International Journal of
Basic and Applied Sciences

(A peer reviewed International Journal)

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International Journal of Basic and Applied Sciences, Vol. 1 No. 3. pp. 218-226 2277-1921. 2012

ISSN *2277 – 1921*

Article type *Full Length Research Article*

Submission date *24 April 2012*

Acceptance date *30 June 2012*

Publication date *15 July 2012*

Article URL *http://www.crdeep.org/category/ijbas*

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International Journal of Basic and Applied Sciences Vol. 1 No. 3. 2012. Pp. 218-226

Full Length Research Paper

Effect of Storage Media and Storage Time on Germination and Field Emergence of *Oxytenanthera abyssinica* Seeds

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Abstract

A study was conducted to determine the effects of different storage media and storage time on the viability of *Oxytenathera abyssinica* (A.Richard) Munro seeds. In 2007 mature seeds were collected from bamboo natural stand in Mandura district, Benshangul Gumuz Regional Sate, north Western Ethiopia. Germination and field emergence experiments were conducted in factorial randomized complete block design using eight traditional storage media (glass bottles, plastic boxes, tin boxes, polythene bags, cotton bags, sisal sacks, clay pots and "kil") and seven storage time (0, 4, 8,12, 16,20 and 24 months after seed collection) as treatments. After conducting initial germination test, sample seeds were taken randomly from each traditional storage media and tested for germination at each four months interval since the time of seed collection. Field emergence test was also conducted by sowing seeds on beds as bare root. Germination percentage, date of 50% germination, date of complete germination, germination energy and field emergence data were recorded. There was highly statistical significant difference on the field emergence and germination percentage of *O. abyssinica* seeds due to storage media, storage time and their interactions (α=0.05, p<0.0001). The germination percentage of the seeds reduced consistency with increasing time. After a year, the germination and field emergence of the seeds was found to be below 50%. Statistically highest germination percentage (70.8) was observed for seeds stored in glass bottle followed by plastic boxes and tin and the minimum germination percentage (17%) was recorded for seeds stored in sisal sacks. Storing bamboo seeds for not more than a year in glass bottle, plastic boxes and tin is better in maintaining the viability of the seeds.

Keywords: *Oxytenanthera abyssinica; Seed germination; Field emergence; Storage time; Storage media*

Introduction

Cultivation of bamboo can provide a cost-effective return in the short term (Shanmughavel & Peddappaiah, 2000), and can provide significant proportion of the national income since it is a multipurpose grass useful for day-to-day life of people. Over 1500 distinct uses of bamboo are recorded and this number is growing rapidly with new development initiatives taking place around the world (Ranjan, 2001).

Ethiopia has the highest bamboo resource in Africa on area basis (Ensermu *et al*., 2000), which accounts about 67% of the bamboo resource in the continent (Kassahun, 2003). According to LUSO consult (1997), the bamboo species found in Ethiopia are the African alpine bamboo (*Arunidinaria alpina*) and the lowland bamboo (*O. abyssinica).* The bamboo resource is playing various roles for the local community. They have a paramount importance and multifaceted use in different parts of the country (Ensermu *et al*., 2000).

However, the bamboo resource in Ethiopia is utilized far below its potential due to lack of knowledge on its management (Kassahun Embaye, 2000) such as lack of technology for its utilization and lack of information on the propagation methods. The resource has also been neglected for many years and even was not included in any forest development endeavors carried out in the country. In Ethiopia, various types and amount of tree seedlings are planted every year in various parts of the country. But none of these reports indicated the production of bamboo seedlings in the nursery and accompanying development activities.

Nowadays, there is a growing interest on the use and development of the bamboo resource in the country by taking the experience of the developed nations, which currently are using their bamboo resource to the maximum and generating a significant amount of income for the livelihood improvement of their society and development of their nation at large. Accordingly, varies efforts have been undertaken in order to develop the propagation methods for the two bamboo species of Ethiopia. At the national level there is also a growing interest and commitment in including bamboo as part and parcel of the development Endeavour.

In line with the bamboo development endeavor, there is a great concern to develop the best propagation methods for the two bamboo species of the nation.

Just before and at the beginning of the current experimental period of the current study, *O. abyssinica* plants found in Metekel Zone of the Benshnagul Gumuz Regional Sate of Ethiopia, were flowering and setting seeds. Since human and animal pleasure in the area is increasing it seemed difficult for bamboo to regenerate naturally. Under such conditions it is of a paramount importance for using artificial regeneration methods for the establishment of artificial bamboo forests.

Although *O. abyssinica* propagates both sexually and asexually from seed and rhizomes respectively (Azene *et al.*, 1993; Dwivedi, 1993; Kassahun *et al.*, 2003). Vegetative method of propagation for *O. abyssinica* has been found unsuccessful and a cumbersome activity for large-scale plantation since it is difficult to extract and transport rhizomes to long distances (Kassahun, 2003). It was also found out that only limited plantation objectives can be achieved by vegetative propagation (Purohit *et al.*, 1998). Hence, if seed is available, propagation by seed should be given due attention (Kassahun, 2003; Demelash Alem, 2006).

Considering the long flowering and seeding cycle of *O. abyssinica*, it is important to collect as much seeds as possible and use either for current or future bamboo plantation establishment (Samora, 1994; Demelash Alem, 2006) since they are hardier in stressful environmental conditions (Samora, 1994). Storing bamboo seeds is also important as they are potential part for tissue culture development (Williams, 1994)

There are two techniques of seed storage depending on the nature of the species. These are under laboratory condition and under normal condition. It is difficult to store large amount of bamboo seeds in the laboratory since facility in most cases is a limiting factor. Because of this reason it is of a paramount importance to develop which is applicable and affordable to the local community.

In line with the selection of the best traditional seed collection methods, it is also important to know for how long the seeds will maintain their viability when they are stored in traditional storage media as the storage time has impact on the viability of seeds. Despite the fact many research outputs are indicating that most bamboo seeds are losing their viability within short period of time, available data to for Ethiopian bamboo seeds is very limited. With this background information the present study was conducted with the following objectives.

Materials and Methods

Geographical Location of the Study Area

The experiment was conducted in Pawe Agricultural Research Center compound (both on station and in laboratory), Pawe special Woreda of the Benishangul Gumuz Regional state in Ethiopia. The research center is located at $11^0$09' N latitude and $36^0$03' E longitude and has an altitude of 1050 m.a.s.l and rainfall of 1555.1 mm. The area has unimodal rainfall which mostly concentrates between March and September. The peak rainfall occurs from July to August. The mean potential evapo-transpiration (PET) is about 1300mm. The mean annual maximum temperature of the area is about 32^oC, where the mean monthly values are 27-37^oC. The soil of the study area includes Haplic Alisols, Eutric Vertisols and Vertic Luvisols (Abayneh Esayas, 2003).

The geology of the area consists of metconglomerate and quartizite of the Precambrian basement complex (Tefera *et al*., 1996). The texture of the soils is is mostly dominated by heavy clay, clay loam, clay and sandy-clay-loam (Abayneh Esayas, 2003).

Seed Collection, Processing and initial germination test

Mature fruits of *O. abyssinica* were collected from Mandura district of the Benishangul Gumuz Regional state of Ethiopia. After transported to Pawe Agricutural Research Center (PARC), the fruits were air-dried, separated in to their single parts and finally threshed manually to extract seeds (Demelash Alem, 2006). The seeds were stored properly until initial germination test was carried out. Initial germination test was conducted using 100 seeds with four replications by taking sample seeds from the bulk sample after thoroughly mixing the bulk seeds and taking randomly by hand. After taking the initial germination test, one kilogram of seed for each treatment type was stored for subsequent germination test. Storage media (glass bottles, plastic boxes, tin boxes, polythen bags, clay pots, cotton cloth bags, sisal bags and "Kil") were prepared to be used as storage media and to see their effect on the viability of the seeds. "Kil" is the the fruit of *Lagenaria abyssinica* used as storing things.

Germination Test

Before conducting the current test, the germinating medium, *i.e.* Petri dishes (9.5 cm diameter) with non-toxic moist blotting paper, were set up in Pawe Agricultural Research Center (PARC).

Pure seeds were mixed, and randomly counted to select seeds for the germination test. Initial germination test was conducted using four replicates of one hundred seeds. Seeds were sown uniformly and watered as needed and kept moist but not wet, as this would have negative effect on seed germination (Kassahun *et al*., 2003). Four hundred seeds in four replications of 100 seed each were used in each test (ISTA, 1996). Field emergence test was also conducted by sowing seeds on beds as bare root. No seed treatment was applied since the seeds of this species do not have seed dormancy (Banik, 1994; Kassahun *et al*., 2003; Demelash Alem, 2006). Seeds were considered to have germinated after the emergence and development of the radicle and plumule from the seed embryo (FAO, 1985). Each day the number of germinated seeds was recorded, and the germinated seeds were removed (Gulzar & Khan, 2001) so as to avoid double counting. Abnormal seeds, seeds infected with fungus, and ingeminated

seeds were considered as non-viable. When no further germination appeared, the total number of germinated seeds for each treatment/ factor combination was added to determine the germination percentage of bamboo seeds (Palzer, 2002).

With the same procedure, seed samples were taken out from each storage media and their germination was tested at intervals of four months using the standard procedure. This experiment was conducted and continued for 24 months, starting from the first germination test period. Since the majority of the seeds did not maintain their viability for more than two years, there was no reason to continue the experiment for more than two years using the remaining seed whose germinating rate has been decreased drastically.

From the collected germination and field emergence test, other parameters were determined.

Germination Energy

The germination energy of bamboo seeds was determined in the seed viability test by recording the germination data until the number of germinated seedlings declined or fall off. According to Schmidt (2000), germination energy can be found in one of the three ways. These include (1) by taking data up to the day of peak germination; (2) so as not to exclude germinable seeds, it was also regarded as lasting until daily germination fall to less than 25% of the peak germination or (3) it was also calculated based on the number of days required to attain 50% of the germination capacity. Therefore, the germination energy of *O. abyssinica* was determined based on these three methods. For the sake of convenience the germination energy for this case was calculated using the third option.

Germination Value

The germination value aims to combine in a single figure total germination together with an expression for germination energy or speed of germination. From the germination test data, the germination value of *O. abyssinica* seeds were computed according to the method of Djavanshir & Pourbeik (1976):

$$GV = (\frac{\sum DGS}{N}) * \frac{GP}{10}$$

Where

GV= Germination value

GP= Germination percent at the end of the test

DGS= Daily Germination Speed, obtained by dividing the cumulative germination percentage by the number of days since sowing.

$\sum DGS$ = The total obtained by adding every *DGS* figure obtained from the daily count

N= the number of daily count, starting from the date of first germination

10= Constant

Germination Speed

According to FAO (1985) germination speed is expressed as peak values or the maximum mean daily germination (cumulative percentage of full seed germination divided by the number of days elapsed since sowing date) reached at the time during the period of the test. The value can was calculated from the germination test data.

Field emergence test

With the same randomization and lay out used for the laboratory work, *O. abyssinica* seeds were also sown on the nursery soil as bare root and their field emergence was determined for each storage time and storage conditions. This test was conducted in temporary nursery which is found in the research centre compound. Except the treatment, all the necessary nursery activities were carried out as usual. Those non-germinated seeds were scrutinized for the cause of failures.

Experimental Design

The laboratory experiment was laid out in factorial randomized complete block design with four replications. Storage media and storage time were taken as treatments. The factors are storage condition with six levels and storage time with seven levels. The details of storage condition and storage period are given below.

Storage media

T_1 = Glass bottles
T_2= Plastic boxes
T_3 = Tin boxes
T_4= Polythene bags
T_5 =cotton bags
T_6= Sisal
T7= Clay Pots
T8= "*Kil*"

Storage time (after seed collection)

P_1= 0 Months
P_2=4 Months
P_3=8 Months
P_4=12 Months
P_5=16 Months
P_6=18 Months
P_7 = 24 Months

Data collection and analysis

Germination percentage, seed viability, date of 50% germination, date of complete germination, germination energy, field emergence of seeds and cumulative germination energy computed form the field and laboratory data were collected. Data were checked for normality and homogeneity of variance. All the data collected were analyzed using SAS software (1999-2001, SAS Institute Inc, USA) and LSD (Least Significance Difference) was used for mean separation.

Results and Discussion

Field Emergence Test

There was highly statistical significance difference on the field emergence of *O. abyssinica* seeds (Table 5.1) due to the storage time (α=0.05, p<0.0001), storage media (α=0.05, p<0.0001) and their interactions (α=0.05, p<0.0001). The current research result was in line with other researches carried out on other bamboo species. For example Banik (1994) indicated that the viability of bamboo seeds varies depending on the species storage condition and storage time.

Table 1 ANOVA for the field emergence of bamboo seeds under different storage time and storage media

Source of Variation	DF	SS	Mean Square	F Value	Pr > F
Storage time	5	58006.98	11601.40	184.07	<.0001**
Storage media	7	24888.54	3555.51	56.41	<.0001**
Replication	3	770.77	256.92	4.08	0.0082
Storage time*storage media	35	13382.81	382.37	6.07	<.0001**
Error	141	8886.98	63.01		
Total	191	105936.08			

The field emergence of the seeds decreased consistency with increasing time in all types of traditional storage media (Table 5.2). When the average field emergence of the bamboo seeds was considered on all types of the containers across the experimental periods, the highest field emergence of the seeds was observed for the seeds stored in glass bottle (43.13%) followed by plastic bottle (40.17%), tin boxes (32.04) and clay pots (36%). The lowest field emergence results (3.42% and 14.37%) were recorded form the seeds that have been stored in sisal sacks and cotton bags respectively (Table 5.2; Fig 5.1).

Table 2: Mean± SD of the field emergence *O. abyssinica* seeds under different storage time and storage media

Storage Media	Experimental periods (Months after Seed Collection)						Average
	4	8	12	16	20	24	
"Kil"	46.0±15.1	27.75±8.2	31.5±7.0	5.0±4.1	25.75±17.5	0.0±0.0	**22.67C**
Tin	71.25±11.0	41.0±6.3	54.25±1.7	20.0±6.8	5.5±11	0.25±0.5	**32.04B**
Cotton bag	47.5±19.5	18.5±4.4	18.25±3.4	2.0±2.16	0.00±.00	0.0±0.0	**14.37D**
Plastic bottle	61.5±4.4	44.5±6.2	58.0±3.9	26.0±10.9	46.25±19.9	4.75±3.30	**40.17A**
Glass bottle	65.25±5.1	44.0±7.10	61.25±7.7	29.0±3.7	53.75±7.5	5.50±3.1	**43.13A**
Polythene tube	56.75±17.1	30.25±9.0	32.75±5.3	15.5±3.9	0.0±0.0	0.25±0.5	**22.58C**
Clay pot	54.75±14.3	30.25±2.8	41.0±10.3	7.5±1.7	34.25±15.4	0.25±0.5	**28.0B**
Sisal sack	41.0±2.4	0.25±0.50	2.75±2.90	0.5±1.0	0.0±0.0	0.0±0.0	**3.42E**
Average	**55.50A**	**29.56C**	**37.47B**	**13.19E**	**20.69D**	**1.38F**	**34.66**

Note: values in the same column and row with the same letters are not statistically different at 5% level of significance

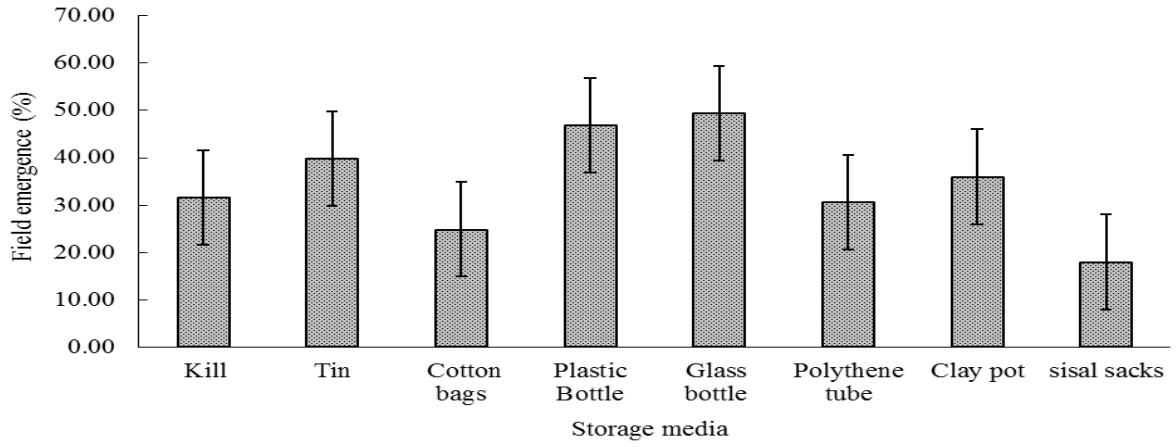

Fig 1: Average Field mergence of the *O. abyssinica* seeds under different storage media across all time periods (the initial field emergence data are also included)

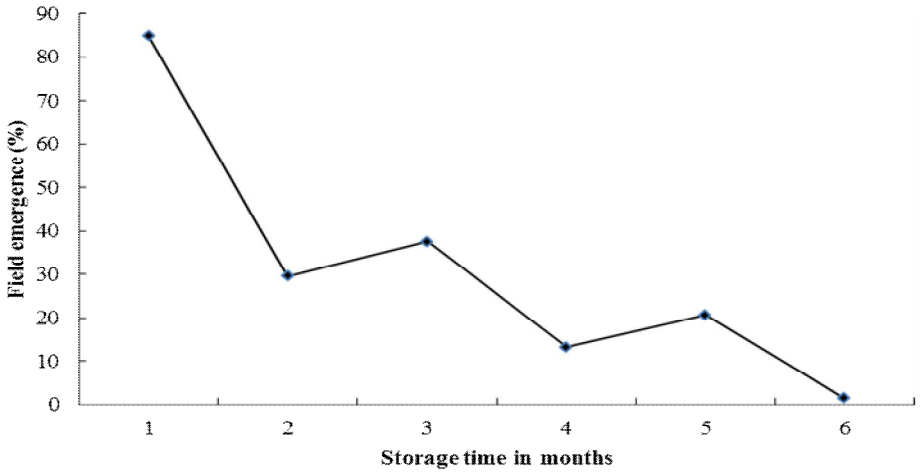

Fig 2: Field emergence of *O. abyssinica* seeds in different storage time

Note: storage time 1= 0 months, 2= four months, 3= eight months, 4= twelve months, 5= sixteen months, 6= twenty months and 7= twenty four months after seed collection

The possible reasons for the lowest field emergence of the bamboo seeds in sisal sacks and cotton bags may be due to the fact that these types of containers absorb more moisture and there may be a number of pests and diseases that enter in to the containers and might have damaged the seeds (Demelash Alem, 2006). Bamboo seeds are sensitive to moisture (Kassahun *et al*., 2003) and lose their viability within short period of time (Banik, 1994). Five months after collection the seeds were observed to be attacked by some seed borers which are common to other crops as well.

Germination Percentage

There was highly statistical significance difference on the germination percentage of *O. abyssinica* seeds (Table 5.3) due to the storage time (α=0.05, p<0.0001), storage media (α=0.05, p<0.0001) and their interactions (α=0.05, p<0.0001). Like that of the field emergence test the germination percentage of the seeds decreased on continuous basis until the end of the experiment period (Fig 5.4). The trend was the same for all types of the germination media used in the experiment.

Table 3 ANOVA for the germination percentage of bamboo seeds in different storage media and storage time

Source of variation	DF	SS	Mean Square	F Value	Pr > F
Storage time	5	62627.92	12525.58	204.53	<.0001**
Storage media	7	63556.58	9079.51	148.26	<.0001**
replication	3	102.92	34.30	0.56	0.6422
Storage time*storage media	35	25705.42	734.44	11.99	<.0001**
Total	191	160627.92			

When the average germination percentage of the seeds was taken in to consideration on all types of the containers across the experimental periods, the highest germination percentage was observed for the seeds stored in glass bottle (70.83%) followed by plastic bottle (66.08%), tin boxes (63.67) and polythene tube (53.83). The lowest field germination percentage results 16.92% and 30.89% were recorded for the seeds stored in sisal sacks and "*kil*" respectively (Table 5.4; Fig 5.3; Fig 5.4). Both low germination percentage and low field emergence results were recorded for the seeds stored in sisal sack and hence the same justification given in the field emergence test applies here.

Table 4: Mean± SD of the germination percentage of *O. abyssinica* seeds under different storage time and storage media

Container type	Experimental periods (Months after Seed Collection)						Average
	4	8	12	16	20	24	
"Kil"	71±14.1	48.0±5.9	47.0±9.6	10.0±2.8	6.5±4.1	0.0±0.0	30.42E
Tin can	79.5±5.5	79.5±7.7	70.0±6.7	70.0±6.9	65.5±7.2	17.5±1.9	63.67B
Cotton bag	70.5±9.15	47.0±7.4	41.5±4.4	21.5±7.7	18.0±2.8	0.0±0.0	33.08E
Plastic bottle	75.0±4.15	70.0±6.3	68.0±4.3	72.0±5.9	58.5±13.9	53.0±6.0	66.08B
Glass bottle	81.0±6.6	70.5±22	74.0±8.5	73.0±3.5	69.5±4.4	57.0±15.4	70.83A

Polythene tube	75.0±6.0	60.5±8.5	65.0±4.2	55.5±10.4	49.5±16.6	16.5±6.8	53.97C
Clay pot	73.5±5.0	67.0±9.5	59.0±5	54.5±4.1	1.0±2.0	0.0±0.0	42.50D
Sisal sack	71.5±11.8	21.0±5.3	7.0±2.6	2.0±1.6	0.0±0.0	0.0±0.0	16.92F
Average	74.63A	57.94B	53.94C	44.81D	33.56E	18.00F	

Note: values in the same column and row with the same letter are not significantly different at 5% level of significance

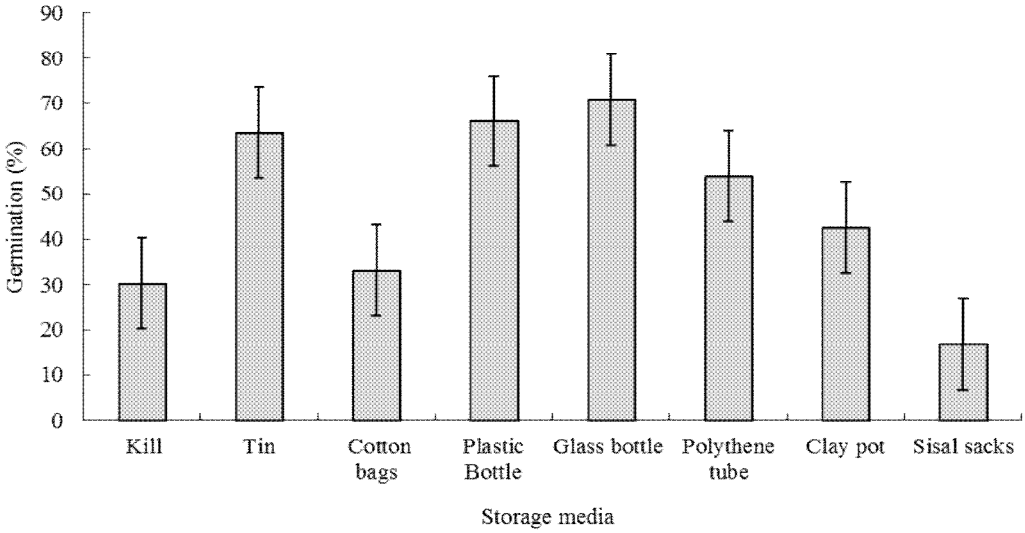

Fig: 3: Germination percentage *O. abyssinica* seeds under different storage media (initial germination percentage data is also included)

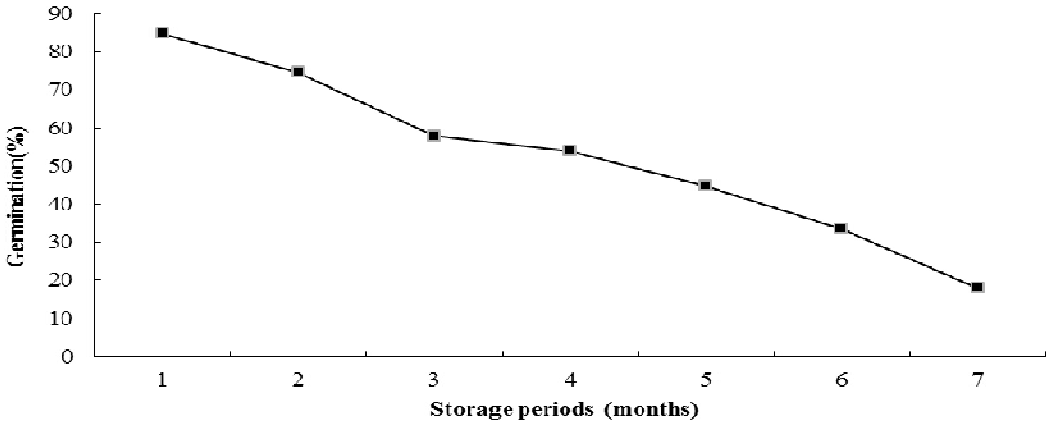

Fig 4: Germination percentage of *O. abyssinica* seeds under different storage time

Note: storage time 1= 0 months, 2= four months, 3= eight months, 4= twelve months, 5= sixteen months, 6= twenty months and 7= twenty four months after seed collection.

Field emergence test of the bamboo seeds did not show the same trend like that of the germination data. The field condition was different from the laboratory condition in that there are many environmental variables such as the nature of the soil which resulted in lower field emergence test results compared with the germination data conducted in the laboratory. Therefore, the field condition is not the reliable way of knowing the full germination potential of the seeds. Since some of the field germination tests were carried out during the rainy season, some of the viable seeds might have been hindered from germination despite the fact that they have the potential to germination if there were some favorable conditions for them.

Germination Value

The germination value of the seeds showed variation due to the storage time and the storage media despite the fact that the trend was not consistent. At the initial germination test, the

maximum germination value (266.25) was recorded in seeds stored in clay pots and the minimum germination value (132.67) was observed in sisal sack stored seeds. The germination value was declining continuously when the seeds were stored for long period of time. After twenty four months, half of the storage media showed germination value of zero ("*kil*", cotton bags, and glass bottle and sisal sacks). In general it is possible to conclude that both the seed storage and storage media has their own impact on the germination value of *O. abyssinica* seeds (Table 5.5). Lack of consistency for the germination value of bamboo seeds were due to the fact the number of days required to take the seeds to complete germination since the time of their initial germination, which was used as a denominator in calculating the germination value based Djavanshir & Pourbeik (1976), was varying across the different testing periods. The inflated germination values with increasing testing period was because of that variation, otherwise the seeds would have showed the same trend like that of the germination percentage and germination values.

Table 5: Germination Value of *O. abyssinica* seeds under different storage time and storage media

Seed storage Media	Seed Storage Time (Months after Seed Collection)						
	0	**4**	**8**	**12**	**16**	**20**	**24**
"Kil"	126.98	81.68	78.23	65.74	42.35	9.87	0
Tin can	174.71	150.35	134.14	176.36	84.60	123.19	7.63
Cotton bag	177.78	128.00	163.05	58.91	131	1.27	0
Plastic bottle	153.62	120.29	14.19	155.84	0.05	136.93	113.02
Glass bottle	172.17	204.68	55.14	214.90	2.15	135.78	0
Polythene tube	154.63	144	176.09	93.62	99.8	0.02	90.05
Clay pot	266.25	148.97	52.41	128.60	5.03	40.74	3.07
Sisal sack	132.67	173.06	123.45	2.45	87.95	0	0

Germination Energy

In the current research, the majority of the seeds in all types of storage media and seed storage time germinated with three days. Only two days were elapsed before the seeds start to germinate in Petri dishes (Table 5.6). The result is in line with some of the previous research outputs conducted on the species (e.g Kassahun, 2003; Demelash, 2006). Under laboratory condition the seeds completed their germination within 4-6 days after they were sown. After the seeds have been stored for about eight and more months from the time of seed collection, the germination percentage falls less than 50%, which made impossible to know the germination energy of the bamboo seeds. The values in table 5.6 designated by NA, stands for germination values which could not be calculated since their germination energy is less than 50%.

Table 6: Germination energy of bamboo seed under different storage time and storage media

Seed storage Media	Seed Storage Time (Months after Seed Collection)						
	0	**4**	**8**	**12**	**16**	**20**	**24**
"Kil"	3	3	3	NA	5	NA	NA
Tin can	3	3	3	2	4	3	NA
Cotton bag	3	3	3	NA	3	NA	NA
Plastic Bottle	3	3	NA	2	NA	3	3
Glass bottle	3	2	NA	2	NA	3	NA
Polythene tube	3	3	3	2	3	NA	3
Clay pot	2	3	NA	2	NA	NA	NA
Sisal sack	3	2	3	NA	3	NA	NA

Conclusion and Recommendation

The traditional storage media and storage time have significant impact on the germination and field emergence of the *O. abyssinica* seeds. Better seed germination and field emergence results were observed in seeds stored in glass bottle and plastic bottles implying that these storage media are better traditional means of storing bamboo seeds. On the contrary, storing bamboo seeds in traditional storage media that has large openings such as such as sisal sacks resulted in high deterioration of the seeds within short period of time. Therefore, airtight traditional storage media are better in maintaining the viability of bamboo seeds for longer period.

Since there is high humidity and temperature in the study area the use of sack and other containers which has many opening resulted in high deterioration of *O. abyssinica* seeds. The current study also showed that seeds of *O. abyssinica* can retain their viability for about two years and hence the local people can collect as much seeds as possible and use for establishing bamboo plantation for any purpose. The seeds stored for the experiment were also observed to be attacked by

some pests and diseases, indicating that there is a need to carry out research to protect the seeds from these pest and diseases so as to extend the storage time of bamboo seeds.

Based on the current research result the following recommendations are given:

- This research result is site specific and similar studies should be conducted in order to identify the best traditional methods that will help to store the bamboo seeds for longer period and use for future bamboo plantation establishment and development activities.
- Storage traditional storage media that are tight and do not allow moisture and other extraneous material to enter are suitable for storing *O. abyssinica* seeds.
- The use of airtight traditional seed storage media seems better for storing bamboo seeds at the local level.
- Since *O. abyssinica* seeds are liable to high moisture and desiccation while germinating, it is best to use regulated watering for better germination of the seeds.
- Some chemical treatments against seed borers seem important in order to prevent the attack of bamboo seeds from the various seed borers.
- The susceptibility of bamboo seeds to fungus during germination is very critical and hence aeration and watering frequency should be regulated during seed germination either in field or in laboratory.
- Awareness creation to the local people is needed so that they will collect and store seeds whenever there is gregarious flowering of the species. This will help them to establish bamboo plantation by themselves.
- Further research has to be done in order to know the effect of other traditional storage media on the viability of *O. abyssinica* seeds.

Acknowledgments

We would like to express our deep appreciation to colleagues of ours at Pawe Agricultural Research Center (PARC) who has contributed in various ways to the finalization of the research project. The administrative staff of Pawe Agricultural Research Center (PARC) is also thanked for their collaboration and facilitation for the smooth running of the research activity. Special thanks also go to Mr. Mulugeta Atnaf (Center Director of PARC) for facilitating the research work. Mr. Gashu Mekonnen also deserves great gratitude for his unreserved contribution during the whole process of the research work. Last but not the least; we are also indebted to Ethiopian Institute of Agricultural Research (EIAR) in financing the research cost.

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