999). Periodic testing of viability is curial to operation of seed banks because it permits the control of genetic erosion during the storage. Genetic erosion of material maintained in gene banks is a problem at the international level (Ruiz, *et al*., 1999; FAO, 1997). For this reason, the monitoring of the main factors causing genetic erosion in ex situ collection is strongly recommended to minimize the loss of genetic diversity. Periodic controls of viability are of vital importance within the routine operation of a seed banks, (Ruiz *et al*., 1999). More studies are needed on seed longevity, nature of seed dormancy, optimal temperature for germination and condition of storage of seeds so as to reduce the loss of viability (Hong *et al*., 1997). *A. tortilis* is widely spread a long seasonal valleys and streams in the desert and semi desert plains of northern and central Sudan (Alamin, 1990). Outside Sudan it is found from Senegal to Cameroon, as far as Somalia, Tropical Africa, North Africa, Middle east and Arabia (Arbonnier, 2004). The tree represents one of the major planting species in the arid zone areas of Sudan due to its multi-purpose uses. Therefore, a continuous supply of good quality seeds is highly demanded.

The objective of this study was to examine the effect of cold and dry storage conditions on seed viability of two subspecies of *Acacia tortilis* (*raddiana and spirocarp*) collected from three provenances across their natural range in Sudan.

MATERIALS AND METHODS

Seed sources

Three provenance across the natural range of the species distribution and where the association of the two subspecies occurs were selected for seeds collection. These sources are: White Nile, Kassala and River Nile States. The description of the three sites is shown in table (1).

TABLE 1. Seed sources of three provenances of *Acacia tortilis* subspecies raddiana and spirocarpa used in study

Provenances	Latitude	Longitude	Attitude (m)	Rainfall (mm)	Maximum (ºC)	Minimum (ºC)
White Nile	13º 30´N	32º 33´E	185	180	37.3	23.3
Kassala	15º 30´N	35º 58´E	458	318.6	37.9	21.7
River Nile	16º 20´N	32º 36´E	178	62.6	37.5	22

* Sources: Modified from Ministry of Sciences and Technology, Meteorological Authority, Khartoum Airport station and Heavens-above.com. 2008.

Seed storage

Cold storage

Subsamples of sound seeds for each subspecies and provenance were stored in cold store (12± 1 °C temperature) in an airtight containers for the 18 months.

Dry storage

Sound seed were stored in normal room temperature (25-30°C) in cotton sack of good aeration.

Germination

Seed germination test was carried out three times every six months in germination room (temperature; 28-32 °C, 24 hours light from the florescent lamps). A subsamples of four hundred seeds were drawn randomly for each subspecies and provenance from each store and divided into four equal replicates. Before germination one seed lot was pre-treated with electric burner to break the dormancy and to enhance the germination and the second lot was not treated as control. Seeds were then sown in germination trays filled with sand and kept moist by daily watering. Germination percentage was recorded every week for four weeks and the final cumulative germination percent was calculated as total germinated seeds divided by total number of sown seeds multiplied with 100.

Moisture content

Seed moisture content was determined on fresh weight basis following constant temperature oven-dry method as described in ISTA rules standards (ISTA, 1993). This was done for all experimental units immediately before germination test.

Seed germination behavior

Seed behavior was determined as germination of untreated seeds to predict the natural seed behavior in the field.

Data analysis

All the data were subjected to Analysis of Variance (ANOVA) and the means were separated by Duncan's multiple range test. Statistical Analysis System (SAS), version 6.12 (SAS institute) was used for data analysis.

RESULTS

Cold storage

The effects of treatments on *Acacia tortilis* subspecies raddiana and subspecies spirocarpa seed germinability under cold storage conditions is given in Table (2). The treatments increased seeds germinability and led to level of germination significantly higher than control through out storage time. However subspecies raddiana treated seeds from Kassala leading the other provenances. In untreated seeds, White Nile showed high seed germinability than others at 6, 12 and 18 months time.

For subspecies spirocarpa the treated seeds from White Nile displayed high germinability than others provenances, where Kassala and River Nile revealed similar germinability and higher than White Nile provenances at 12 month storage time. River Nile showed high seeds germinability than other provenances at 18 months Table (2).

Dry storage

The treatments effects led to level of germination percentage significantly higher than control (Table 3). For both treated and untreated seeds White Nile obtained higher germination (3).

For subspecies spirocarpa treated seeds, the three provenances displayed very high germinability in all storage time, but Kassala exhibited more than others at 6, 12 and 18 months. However for control River Nile exhibited more germinability at 6 and 12 months, where Kassala and White Nile at 18 months. The result showed that by increasing storage time the seed released part of its dormancy and increased seeds germinablity in dry storage in the three provenances..

Seeds behaviour of the two subspecies of the same provenance

Seed behaviour for various subspecies and provenances are shown in (Figs. 1,2,3 and 4). Seed behaviour reflects germination trend of untreated seed in their natural habitats. The results indicates for both species and provevnace resulted in higher untreated seed germination. The effect was increased with increasing storage period. Subspecies spirocarpa displayed more seed germinability than raddiana in untreated seeds.

Seed moisture content

The two subspecies in three provenances exhibited seeds moisture content percentage range from (3.48- 6.16) in cold storage, while in dry storage the two subspecies exhibited seeds moisture content range from (3.70-6.41) (Table 4) and (Table (5).

At the three provenances the subspecies spirocarpa exhibited more seed moisture content than raddiana, but the two subspecies did not reduced or drop their seed moisture content after 18 month of storage time in the two storage condition Table (6) and (7).

TABLE 2. The effect of the cold and dry store on the subspecies raddiana treated and untreated seeds germination % from the three provenances for 6, 12 and 18 months storage time

Provenance	Cold store						Dry store					
	Treated seeds			Control			Treated seeds			Control		
	6 month	12 month	18 month	6 month	12 month	18 month	6 month	12 month	12 month	6 month	12 month	18 month
White Nile	94.0^{a}	95.5^{a}	91.0^{b}	17.5^{a}	11.5^{b}	9.0^{a}	93.5^{a}	97.0^{a}	94.5^{a}	8.0^{a}	16.5^{a}	55.5^{a}
Kassala	96.0^{a}	96.5^{ab}	94.0^{a}	3.0^{b}	3.5^{c}	1.5^{b}	83.5^{b}	93.5^{a}	94.5^{a}	1.0^{b}	6.5^{a}	30.5^{b}
River Nile	91.0^{a}	90.0^{b}	85.0^{b}	6.0^{b}	17.0^{a}	7.7^{a}	88.5^{ab}	93.5^{a}	88.5^{a}	7.0^{a}	12.5^{a}	45.5^{a}

*Means with same letter in same column for same germination time for same store for same treatment are not significantly different at p=0.05 using Duncan New Multiple Range Test.

TABLE 3. The effect of the cold and dry store on the subspecies spirocarpa treated and untreated seeds germination % from the three provenances for 6, 12 and 18 months storage time

Provenance	Cold store						Dry store					
	Treated seeds			Control			Treated seeds			Control		
	6 month	12 month	18 month	6 month	12 month	18 month	6 month	12 month	18 month	6 month	12 month	18month
White Nile	93.0a	87.5^{a}	79.5^{b}	9.0^{b}	11.5^{a}	7.5^{b}	74.5^{b}	91.0^{ab}	88.0^{a}	14.5^{a}	51.0^{a}	61.5^{a}
Kassala	89.0^{a}	92.5^{a}	91.5^{a}	7.0^{b}	20.0^{a}	10.0^{b}	97.0^{a}	99.0^{a}	93.0^{a}	15.0^{a}	35.0^{ab}	51.0^{a}
River Nile	97.0^{a}	92.5^{a}	93.5^{a}	18.5^{a}	18.5^{a}	16.0^{a}	92.5^{a}	98.0^{a}	85.0^{a}	19.0^{a}	51.5^{a}	61.5^{a}

*Means with same letter in same column for same germination time for same store for same treatment are not significantly different at p=0.05 using Duncan New Multiple Range Test.

TABLE 4. *Acacia tortilis* subspecies raddiana and spirocarpa seeds moisture content % during storage time at cold store in three provenances

Subspecies	Raddiana			Spirocarpa		
Provenance	Mc 6 month	Mc 12 month	Mc18 month	Mc 6 month	Mc 12 month	18 month
White Nile	3.48^{a}	4.79^{a}	6.01^{a}	5.12^{a}	5.33^{a}	5.96^{a}
Kassala	4.44^{a}	4.5^{a}	4.85^{b}	4.61^{a}	4.94^{b}	6.16^{a}
River Nile	3.66^{a}	2.84^{a}	3.79^{c}	4.15^{a}	4.47^{c}	3.81^{a}

*Means with same letter in same column for the same subspecies for same storage time are not significantly different using at P=0.05 using Duncan New Multiple Range Test.

TABLE 5. *Acacia tortilis* subspecies raddiana and spirocarpa seeds moisture content % during storage test time at dry store in three provenances.

Suspecies	Raddiana			Spirocarpa		
Provenance	Mc 6 month	Mc 12 month	Mc 18 month	Mc 6 month	Mc 12 month	Mc 18 month
White Nile	3.48^a	4.89^a	5.82^a	5.12^a	5.39^a	5.48^a
Kassala	4.44^a	3.71^b	4.93^a	4.61^a	5.37^a	6.41^a
River Nile	3.56^a	3.70^b	4.41^a	4.15^a	5.35^a	5.66^a

*Means with same letter in same column for the same subspecies for same storage time are not significantly different using at P=0.05 using Duncan New Multiple Range Test.

TABLE 6. *Acacia tortilis* subspecies raddiana and spirocarpa seeds moisture content during storage time at cold store within the same provenance

Moisture test time	6 month		12 month		18 month	
Provenance	*raddiana*	*spirocarpa*	raddiana	*spirocarpa*	*raddiana*	*Spirocarpa*
White Nile	3.48^b	5.12^a	4.79^b	5.33^a	6.01^a	5.96^a
Kassala	4.44^a	4.61^a	4.5^a	4.94^a	4.85^a	6.16^a
River Nile	3.66^a	4.15^a	2.68^b	4.47^a	3.79^a	3.81^a

*Means with same letters for same provenance for same storage test time are not significantly different at P= 0.05 using Duncan Multiple Range Rest.

TABLE 7. *Acacia tortilis* subspecies raddiana and spirocarpa seeds moisture content during storage time at dry store within the same provenance

Moisture test time	Mc at 6moth		Mc at 12 month		Mc at 18 month	
Provenance	raddiana	spirocarpa	raddiana	spirocarpa	raddiana	Spirocarpa
White Nile	3.48^b	5.12^a	4.89^a	5.39^a	5.85^a	5.48^a
Kassala	4.44^a	4.61^a	3.71^a	5.37^a	4.93^b	6.41^a
River Nile	3.56^a	4.15^a	3.70^a	5.35^a	4.41^a	5.66^a

*Means with same letters for same provenance for same storage test time are not significantly different at P= 0.05 using Duncan New Multiple Range Test.

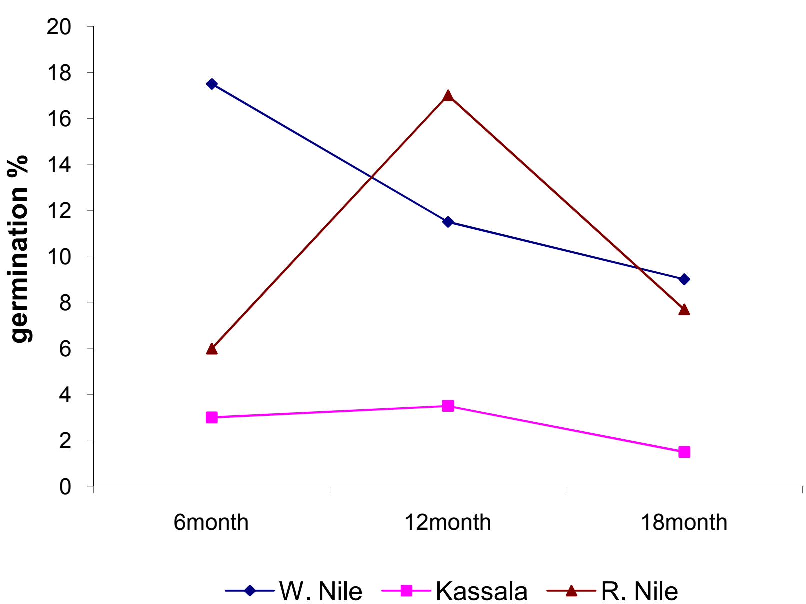

FIGURE 1: *A. tortilis* subspecies raddiana seed germination behaviour at cold storage.

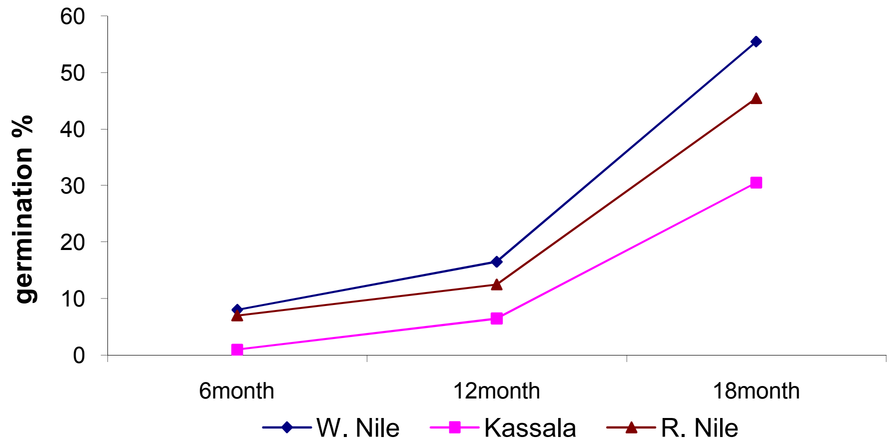

FIGURE 2: *A. tortilis* subspecies raddiana seed germination behaviour at dry storage.

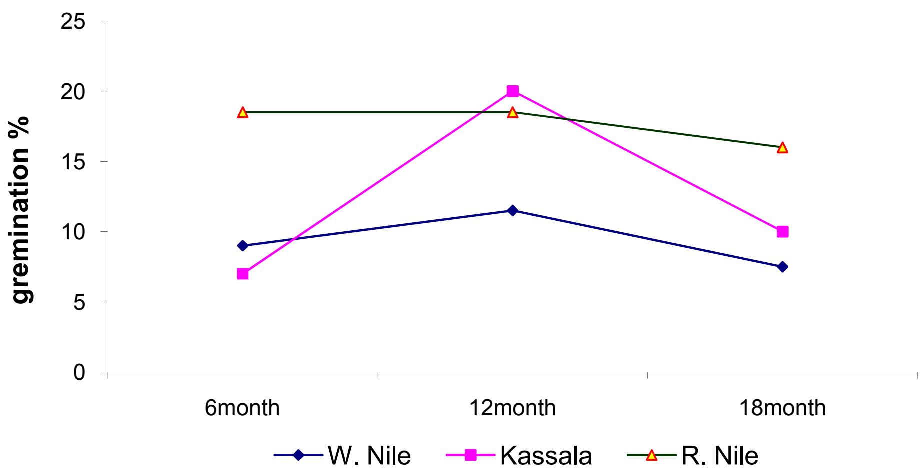

Figure 3: *A. tortilis* subspecies spirocarpa seed germination behaviour at cold storage.

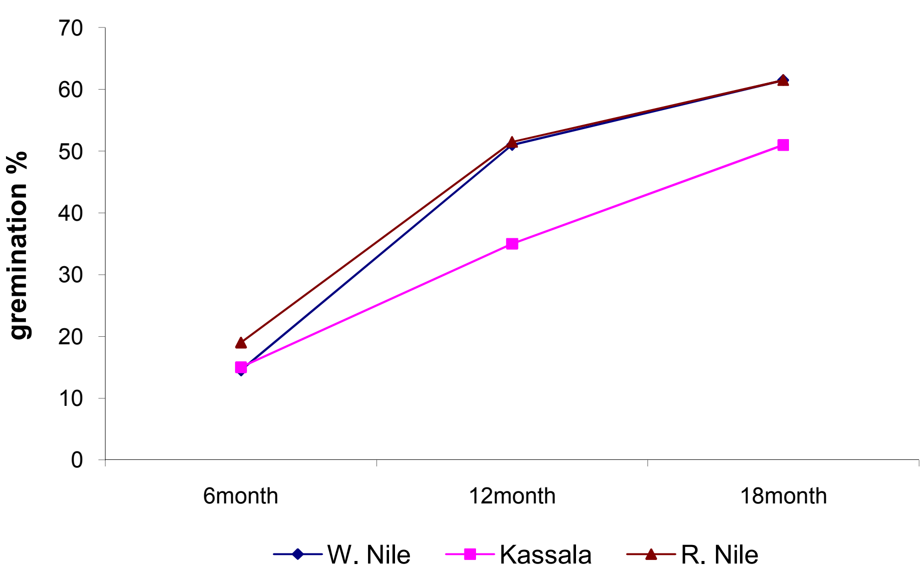

FIGURE 4. *A. tortilis* subspecies spirocarpa seeds germination behaviour at dry storage.

DISCUSSION

Periodic testing of seed viability is curial to operation of seed banks for the control of genetic erosion during storage. Lower seed moisture content and improved storage condition may maintain seed longevity and viability loss depends on moisture content and temperature (Ruiz *et al*., 1999; Roberts, 1981). The significant difference occurred between the two stores in seed viability at 12 and 18 months of storage time, may be due to the difference in storage conditions and variation in seeds moisture content. Temperature and moisture content are most important factors influencing the seeds longevity and ageing (Spano *et al*., 2006; Govender *et al*., 2007).

The similarity observed in this study between provenances in seeds viability under cold and dry storage might reflect adaptation to near similar environmental conditions. The same result was recorded by (Teketay, 1997) for seeds of *Juniperus*, *Allphylus* and *carissa* species in dry storage conditions. Seed viability was not declined throughout the storage period in both stores. This is consistent With (Teketay, 1997) who report that less pronounced decline of germinability in seeds of *A. tortilis* with time of dry storage. Also, in line with (Heatherly, *et al*., 1995), under cold store. Mechanical scarification of seed by electric burner improved germination over untreated seeds of the two subspecies in the three provenances. It is known that seeds of this species possess physical dormancy. The results can provide an insight into factors governing the regeneration ecology of the subspecies. Untreated seeds in cold storage showed low germinability compare to dry storage. This indicated that the low temperature induces seeds dormancy, thus seeds retained viability for long period. Evidence that cold storage reduces or prevents the effects of ageing was indicated by the slower rate of damage caused by low temperature (Spano *et al*., 2006). Germination of seeds in a particular situation and season is determined by interaction between the dormancy releasing factors such as temperature, light and moisture, (Teketay, 1997). The increase in germinability in dry storage over cold storage for untreated seeds could be due to an initial dormancy which is broken with a time of storage in dry conditions as the result of temperature rise. This indicates that dry storage is not optimum condition for long period storage due to temperature and moisture changes. In natural environment, summer high temperature break dormancy in many species while winter low temperatures induce dormancy (Baskin and Baskin, 1998; Qaderi *et al*., 2004). Gutterman, (1996) reported that the period of dry storage influence the start, speed and level of *Spergularia diandra* germination. The study results provide evidence that dormancy plays a major role in regulating germination in dry forest species. The dry environment is evidently characterised by the larger variation, but unpredictable seasonal changes in moisture and relatively small changes in temperatures. The effects of storage on seeds have been reported for many species like *Avena fatua*, *Bromus rubens*, *Cirsium vulgre*, (Qaderi *et al*., 2004). It has been reported that dry storage increased germination of stored over fresh seeds in many species including *Spergularia diandra*, *Pottulaca olercea*, *Lonicera* sp. *Prosopis juliflora*, (Gutterman, 2000; El.keblawy and Al-Rawai 2006). The variation that existed between the two subspecies at 12 and 18 months in seed viability, may be due to variations in seed moisture content at 12 and 18 test and seeds can have different ecological requirements for dormancy breakage and therefore may vary in response to the same season cues. The significant effects for time of seeds storage, temperature on final germination percentage of *Prosopis juliflora* within a species or population, indicated that seeds can have different requirements for dormancy breakage and therefore may vary in response to the same season cues (Baskin and Baskin, 1998; El.keblawy and Al-Rawai, 2006). Thus it is possible that seeds within population, as well as different population of given species, have evolved different germination requirement, based on their particular environmental and evolutionary history, (Evans *et al*., 1995).

As viability loss depends on moisture content and temperature (Robert, 1981; Ruiz *et al*., 1999), it seems that the two subspecies maintain their seeds viability and moisture content for reasonable storage time. *Acacia tortilis* seeds retained their viability for many years and present few storage problem by virtue of their hard seed coat which restricted moisture exchange and loss of stored reserves through respiration (Ruiz *et al*., 1999; Doran *et al*., 1983; Govender, *et al*., 2007). Similar strategies of seed germinations have been also found in other plant species occurring in deserts and arid zones (Huang and Gutterman, 1999; Gozlan *et al*., 1999).

In this study, results confirmed that *Acacia tortilis* seed would be stored safely at cold and dry storage and it maintained acceptable germination percentage even after storage under suboptimum conditions. The results provide evidence that dormancy plays a major role in regulating germination in *Acacia tortilis* and prevent seeds from dropped their viability during storage for reasonable period of time. The two subspecies in the cold and dry storage did not show seeds viability decline after 18 month. However, this is not a long period of storage time to give clue results, of this type of seeds which is characterised by hard seed coat dormancy which regulate the seed germinablity and prevents the seed from germination during unfavourable conditions. Consequently, studies about long-term viability are needed to determine the storability of seed material in seed gene bank. The results obtained from tests revealed that cold and dry storage conditions are suitable for *Acacia tortilis* seeds preservation in the three provenances. But the cold store excelled the dry storage since the seed did not break dormancy with increasing time.

CONCLUSION

The results revealed that *Acacia tortilis* under cold and dry storage conditions retained full seeds viability for 18 months of storage. The provenances responded similarly to storage conditions. The variation that appeared within the two subspecies of the same provenance, may be due to different degree of seed coat dormancy and genetic gain they have.

Dry storage conditions increased untreated seeds germinability by increasing storage time for both subspecies in the three provenances. By increasing storage time subspecies spirocarpa showed more seeds germinability than subspecies raddiana. The increase in seeds germinability could be due to dormancy which was broken after considerable time of dry storage.

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