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RESEARCH ARTICLE

Variability in seed longevity and viability of two *Acacia tortilis* subspecies under artificial burial soil storage from three provenances

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Abstract

The aim of this study was to examine the variability in seed longevity and viability of artificially buried seeds of *Acacia tortilis* subspecies raddiana and subspecies spirocarpa in soil for 18 months among and within three provenances. Burial technique was used to examine seed viability and longevity of two subspecies from three provenances in Sudan. The seeds were collected from three provenances, White Nile, Kassala and River Nile states. 2000 viable sound seeds of both subspecies of the three provenances were buried in soil at 5 cm depth in plastic container in open yard. Artificially buried seeds were followed by 6 months periodic exhumation and germination test during 18 months. The provenance did not show significant differences after 6, 12 and 18 month test time, but the subspecies showed significant differences ($P \leq 0.003$) at 6 month test. Both *Acacia tortilis* subspecies had persistent soil seed banks in the three provenances. The seeds can remain viable in the soil for two rainy seasons and reach its maximum germination after 12 month and viability started to decline with increasing burial time in soil. Both subspecies in the three provenances showed above 50% of decayed seeds from total seeds buried in soil during 18 month. The seed viability decreased with increased burial time in soil for the second year and most seeds lost its viability by 18 month burial time. Very little differences was observed between the rate of seed germination, viability and decayed seed between the *Acacia tortilis* subspecies.

INTRODUCTION

Acacia trees are key elements in African savannas, the distribution of these trees are governed by the variation in soil characteristics and rainfall (Bond and Midgley 2001). The life histories of plant species have been subjected to selective forces that determined whether a young plant of given species is at the right spot at the right time to develop into adult (Loth et al., 2005).

Seed longevity and persistence in soil seed banks can compensate for effects of unfavorable environmental conditions on seedling germination over the long term (Gutterman, 1994; Holmgren et al, 2006; Wijayratne and Pyke, 2012). Seed banks may be especially important for population persistence in ecosystems where opportunities for seedling establishment and disturbance are unpredictable (Baskin and Baskin, 2001; Fenner and Thompson, 2005).

The seed bank is the collection of viable seeds present on or within the soil and associated litter at any given time and represented the stock of regeneration potential (Simpson et al., 1989). Seed banks of most habitats contain both transient and persistent components, the transient component is composed of short- lived, non dormant seed that may not be viable at the onset of the second growing season, while current years seeds that remain viable at the onset of the second growing season, as well as viable seed dispersed in previous years, constitute the persistent component of the seed bank (Baskin and Baskin, 1998 and Araujo, C.G. and Cardoso, 2006). The size of the seed bank of a species is determined by the seed production, extent of seed rain, mortality of seeds in the soil and number of seed germinating (Roberts, 1981).

Buried seeds of many species can retain their viability and survive in soil for long periods (Shaukat and Siddiqui, 2004). Seed dormancy in the higher plants is a mean of avoiding unfavorable environmental conditions by arresting growth and development (Evans and Cobin, 1995).

The wide spread occurrence of between-years dormancy has led to the existence of seed banks- large populations of dormant viable seed in the soil of most of the world major ecosystems, (Thompson, 1992). Theoretical considerations assume that maternal plants can reduce the risk of reproductive failure of their seed crops by spreading germination over the time, seed dormancy and seed dispersal enable escape from unfavorable condition (Skordidis and Thanos, 1995). An understanding of the population dynamics of buried viable seeds, composition of the seed banks and spatial pattern of seeds of the species involved of great value in providing abases for management and control strategy (Shaukat and Siddiqui, 2004).

Soil seed banks play a crucial role in the dynamics of buried viable seeds of some practical importance not only in agriculture, but also in forestry and conservation, (Teketay and Granstrom, 1995). Seed longevity and persistence in soil seed banks may be especially important for population persistence in ecosystem where opportunities for seedling establishment and disturbance are unpredictable. Seed longevity is defined as seed viability after seed dry storage (storability) and therefore describes the total seed life span (Rajjou and Debeaujon, 2008).

There is no information regarding seed longevity of *Acacia tortilis* for both subspecies in semi-arid area in the Sudan. Therefore the aim of this study was to evaluate and determine the longevity and viability of seed and variation of seeds between and within *Acacia tortilis* subspecies sources.

Materials and Methods

Seed sources

The study deals with *Acacia tortilis* subspecies raddiana and subspecies spirocarpa, where the association of the two subspecies occurs in their natural habitats in three sites. The seeds for each subspecies were collected during April-May 2005 from + 30-40 trees, 100-200 m apart from each other. The collection was done from the three geographical locations, White Nile, Kassala and River Nile. The description of the seed sources is shown in Table 1.

Table (1). Seed sources of the three locations of *Acacia tortilis* subspecies raddiana and subspecies spirocarpa used in study.

Locations	Latitude	Longitude	Altitude (m)	Rainfall (mm)	Max (◦C)	Min (◦C)
White Nile	13˚ 30´N	32˚ 33´E	185	180	37.3	23.3
Kassala	15˚ 30´N	35˚ 58´E	458	318.6	37.9	21.7
River Nile	16˚ 20´N	32˚ 36´E	178	62.6	37.5	22

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

Experimental design

Two thousand viable sound seeds were drawn from the seed lot for each subspecies and locations and were divided into three replicates and each replicate was buried in plastic barrel containers filled with soil brought from the site where seeds were collected. Soil was sieved to remove soil seed bank. Seeds were buried at 5cm soil depth. The plastic containers were kept in the open yard of National Tree Seeds Centre at Soba, Sudan. Seed viability was assessed every six months by germination test in germination room. Two hundred seeds were taken from the three containers for each subspecies for each provenance and were divided into four replicates in a complete randomized design.

Seeds longevity assessment

The recovered seeds were washed by water to remove the soil, then the seeds were germinated in germination room under controlled environmental conditions (Temperature was 28-32 $^{\circ}$C, 12hours light from florescent lamps). Two hundred seeds were divided into four replicates of 50 seeds. Seeds were sown in germination trays filled with sand and were kept moist by daily watering. Germination was evaluated weekly for period of four weeks.

The germination % was calculated by dividing the number of germinated seeds by total the number of sample seeds × 100. Seed viability was assessed every six month for 18 months.

Decayed seeds (seed mortality)

After 18 month the seed viability was evaluated by counting the seed germinated by the rainfall in addition to the seeds germinated in germination room, the remaining buried seeds which were not found in soil were considered as decayed seed (loss seeds). Then the total % for each of seeds kind was recorded (room germinated, rainfall germinated and decayed).The decayed seeds % were calculated as follow:

$$\frac{\text{Total buried seeds} - (\text{seeds test for viability} + \text{rain germinated seeds}) \times 100}{\text{Total buried seeds}}$$

Analysis of the data

The data were subjected to analysis of variance (ANOVA) to confirm the differences between the locations and subspecies. Duncan's Multiple Range test was used to separate the means. Statistical Analysis System (SAS), version 6.12 (SAS institute) was used for analysis.

Results

Seed longevity

Seed longevity was evaluated as germination ability of seed every six month under artificial burial in soil.

NO significant differences were found among provenances in seed viability at 6, 12 and 18 months burial time, but the subspecies showed highly significant variation ($p \leq 0.003$) at 6 month burial time. However at 6 months period the subspecies raddiana provenances showed the highest germination %, while the River Nile revealed the lowest germination % Table (2). On the other hand for the subspecies spirocarpa the three provenances displayed highly germination %, River Nile and Kassala provenances displayed higher germination % than White Nile which displayed the lowest germination % Table (2). Therefore the two subspecies showed seed dormancy and maintained high seed viability, but very little differences was observed between rate of seed germination and seed viability among provenances and within subspecies Table (2). At12 month duration test, the three provenances obtained high germination % and seed viability that range from 67.5- 84.5 % more than 6 month test Table (2). Therefore this result suggested that the seeds released most of its dormancy and intended to germinate more seed than 6 month burial duration test.

Whereas at 18 month burial duration test, subspecies raddiana in the three provenances showed very low germination % compared to 6 and 12 months. However the River Nile showed better germination % than other provenances, therefore the seed viability was dropped to less than 29% Table (2). While within the two subspecies in same provenance subspecies pirocarpa indented to germinate more seed than subspecies raddiana at 6 months test time Table (3). Therefore at 12 months burial duration test the two subspecies achieved high seed viability except subspecies spirocarpa in White Nile obtained the lowest germination 43.5 % Table (3). While at 18months duration test the two subspecies showed very low seed viability% compared to 6 and 12 month burial time test, but subspecies raddiana showed better seed viability % than subspecies spirocarpa Table (3). Figure 1and 2 showed the relationship between seed viability, dormancy and longevity for both subspecies.

The result suggest that both *Acacia tortilis* subspecies in the three provenances have persistent soil seed banks and seed remain viable in soil for two years of one set of seed dispersal. Whereas the seeds viability was decreased with increased burial time after 12 month.

Table (2).Variation in seeds viability % of the two *Acacia tortilis* subspecies from the three provenances at 6, 12 and 18 burial duration test.

Germination time	raddiana			spirocarpa		
	White Nile	Kassala	River Nile	White Nile	Kassala	River Nile
6 month	66.5^{a}	68.0^{a}	39.5^{b}	66.5^{b}	80.5^{a}	81.5^{a}
12 month	84.0^{a}	78.0^{ab}	67.5^{b}	43.5^{b}	87.5^{a}	77.0^{a}
18 month	26.22^{a}	20.0^{a}	29.0^{a}	12.5^{b}	50.0^{a}	25.0^{b}

*Means with same letters in same row for the same germination time for same subspecies are not significantly different at p= 0.05 using Duncan New Multiple Range Test.

Table (3). Variation in seeds viability% of the two *Acacia tortilis* subspecies within the same provenance at 6, 12 and 18 month burial time**.**

Germination Time	White Nile		Kassala		River Nile	
	raddiana	spirocarpa	raddiana	spirocarpa	raddiana	spirocarpa
6 month	66.5^{a}	66.5^{a}	68.0^{a}	80.5^{a}	39.5^{b}	81.5^{a}
12 month	84.09^{a}	43.5^{b}	78.0^{a}	87.5^{a}	67.7^{a}	77.0^{a}
18 month	26.22^{a}	12.5^{b}	20.0^{b}	50.0^{a}	29.0^{a}	25.0^{a}

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

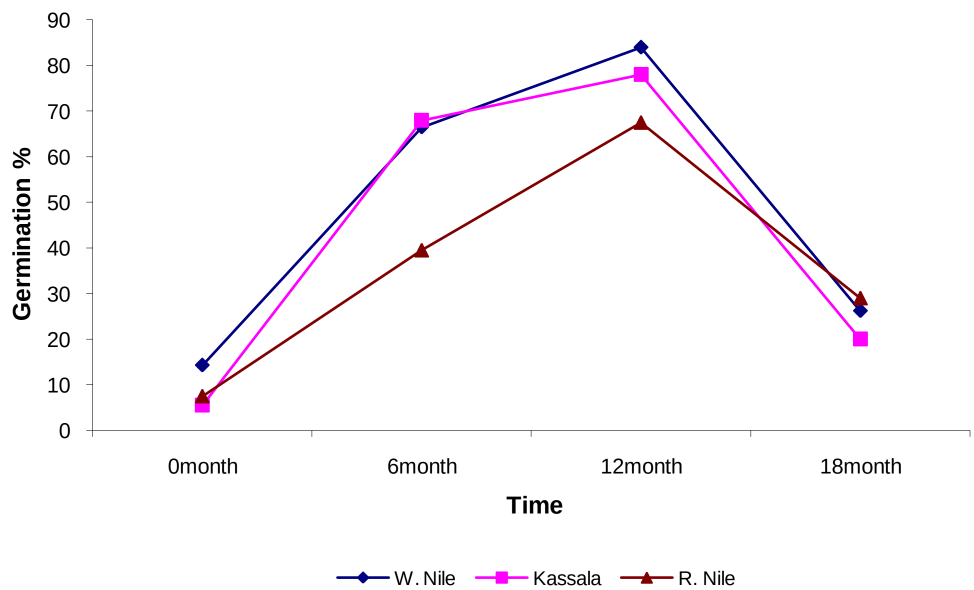

Fig. (1):*A. tortilis* subspecies raddiana buried seeds germination behavior and dormancy released through burial time in soil.

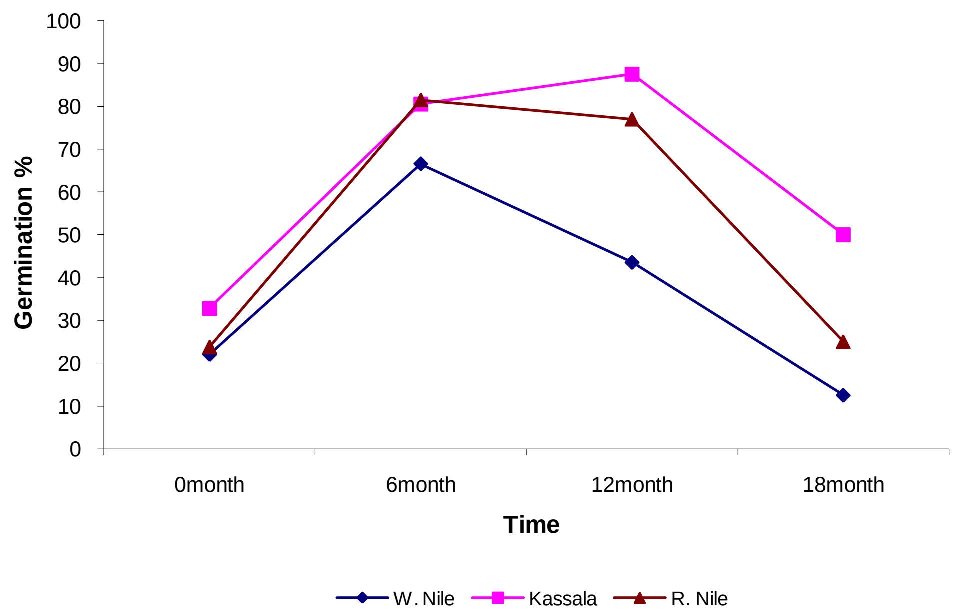

Fig. (2): *A. tortilis* subspecies spirocarpa buried seeds germination behavior and dormancy released through burial time.

Decayed seeds

The decayed seeds % was calculated by (room germinated seeds %+ the rain germinated %) subtracted from the total seeds used in the experiments, the remaining seeds were considered as decayed. Decayed seeds were the seeds buried in the soil and did not germinate in the lab or by the rainfall. There was no significant differences existed among the provenances and subspecies, but provenance× subspecies interaction showed significant differences ($P \leq 0.007$). However subspecies raddiana among provenances, Kassala showed the highest decayed seeds and the lowest decayed seeds was shown by the White Nile Provenance Table (4).

While the subspecies spirocarpa among the provenances, the highest decayed seeds % was shown by White Nile and the lowest decayed seeds % was shown by Kassala Table (4). Within subspecies in same provenance, the subspecies spirocarpa showed high decayed seeds than raddiana in White Nile, but subspecies raddiana showed high decayed seeds % than subspecies spirocarpa in both Kassala and River Nile provenance Figure (3). The mortality of buried seed at the end of the experiment ranged from 45.57% - 64.31% in subspecies raddiana and from 55.92% - 69.36% in subspecies spirocarpa Figure (3).

Table(4). Variation in decay seeds of two *Acacia tortilis* subspecies from the three provenances.

Seed parameters	raddiana			spirocarpa		
	White Nile	Kassala	River Nile	White Nile	Kassala	River Nile
Decayed seeds	54.57^{b}	66.36^{a}	$64.31a^{b}$	69.36^{a}	55.92^{b}	58.72^{b}

*Means with same letters in same row for same parameter for the same subspecies are not significantly different at p= 0.05 using Duncan New Multiple Range Test.

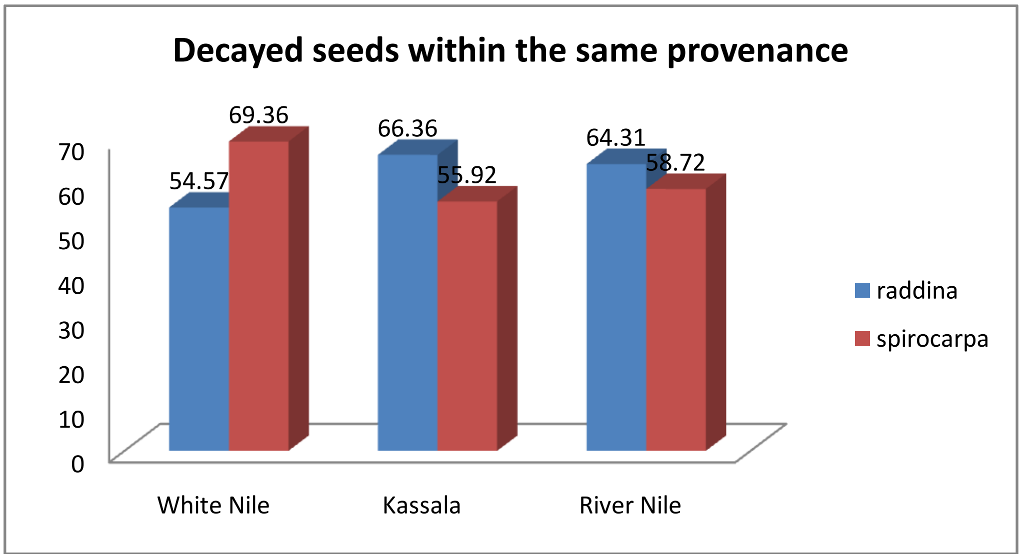

Fig.(3). The variation in decayed seeds of *Acacia tortilis* subspecies raddiana and subspecies sprocarpa within the same provenance.

Discussion

The results showed that both *Acacia tortilis* subspecies raddiana and spirocarpa had a persistent soil seed banks in the three provenances, the seed can remain viable for two rainy seasons, while current year seeds that remain viable at the onset of second growing season. In general, seed capable of forming persistent seed banks have dormancy mechanism through which germination is arrested even if the environmental conditions seem to be suitable for embryo growth.

The seeds of the two subspecies in the three provenances reach its maximum germination in 12 months burial time and started to decline their viability with increasing burial time in soil. Persistent soil seed banks ensure the sustainable supply of viable seeds thus contributing to seedling recruitment in disturbed environment (Matus et al., 2005; Urban, 2005; Liu et al., 2007). The presence of buried viable seeds in soil is commonly associated with phenomenon of dormancy which prevents the seed from germinating during unfavorable environmental variability conditions and uncertainty (Degreef et al., 2002). Environmental factors are generally required to break seed dormancy in legume, (Teketay and Granstrom, 1997; Degreef et al., 2002).

In first six months the high germination % scored by the three provenances for the two subspecies, provided the clues concerning the regeneration of the two subspecies. Species with relatively low seed germination in soil and very little no evidence of seeds deterioration during the first six months of seeds buried in soil, temperature and humidity are the most important factors affecting the longevity of stored seeds (Croker and Borton, 1957; Mayor et al., 2007). White Nile and Kassala provenances scored more germination % than the River Nile, the results provide clues concerning the regeneration ecology of subspecies raddiana, White Nile and Kassala tend to release portion of their dormancy in the first six months after burial than River Nile and seeds of the three provenances retained high seed viability.

The dormancy pattern present in seeds ensures that even when the rainy season starts early, a large fraction remain viable to germinate later (Veenedaal and Ernst, 1991 and 1996). The acquisition of seed dormancy of buried depth would be ecologically advantageous, because seeds would be survive in the dormant state in seed bank, (Baskin and Baskin, 1998; Huang and Gutteman, 2002; Ren et al., 2002).

At 12 month burial time, subspecies raddiana, from the three provenances increased their buried seeds germinability and showed high seed viability, but the White Nile released most of their seed dormancy than Kassala and river Nile provenance, the three provenances still retain their seed viability. Extremely high germination rates such those recorded with early rain are responsible for deficit in the proportion of dormant seeds forced in soil and provoke a lack of balance in the whole population dynamics that threatens its survival (Degreef et al., 2002).

Subspecies spirocarpa, Kassala and River Nile seem to release most of their seeds dormancy, but the White Nile released their seed dormancy and started to lose part of their seed viability. This may be indicate that the White Nile can lose seed longevity more rapidly than the other provenances, that is may be due to the soil type or ecological adaptation. The life history of plant species have been subjected to selective forces that determined whether a young plant of given species is at the right spot at the right time to develop into adult (loth et al., 2005).

The fact that a substantial degree of germination took place in situ in the burial experiment suggest no particular environmental clue is triggering germination in the field, and that the seed populations will turn over within few years.

The results showed that the seed populations of the two subspecies studied in the three provenances retained some of their viability for considerable time in soil. The increase in germination could be due to an initial dormancy which is broken after a time of burial, one or two years. This indicates that dormancy is importance phenomenon in *Acacia tortilis* which may result in avoiding the long dry seasons and unreliable rainy seasons.

At 18 month of burial, after the second rainy season the two subspecies in the three provenances, lost most of their viability and showed high seed deterioration and viability declined almost to (20-29 %). The seeds coat is mechanically or biologically degraded, drastic temperature change combined with rainfall typically breaks the dormancy. The variation in their viability % may suggest that they have different ecological adaptation mechanism to the soil factors treatment and rainfall in their natural habitat. From results obtained seeds longevity for the two subspecies declined with increasing burial time in soil after one year of seed banks storage. The results provide clues concerning the regeneration ecology of *Acacia tortilis*. Most studies suggest that germination is primary cause of the depletion of buried seeds and usually more important than the deterioration (Teketay and Granstrom, 1997). Results also indicated that seeds were gradually released from their dormancy and decreased the seeds viability with increasing time during storage. Although the rain intensity and distribution are important factor in soil buried seeds and also predation can be an important depletion factor for seed lying on soil surface (Ren et al., 2002).

Most plant germination studies have concerned with the effects of specific environmental factors on seed germination and seedling establishment, (Wang et al., 1997; Zang, 2001).

In this study the three provenances for both subspecies showed above 50 % of decayed seed during 18 month of seed buried in soil, this result revealed that a high proportion had died during experiment period and seeds lose their viability and fail to germinate. The seed viability declined with increasing storage time in soil after one year of seed dispersal. The mortality of seeds may be caused by fungal infection, unfavorable micro-environment, incomplete germination due to low moisture in soil and seed imbibed in and fail to germinate. Although the rain intensity and distribution are important factors in soil burial seeds and also predation can be an important depletion factor for seeds lying on soil surface. Many seeds in the soil seeds banks are close to the surface of the soil where soil temperatures may be high in un-shade micro-site, the upper surface of the soil dries rapidly during the hot summer days and may inhibit germination, (Wilson and Witkowski, 1998).

The favorable micro- organism activity in the soil, affecting a greater number of buried seed and observation of dead seeds showed evidence of fungal activity directly related to the relative humidity of the environmental surrounding the seeds (Bewley and Black, 1982; Mayor et al., 2007).

The overall results provide clues concerning the regeneration ecology of the subspecies of *Acacia tortilis* (raddiana and spirocarpa studied in the three provenances, the seeds induce dormancy due to harsh environment and erratic

rain fall, they retain their seed viability about two year in soil from one year seed dispersal. Results indicated that seeds were gradually released from their dormancy with increasing storage time and seed population of the two subspecies in the three provenances retained their seed viability for considerable time in soil.
The total number of seed produced by plant, only small fraction becomes seedlings, large losses occur between seed dispersal and seedling emergence because of various reasons, (Wang et al., 1997; Zang 2001). The study found that all buried seeds may showed one of the three responses, (1) they may germinate and emerge as seedlings (2) They may germinate but the seedling are unable to emerge above the soil surface or (3) they may succumb to various mortality factors.
From the experiment observation that a proportion of seeds had germinated but the seedling failed to emerge above the soil surface may be due to lack of enough soil moisture and erratic rainfall. Seed dormancy of buried depth would be ecologically advantageous, because seeds would be survive in the dormant state in seed bank.
The germination and dormancy mechanism are of great adaptive importance to plants by assuring the seedling emergence to occur at the most advantageous time and place.
The high burial decayed seed % revealed by the three provenances indicated that when the shower of rain falls, the seed imbibe water and there is no enough moisture in the soil and upper layer of the soil dried rapidly soon then seed fail to germinate. The differential seed behavior observed during burial in soil can be an indicator of the germination strategy of species studied.

Conclusions

The two subspecies of *Acacia tortilis* in the three provenances showed persistent soil seed banks, the buried seeds can remain viable in soil for about two years in soil from one year seed dispersal. After the second rainy season the two subspecies loosed most of seeds viability. Subspecies spirocarpa tend to germinate burial seeds more rapidly and loose its seed viability than subspecies raddiana.
Therefore, a persistent seed bank is one of the mechanisms for the pioneering plant species to adapt to dry areas. Very little differences were observed between rate of seed viability and longevity among provenances and within subspecies.
Seed dormancy played important role in regulated both *Acacia toritils* subspecies seed viability and longevity buried in the soil. Both subspecies will lose their seeds viability and longevity buried in soil within two years. The three provenances of both subspecies showed above 50 % of decayed seed during 18 months of seed burial in soil. Although 50 % of buried seeds were loosed their viability and fail to germinate.

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