Seed dormancy in *Cassia fistula* Linn. population from Nigeria.

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Abstract: Seed dormancy in the population of *Cassia fistula* from Covenant University, Ota, Ogun State, Nigeria was investigated through laboratory experiments with a view to elucidate the best method that can be used to enhance maximum germination of the seeds. The treatments were mechanical scarification, aeration, hot water immersion, chemical methods and a combination of the treatments on seeds of different storage times. Percentage germination, mean germination time and germination rate were calculated at the end of the 20-day experiments. Results showed that mechanical scarification with wood sand paper, hot water immersion and concentrated sulphuric acid were the best treatments that significantly enhanced seed germination. Treatments with aeration, dilute sulphuric acid, 10% and 20% potassium permanganate and copper sulphate solutions did not significantly enhance germination in both the scarified and unscarified seeds. Dormancy in *C. fistula* appears to be primary exogenous type. The freshly harvested seeds germinated best with germination decreasing with storage time. Mechanical scarification in conjunction with either hot water immersion or concentrated sulphuric acid scarification are the most efficient methods for rapid and uniform germination of *C. fistula* seeds for use in afforestation, landscaping and pharmacological programmes in Nigeria.
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1. Introduction

Seed dormancy refers to a state in which the viable seeds fail to germinate when provided with conditions normally favourable to germination such as adequate moisture, appropriate temperature regime and light (Schmidt, 2000). Seed dormancy is also defined as a temporary failure of a mature viable seed to germinate under environmental conditions that would normally favour germination (Hilhorst, 1995, Li and Foley, 1997). Seed dormancy is an important evolutionary factor in plants, ensuring their survival in unfavourable conditions and allowing them to germinate when the chances of survival for the young seedlings are at the greatest (Johnson and Raven, 2002).

Dormancy can be classified, based on development into two types. (Crocker 1916, Nikolaeva 1977 and Schmidt 2000). They are (i) innate or primary dormancy; in which dormancy is present when the seeds are ready for dispersal from the mother plant(s); and (ii) induced or secondary dormancy; in which dormancy develops as a response to external environmental factors. Also the location of dormancy in different parts of the seed can be used in classifying dormancy (Verma et al. 1995). These are (i) endogenous or embryo induced dormancy which is due either to rudimentary or immature development or to the presence of chemical inhibitors in the embryo and (ii) exogenous or seed-coat induced dormancy which may be due to various factors such as mechanical resistance of seed coat to water and gas permeability, light sensitivity of seed coat and the presence of germination inhibitors (e.g, phenolics, tannins, alkaloids, indoles and abscissin) in seed coats.

Pretreatment is a term used for conditions or processes applied to break dormancy prior to germination. The purpose of pretreatment is to ensure that seeds will germinate and that germination is fast and uniform. Although pretreatment methods have been developed and described for many species, dormancy still causes problems of low germination percentages and rates for several tropical species. This is due to lack of general knowledge of their seed physiology and to variation in dormancy rate (Schmidt, 2000).

Furthermore, taxonomically related species often share the same type of dormancy, for example, most species of the family Fabaceae exhibit physical dormancy caused by advanced morphological structures of the seed coat. The seed coat consists of four distinct layers namely; the cuticle, the macrosclereids, osteosclereids and parenchyma layer (Schmidt, 2000). The cuticle is the outer layer which has a waxy and water-repellent character. Macrosclereids or palisade layer consists of long, narrow, tightly packed vertical cells. Osteosclereids is a layer of more loosely packed cells and parenchyma layer is made up of little differentiated cells. Impermeability of the leguminous seed coat is ascribed to the two outer layers of cuticle and macrosclereids.

Cassia fistula Linn. is an ornamental tree commonly known as the "golden shower tree" and "Indian Laburnum". It is one of the 400 different species that comprise the genus *Cassia* and it belongs to the subfamily Caesalpiniaceae and the family Fabaceae (Sartorelli et al. 2009). It is a native of the Indian subcontinent and is distributed in various tropical regions including Asia, West Indies, South America, Australia and Africa (Orwa et al. 2009). One of the authors (OTO) found it growing at the zoological garden in Waikiki, Oahu, Hawaii.

It is known for its medicinal properties. Phytochemical investigations have proven *C. fistula* to be an important valuable medicinal plant due to the presence of secondary metabolites it contains (Sen and Shukia, 1968; Danish et al. 2011). It is a rich source of phenols, tannins, flavonoids and glycosides. Pharmacological activities of various parts of *C. fistula* include antibacterial, anti-diabetic, anti-fertility, anti-inflammatory, antioxidant, hepato-protective, antitumor and antifungal activities (Kirtikar and Basu 2006; Danish et al. 2011).

Apart from being widely used for landscaping because of its golden yellow flowers which last for several months, it is known for its landscaping attributes like tolerance to drought conditions and low maintenance requirements (Ghouse et al. 1980). It is as a result of these that *C. fistula* was planted in Covenant University, Ota, Ogun State, Nigeria at its founding in 2002 as the dominant landscaping tree species. There are about 250 trees in the population on the campus. We have recorded only few trees at the Universities of Lagos and Benin. Other new Universities and some older ones, State governments and even the Federal government of Nigeria are in the process of imitating Covenant University. Since *C. fistula* is drought tolerant, it might be useful against desert encroachment in the Northern parts of Nigeria while it also serves as beautiful landscape.

So there is the need to raise lots of seedlings at minimum cost since *C. fistula* seeds exhibit dormancy (Al-Menaie et al. 2010, Karaboon et al. 2005). Our objective is to find out the best and cheapest method(s) to break dormancy in the seeds of *C. fistula* from Covenant University, Ota. Ogun State. Nigeria. The results, which will be the first in sub-Saharan Africa, will be compared with other research work from other parts of the tropical region. There may also be need to store seeds for use at a later time, so we intend to find out the state of the seeds with storage time.

2. Materials and Methods

To determine the best method to break the seed dormancy of *C. fistula,* two sets of experiments were carried out using seeds of different storage periods of twelve months, six months and freshly harvested seeds designated as 12M, 6M and 0M.

Experiment 1 - Effects of mechanical scarification, aeration and hot water treatments on the germination of *C. fistula* seeds.

Healthy mature pods of *C. fistula* that had fallen from the trees were collected from the population in Covenant University, Ota, Ogun State, Nigeria. The pods were gently crushed and the seeds removed. The seeds were thoroughly washed with water to remove adhering pericarp parts, air-dried for two days and the individual seeds were first sorted by size into small and large and then weighed using an electronic balance. The seeds were graded into light and heavy seeds based on their weight; with light seeds weighing less than 0.15g and heavy seeds weighing more than 0.15g (Al-Menaie et al. 2010).

Seeds were tested for viability by floatation method in distilled water; this involves separating the seeds into "floaters" and "sinkers". Viable seeds were considered to be those that sank. Prior to the experiment, seeds were surface sterilized in 20% sodium hypochlorite solution for ten minutes to prevent fungal infection during germination. Half of the seeds were mechanically scarified using wood sand paper to partially reduce the thickness of the seed coats and the other half was left unscarified. Other treatments were aeration and hot water immersion of the light and heavy seeds. The aeration treatment was done by using an aerator to bubble air into cold distilled water at room temperature for 120 minutes in a beaker containing the seeds. The hot water treatment was carried out by immersing the seeds in hot water at 84^0C and allowed to remain for 24 hours in the cooling hot water.

There were 10 treatments apart from the control. These are denoted as follows:

T0: Control, light seeds unscarified.

T1: Light seeds unscarified and aerated.

T2: Light seeds unscarified and hot water treatment.

T3: Light seeds scarified.

T4: Light seeds scarified and aerated.

T5: Light seeds scarified and hot water treatment.

T6: Heavy seeds unscarified.

T7: Heavy seeds unscarified and aerated.

T8: Heavy seeds unscarified and hot water treatment.

T9: Heavy seeds scarified.

T10: Heavy seeds scarified and aerated.

T11: Heavy seeds scarified and hot water treatment.

The experiment was a completely randomised design with each treatment containing five replicates of twenty five seeds each. The seeds were sown in 9 cm sterile disposable Petri dishes lined with two layers of moistened filter paper and the Petri dishes were kept in a germination chamber at room temperature.

Experiment 2: This was to determine the effects of different concentrations of sulphuric acid, potassium permanganate and copper sulphate solutions on the germination of *C. fistula.*

Only heavy seeds were used for this experiment as there were no more light seeds. Half of the seeds were mechanically scarified using wood sand paper and the other left unscarified. There were 12 treatments with five replicates of twenty five seeds for each set. Different sets of *C. fistula* seeds were immersed in concentrated sulphuric acid for periods of one, two and 10 minutes and in dilute (10%) sulphuric acid for 30, 60 and 120 minutes respectively. The seeds were then thoroughly rinsed with distilled water to remove any trace of the acid. In another set of treatment, scarified and unscarified seeds of *C. fistula* were dipped in 10% and 20% copper sulphate solution for 30, 60 and 120 minutes. Another set of scarified and unscarified seeds were dipped in 10% and 20% potassium permanganate solution for 30, 60 and 120 minutes. The seeds were then thoroughly rinsed with distilled water to remove any trace of the solutions.

Table 1. The treatments, duration and type (scarified/unscarified) of seeds used in this experiment.

TREATMENT	DURATION	UNSCARIFIED SEEDS	SCARIFIED SEEDS
100% H_2SO_4	1 minute	CT1	CT4
	2 minutes	CT2	CT5
	10 minutes	CT3	CT6
10% H_2SO_4	30 minutes	CT7	CT10
	60 minutes	CT8	CT11
	120 minutes	CT9	CT12
10% $KMnO_4$	30minutes	CT13	CT16
	60 minutes	CT14	CT17
	120 minutes	CT15	CT18
20% $KMnO_4$	30 minutes	CT19	CT22
	60 minutes	CT20	CT23
	120 minutes	CT21	CT24
10% $CuSO_4$	30 minutes	CT25	CT28
	60 minutes	CT26	CT29
	120 minutes	CT27	CT30
20% $CuSO_4$	30 minutes	CT31	CT34
	60 minutes	CT32	CT35
	120 minutes	CT33	CT36

In each experiment, treated seeds were sown in 9 cm sterile disposable Petri dishes lined with two layers of moistened filter paper. The labelled Petri dishes were kept in a germination chamber at room temperature. Additional water was added to the Petri dishes when required during the course of the experiments so as to provide adequate moisture.

Germination Parameters Estimation

Germination study was carried for 20 days for each experiment and the number of germinated seeds in each treatment was recorded daily. Germination was regarded to have occurred when the radicle was observed. At the end of the germination period, the germination percentage, germination rate (Maguire, 1962) and mean germination time were calculated using the following equations:

Mean germination rate (MR) was calculated as the reciprocal of the mean germination time.

$$MR = \frac{1}{\text{Mean Germination Time}}$$

Mean germination time (MT) was calculated according to Scott et al. (1984) and Czabator (1962).

$$MT \text{ (days)} = \frac{\Sigma \text{ Ti Ni}}{\Sigma \text{ Ni}}$$

where Ti is the number of days from the beginning of the experiment,

Ni is the number of seeds germinated per day and

Σ Ni is the sum of seeds that germinated.

Statistical Analyses

Data collected from the experiments were subjected to statistical analyses using the Graph Pad Prism 5.0. Comparisons of mean germination percentage, mean germination time and mean germination rate were made for each experiment using the analysis of variance (ANOVA). Tukey's test was used to separate means that were significantly different at $P < 0.05$.

3. Results

The major results discussed are for the freshly harvested seeds (0M).

Light seeds

Scarification: Unscarified light seeds had poor germination (12%). With scarification, there was a significant increase ($p < 0.05$) to 41%. Unscarified light seeds had a mean germination time of 12 days while scarification significantly ($p < 0.05$) reduced the mean germination time to 6 days. Scarification nearly doubled the mean germination rate (tables 2a-c).

Aeration: Aeration alone had no significant increase on germination percentage and germination time but with scarification there was significant increase in germination ($p < 0.05$) (table 2a). There was no significant difference between the mean germination time of the aerated seeds and the control (table 2b). But with scarification, the time taken to germinate was reduced significantly to 6 days from 12 days. Aeration had no effect on the mean germination rate. However, the mean germination rate was doubled in the scarified seeds (table 2c).

Hot water treatment: Hot water at 84^0C had no effect on the germination percentage of unscarified light seeds. With scarification, there was a significant increase ($p < 0.05$). Comparison of the result of light scarified seeds which were immersed in hot water with light scarified seeds only, showed a significant increase ($p < 0.05$) in germination (table 2a). Hot water treatment slightly reduced the mean germination time from 13.7 days to 12.6 days. This was further reduced to 6 days with scarification (table 2b). Hot water had no effect on the germination rate of unscarified seeds.

With scarification, the germination rate was doubled (table 2c).

Table 2A: Mean germination percentage (%) of 12M, 6M and 0M scarified and unscarified light seeds of *Cassia fistula* Linn. treated with aeration and hot water.

Treatment	Mean germination percentage (%) 12M	6M	0M
T0	1.60	8.00	12.00
T1	2.40	8.00	10.40
T2	4.00	11.20	13.60
T3	3.20	29.60	40.80
T4	3.60	44.80	51.20
T5	4.80	52.00	58.40

Table 2B: Mean germination time (days) of 12M, 6M and 0M scarified and unscarified light seeds of *C. fistula* treated with aeration and hot water.

Treatment	Mean germination time (days) 12M	6M	0M
T0	11.50	13.50	11.72
T1	9.00	13.43	12.60
T2	9.80	14.25	12.22
T3	9.25	8.19	6.29
T4	5.31	6.14	5.97
T5	6.34	6.34	6.12

Table 2C: Mean germination rate (d^{-1}) of 12M, 6M and 0M scarified and unscarified light seeds of *C. fistula* treated with aeration and hot water.

Treatment	Mean germination rate (d^{-1}) 12M	6M	0M
T0	0.09	0.08	0.09
T1	0.11	0.08	0.08
T2	0.10	0.07	0.08
T3	0.11	0.12	0.16
T4	0.19	0.16	0.17
T5	0.16	0.16	0.16

Heavy seeds

Scarification: Unscarified heavy seeds had poor germination of 4%. Scarification significantly ($p < 0.05$) increased the germination percentage to 50% (table 3a). Scarified heavy seeds took about half the time of the unscarified seeds to germinate (table 3b), consequently, scarification nearly doubled the mean germination rate (table 3c).

Aeration: The unscarified aerated heavy seeds had low germination percentage of 7%. Scarification significantly ($p < 0.01$) increased the germination percentage of aerated seeds to 63% (table 3a). Aeration reduced the time of germination in unscarified seeds to 10 days and with scarification was further reduced to five days (table 3b). Scarification doubled the mean germination rate (table 3c).

Hot water treatment: Unscarified heavy seeds treated in hot water had low percentage germination of 10%. There was no significant increase between this and the untreated unscarified heavy seeds. Scarified heavy seeds immersed in hot water had significantly enhanced germination ($p < 0.05$) and reduced germination time when compared with unscarified heavy seeds immersed in hot water. The mean germination rate of scarified seeds was more than doubled that of unscarified seeds (table 3a-c).

Table 3A: Mean germination percentage (%) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with aeration and hot water.

Treatment	Mean germination percentage (%) 12M	6M	0M
T6	0.00	0.00	4.00
T7	0.00	5.60	7.20
T8	0.00	12.80	10.40
T9	8.00	44.80	49.70
T10	8.60	58.40	63.20
T11	8.80	70.00	72.80

Table 3B: Mean germination time (days) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with aeration and hot water.

Treatment	Mean germination time (days) 12M	6M	0M
T6	0.00	0.00	12.63
T7	0.00	10.00	10.07
T8	0.00	9.40	11.40
T9	6.73	6.99	6.99
T10	5.13	6.43	4.91
T11	7.19	6.63	5.09

Table 3C: Mean germination rate (d^{-1}) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with aeration and hot water.

Treatment	Mean germination rate (d^{-1}) 12M	6M	0M
T6	0.00	0.00	0.08
T7	0.00	0.10	0.10
T8	0.00	0.11	0.09
T9	0.15	0.14	0.14
T10	0.20	0.16	0.20
T11	0.14	0.15	0.20

Concentrated sulphuric acid (1, 2 and 10 minutes)

Unscarified heavy seeds treated with concentrated sulphuric acid for time periods of 1-10 minutes had mean germination percentages of 43-53%. This showed a significant increase ($p < 0.01$) when compared with the unscarified heavy seeds without treatment. The scarified heavy seeds treated with concentrated sulphuric acid had the highest germination percentage (57-82%) of all treatments (table 4a). The mean germination time for the heavy scarified seeds treated for 10 minutes with concentrated sulphuric acid was only 3 days as compared to 12 days in untreated unscarified seeds (table 4b). The mean germination rates for both the scarified and unscarified seeds were the same (table 4c).

Table 4A: Mean germination percentage (%) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with concentrated sulphuric acid.

Treatment (Concentrated sulphuric acid)	Mean germination percentage (%) 12M	6M	0M
CT1	12.00	23.20	43.00
CT2	17.60	27.20	44.80
CT3	20.80	38.40	52.80
CT4	16.80	52.00	56.80
CT5	18.40	56.00	68.00
CT6	20.80	70.04	82.40

Table 4B: Mean germination time (days) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with concentrated sulphuric acid.

Treatment (Concentrated sulphuric acid)	Mean germination time (days) 12M	6M	0M
CT1	6.71	4.12	3.50
CT2	5.31	4.17	3.32
CT3	5.20	3.64	3.03
CT4	6.81	3.54	3.50
CT5	6.16	3.50	2.79
CT6	6.19	3.14	3.01

Table 4C: Mean germination rate (d^{-1}) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with concentrated sulphuric acid.

Treatment (Concentrated sulphuric acid)	Mean germination rate (d^{-1}) 12M	6M	0M
CT1	0.15	0.24	0.29
CT2	0.19	0.24	0.30
CT3	0.19	0.28	0.33
CT4	0.15	0.28	0.29
CT5	0.16	0.29	0.36
CT6	0.16	0.32	0.33

Dilute sulphuric acid (30, 60 and 120 minutes)

Heavy unscarified seeds treated with dilute sulphuric acid had low germination of 3-9%. With scarification, there was a significant ($p < 0.05$) increase in the germination percentage of the heavy seeds treated with dilute sulphuric acid (table 5a). The time taken to germinate by the unscarified seeds was 10 days. This was reduced to 7 days by scarification (table 5b). The mean germination rate increased from 0.10 to 0.15 d^{-1} for the scarified seeds (table 5c).

Table 5A: Mean germination percentage (%) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with dilute (10%) sulphuric acid.

Treatment (Dilute sulphuric acid)	Mean germination percentage (%) 12M	6M	0M
CT7	0.00	3.20	3.20
CT8	0.00	3.20	4.80
CT9	0.00	5.60	8.80
CT10	3.20	44.00	49.60
CT11	4.80	46.40	55.20
CT12	6.40	56.80	60.00

Table 5B: Mean germination time (days) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with dilute (10%) sulphuric acid.

Treatment (Dilute sulphuric acid)	Mean germination time (days) 12M	6M	0M
CT7	0.00	9.50	10.00
CT8	0.00	9.50	9.75
CT9	0.00	8.60	9.13
CT10	5.00	6.58	6.57
CT11	5.00	7.82	6.72
CT12	8.40	6.06	6.57

Table 5C: Mean germination rate (d^{-1}) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with dilute (10%) sulphuric acid.

Treatment (Dilute sulphuric acid)	Mean germination rate (d^{-1}) 12M	6M	0M
CT7	0.00	0.11	0.10
CT8	0.00	0.11	0.10
CT9	0.00	0.12	0.11
CT10	0.20	0.15	0.15
CT11	0.20	0.13	0.15
CT12	0.12	0.17	0.15

Potassium permanganate solutions (30, 60 and 120 minutes)

Heavy seeds treated with 10% potassium permanganate solution had germination percentage of about 10-14% and 19-22% in the unscarified and scarified seeds. With 20% solution, there was no significant difference ($p>0.05$) between the germination percentages of the unscarified and scarified heavy seeds and between the seeds treated with 10% potassium permanganate solution (tables 6 and 7). Potassium permanganate solution significantly decreased ($p < 0.05$) the germination percentage of the scarified heavy seeds when compared with other scarified seeds (tables 3a-7a). There was no difference in the germination time of the heavy unscarified seeds and scarified seeds treated with 10% solution (table 6b), but the germination time was slightly shorter with the scarified seeds treated with 20% solution (table 7b). There was no difference in the germination rates of scarified and unscarified seeds treated with 10% potassium permanganate solution. However, scarified seeds treated with 20% potassium permanganate had faster germination rate (table 7c).

Table 6A: Mean germination percentage (%) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 10% potassium permanganate solution.

Treatment (10% $KMnO_4$)	Mean germination percentage (%) 12M	6M	0M
CT13	0.00	6.40	9.60
CT14	0.00	8.00	11.20
CT15	0.00	9.60	13.60
CT16	5.60	14.40	19.20
CT17	8.80	14.40	22.40
CT18	10.40	16.80	21.60

Table 6B: Mean germination time (days) of 12M, 6M and 0M scarified and unscarified heavy seeds of*C. fistula* treated with 10% potassium permanganate solution.

Treatment (10% $KMnO_4$)	Mean germination time (days) 12M	6M	0M
CT13	0.00	9.50	10.25
CT14	0.00	11.60	10.23
CT15	0.00	12.10	10.60
CT16	13.30	8.00	11.44
CT17	14.57	8.17	11.40
CT18	14.83	8.15	11.01

Table 6C: Mean germination rate (d^{-1}) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 10% potassium permanganate solution.

Treatment (10% $KMnO_4$)	Mean germination rate (d^{-1}) 12M	6M	0M
CT13	0.00	0.11	0.10
CT14	0.00	0.09	0.10
CT15	0.00	0.08	0.10
CT16	0.08	0.13	0.09
CT17	0.07	0.12	0.09
CT18	0.07	0.12	0.09

Table 7A: Mean germination percentage (%) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 20% potassium permanganate solution.

Treatment (20% $KMnO_4$)	Mean germination percentage (%) 12M	6M	0M
CT19	0.00	7.20	10.40
CT20	0.00	5.60	14.40
CT21	0.00	8.80	14.40
CT22	8.00	8.00	16.00
CT23	7.20	8.40	21.60
CT24	8.80	8.00	24.00

Table 7B: Mean germination time (days) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 20% potassium permanganate solution.

Treatment (20% $KMnO_4$)	Mean germination time (days) 12M	6M	0M
CT19	0.00	11.00	10.33
CT20	0.00	10.90	10.74
CT21	0.00	10.50	11.63
CT22	11.93	6.54	8.22
CT23	12.40	8.69	8.39
CT24	13.40	9.42	8.50

Table 7C: Mean germination rate (d^{-1}) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 20% potassium permanganate solution.

Treatment (20% $KMnO_4$)	Mean germination rate (d^{-1}) 12M	6M	0M
CT19	0.00	0.09	0.10
CT20	0.00	0.09	0.09
CT21	0.00	0.10	0.09
CT22	0.08	0.15	0.12
CT23	0.08	0.12	0.12
CT24	0.08	0.11	0.12

Copper sulphate solution (30, 60 and 120 minutes)

The effects of the 10% and 20% copper sulphate solutions on germination were about the same as the effects of the potassium permanganate solutions as shown in tables 8 and 9. However, the average time taken to germinate by the unscarified heavy seeds was longer than for the scarified seeds. With scarification, there was an increase in the mean germination rate (tables 8c and 9c).

Table 8A: Mean germination percentage (%) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 10% copper sulphate solution.

Treatment (10% $CuSO_4$)	Mean germination percentage (%) 12M	6M	0M
CT25	0.00	6.40	8.00
CT26	0.00	9.60	10.40
CT27	5.60	8.00	12.00
CT28	5.60	15.20	15.50
CT29	8.00	13.60	16.60
CT30	10.40	17.60	18.00

Table 8B: Mean germination time (days) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 10% copper sulphate solution.

Treatment (10% $CuSO_4$)	Mean germination time (days) 12M	6M	0M
CT25	0.00	10.17	9.50
CT26	0.00	11.20	10.00
CT27	10.60	10.87	10.00
CT28	9.00	7.88	8.73
CT29	8.70	8.95	9.20
CT30	6.60	7.82	9.66

Table 8C: Mean germination rate ((d^{-1}) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 10% copper sulphate solution.

Treatment (10% $CuSO_4$)	Mean germination rate (d^{-1}) 12M	6M	0M
CT25	0.00	0.10	0.11
CT26	0.00	0.09	0.10
CT27	0.09	0.09	0.10
CT28	0.11	0.13	0.12
CT29	0.12	0.11	0.11
CT30	0.15	0.13	0.10

Table 9A: Mean germination percentage (%) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 20% copper sulphate solution.

Treatment (20% $CuSO_4$)	Mean germination percentage (%) 12M	6M	0M
CT31	4.00	8.80	11.20
CT32	4.00	13.60	15.20
CT33	6.40	11.20	17.60
CT34	8.00	16.00	21.60
CT35	10.40	16.80	24.00
CT36	10.40	19.20	25.60

Table 9B: Mean germination time (days) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 20% copper sulphate solution.

Treatment (20% $CuSO_4$)	Mean germination time (days) 12M	6M	0M
CT31	10.63	10.10	10.43
CT32	12.80	10.50	11.55
CT33	11.90	9.80	9.87
CT34	7.17	7.00	7.61
CT35	6.18	7.74	7.09
CT36	6.97	7.62	7.76

Table 9C: Mean germination rate (day^{-1}) of 12M, 6M and 0M scarified and unscarified heavy seeds of *C. fistula* treated with 20% copper sulphate solution.

Treatment (20% $CuSO_4$)	Mean germination rate (d^{-1}) 12M	6M	0M
CT31	0.09	0.10	0.10
CT32	0.08	0.10	0.09
CT33	0.08	0.10	0.10
CT34	0.14	0.14	0.13
CT35	0.16	0.13	0.14
CT36	0.14	0.13	0.13

Storage

In all the experiments using seeds of different storage periods, the freshly harvested seeds (0M) had significantly higher germination percentage, shorter germination time and faster germination rate than the twelve months-stored (12M) seeds ($p < 0.05$) (tables 3-8). In fact, many twelve months-stored seeds did not germinate. For the six months stored-seeds (6M), even though there was no significant difference between their results and those of the freshly harvested seeds (0M), the freshly harvested seeds consistently had higher germination percentages and faster germination time (tables 3-5).

5. Discussion

In *C. fistula*, mechanical scarification resulted in significantly ($p < 0.05$) improved germination in the light and heavy scarified seeds when compared to the unscarified seeds (tables 3-5). This is probably due to

the fact that mechanical scarification might have helped in physically weakening the impermeable layer in the seed coat allowing water and air to enter the seeds thereby permitting the embryo to overcome the mechanical restriction of surrounding tissues.

Studies carried out by Todaria and Negim (1992) showed that mechanical scarification was effective in breaking the dormancy of the related species, *Cassia nodosa* Buch-Ham ex. Roxb. seeds. This result is also consistent with the results of other authors such as Al-Menaie et al. (2010) for *C. fistula* in Kuwait, Babely and Kandya (1998) for *C. fistula* in Egypt, Karaboon et al. (2005) for *C. fistula* in Thailand and Nalawadi et al. (1977) for *C. fistula* in India. Consequently, mechanical scarification may be an excellent, cheap and practical treatment to be used to break seed coat dormancy in tropical plant species. Scarified seeds also had higher mean germination rates which is indicative of rapid and uniform germination of seeds (tables 3-5).

The low germination percentages in unscarified light seeds bubbled with air in cold distilled water (table 2a) suggests that aeration had no effect on breaking the seed dormancy. This is in agreement with Amusa (2010) who observed that soaking seeds in cold water reduced the germination of *Afzelia africana* Sm. seeds due to oxygen deficiency. Scarified heavy seeds germinated better when aerated compared to both the control and the scarified and aerated light seeds. Heavy seeds are probably more viable and contain more food reserves than the smaller ones, which is helpful in germination by providing more energy (Lusk, 1995).

The low germination percentage for both the light and heavy unscarified seeds (T2 and T5) treated with hot water (tables 2a and 3a), could be due to the fact that the hot water did not act sufficiently enough to break or soften the impermeable hard seed coats thereby imbibition of water and air is still prevented. Consequently, the growth of the seed's embryo is inhibited. The significantly high germination in scarified heavy seeds in the 0M and 6M seeds in hot water at 84°C supports the assertion. This is in agreement with the studies of Agboola and Etejere, (1991) and Agboola and Adedire, (1998) who reported that immersion of dry seeds of tropical tree species in boiling water enhanced seed germination. Immersion of scarified seeds in hot water may lead to the quick and easier rupture of the coat wall allowing water to permeate the seed tissues causing physiological changes and subsequent germination of the embryo (Sabongari, 2001).

Heat damage was observed for *Cassia sieberiana* Dc. (Todd-Bockarie et al. 1993) although the seeds of this species still maintained high viability (75%) after 2 minutes' boiling; longer boiling and any dry heat treatment rapidly reduced viability. In *C. fistula*, a quick dip in boiling water (100^0C) killed 50% of the seeds and 68% were killed after 5 minutes in boiling water (Babeley and Kandya, 1988). Thus, boiling the seeds for a long time may be deleterious to germination. For *C. fistula*, immersion of seeds in hot water may be a quick and cheap method to break dormancy and produce large amount of uniform seedlings for afforestation and landscaping in Nigeria.

Treatment with concentrated sulphuric acid for a period of 10 minutes gave the highest percentage germination (82.4%) for the freshly harvested scarified seeds (table 4a) indicating that the seed coats were quickly ruptured, thereby leading to a faster germination rate. The medium percentage germination 42 – 53% found in unscarified seeds, CT1, CT2 and CT3 (table 4a) suggests that the time of immersion was not long enough for adequate rupture of their seed coat. Sulphuric acid is believed to rupture the seed coat and expose the lumens of the macrosclereids cells thereby allowing imbibition of water which triggers germination (Nikolaeva, 1977). In the unscarified seeds, water and air are not available to the embryo due to the impervious testa. Mechanical scarification combined with the effect of concentrated sulphuric acid made the seed coats more permeable to water and air thereby resulting in the best germination percentage (table 4a).

Duration of acid pretreatment should aim at reaching a balance in which the seed-coat (or pericarp) is sufficiently ruptured to permit the seed to imbibe, but without the acid itself reaching the embryo. Kobmoo and Hellum (1984) found that 15-45 minutes soaking in concentrated sulphuric acid was highly effective in *Cassia siamea* Lam. resulting in about 98% germination, while germination was lower for both shorter and longer exposure. Ten minutes immersion in concentrated sulphuric acid which produced the highest germination in this study was apparently not long enough to fully overcome dormancy in *C. fistula*. In using concentrated sulphuric acid to break seed dormancy, caution must be taken not only of the concentration of the acid, as some scientists regard 50% as being concentrated, but also of the thickness of the testa.

The low germination of the scarified and unscarified heavy *C. fistula* seeds treated with potassium permanganate and copper sulphate solutions indicates the ineffectiveness of copper sulphate and potassium permanganate solutions in breaking its seed dormancy. Similar result was reported for *Uraria picta* Jacq., a tropical species of hard coat family by Okusanya et al. (1992). The significantly low germination with scarified seeds treated with potassium permanganate and copper sulphate in comparison with other scarified seeds (tables 6-9) indicates that these solutions and/or the concentrations used could possibly be toxic to the embryo.

Comparison of various treatments revealed that treatments such as mechanical scarification with sand paper and application of concentrated sulphuric acid enhanced germination in *C. fistula* seeds (table 4a). It can therefore be deduced that seed dormancy in *C. fistula* is seed coat induced, since the treatments which enhanced germination and reduced the mean germination time as well as increased the germination rates were those that degraded or ruptured the seed coat. When seed coat is softened, the process of hydrolysis could commence to release simple sugars that could be readily utilized in protein synthesis as well as the release of hormones such as auxins and ethylene, which could increase nucleic acid metabolism and protein synthesis (Irwin 1982, Jackson 1994).

The ecological significance of dormancy in *C. fistula* is that the impermeability of seed coat to water ensures good chances of survival for the plants in tropical regions. Dormancy has evolved as a strategy to avoid germination under conditions where seedlings survival is likely to be low. By producing seeds with different degrees of dormancy, or dormancy which is gradually broken by environmental factors, e.g. gradual abrasion of hard seed-coats by sand, leaching of inhibitors by rainwater (Brown 1972, Villiers 1972), the plant species saves part of the seed pool, so that some of the seeds are likely to germinate when conditions are favourable for seedling establishment (Mayer and Poljakoff-Mayber, 1982).

Physical dormancy is also related to dispersal. For most species, germination must be delayed until the seeds have reached a safe site, i.e. after dispersal. A hard seed-coat in legumes or endocarp in drupes usually protects fruits or seeds adapted to dispersal by ingestion. These coverings also often restrict water uptake and thus imbibition, but ingestion usually partly abrades the seed-coats (Halevy 1974, Winer 1983). As the fruit pulp is eaten by animals, decomposed by bacteria and fungi, or washed away by rain, the pulp and inhibitors are removed.

Dormancy is influenced by developmental, genetic and environmental factors and interaction between the three. Within any species, dormancy may vary from very shallow to very deep between different seed lots and between individual seeds in the lot (Lacerda et al. 2004). The production of seeds with variable levels of dormancy leads to a diverse seed bank in the soil, reduces seedling competition and also helps to avoid the simultaneous development of all seedlings within a given population, which would be undesirable in a given year where growing conditions happen to be inadequate (Andersson and Milberg, 1998).

The germination percentages, germination time and rate of the seeds used in these experiments are an indication of the degree and variation of dormancy in the population of *C. fistula* in Covenant University. Other factors that may lead to differences in germination percentages among or within populations are the age and the nutritional status of the mother plant during seed maturation (Fenner, 1991), the environmental conditions prevailing at the time of seeds production (Okusanya and Ungar 1983), seed position on the mother plant (Tieu et al. 2001), seed size and shape (Baloch et al. 2001, Jones and Nielson 1999), the time since seed harvest and the duration of seed storage (Santarém and Aquila 1995). It is not clear which ones of the above apply to the *C. fistula* population in Covenant University, but nutritional status of the mother plants during seed maturation seems most plausible. This is because the population which occupies a large area on the vast Covenant University campus is on different soil types (Okusanya, unpubl. data). Also, the population is of the same age, seed sizes are fairly uniform since they were initially sorted by weight and the seeds were harvested at the same time.

C. fistula seeds from Covenant University population obviously lost viability with time of storage at room temperature because of reduced germination percentage and increased germination time as period of storage increased (tables 2 – 5). This is similar to the result for *Leucaena leucocephala* Lam. (Duguma et al. 1988). The results however indicate that seeds of *C. fistula* can be stored well for six months at room temperature since there was no significant difference between the results of seeds stored for six months (6M) and those of freshly harvested (0M) seeds (tables 2 - 5). Schmidt (2000) reported that there is loss of seed viability in Fabaceae as seed moisture content decreased. In tropical condition as in Nigeria, the seeds of *C. fistula* would lose moisture with time at room temperature and consequently viability. Another experiment is being planned to determine the best storage conditions for the seeds of *C. fistula* in Nigeria.

It is concluded that mechanical scarification in conjunction with concentrated sulphuric acid is probably the most efficient method of breaking seed dormancy in *C. fistula* from the population in Covenant University, but hot water immersion in conjunction with physical scarification is recommended as the most cost effective method for rapid and uniform seed germination in nurseries for use in afforestation, landscaping and pharmacological programmes in Nigeria.

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