

GERMINABILITY AND VIABILITY OF *ALBIZIA SAMAN* SEEDS SUBJECTED TO THREE SCARIFICATION METHODS

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ABSTRACT

Three trials were conducted at the Teaching and Research Farm of the University of Agriculture, Abeokuta, Nigeria, on germination and viability of rain tree (*Albizia saman*) seeds. In the first trial, whole *A. saman* pods containing an average of 1000 seeds were fed to three breeds of cattle (N'dama, Muturu and White Fulani). In trial 2, uningested whole seeds were treated with hot water at different temperatures (30 - 75°C) for varying length of time (5 - 40 minutes) and the untreated seeds as control. The last trial involved the use of uningested seeds treated with concentrated sulphuric acid for similar periods of time as with hot water. The seeds that did not germinate after 50 days were treated with tetrazolium to determine their viability. The results of this study showed that the number of germinated seeds was significantly ($p < 0.05$) influenced by both the breed of cattle and the length of seed ingestion. There was improvement in seed germination after ingestion compared to uningested seeds. Maximum germination was obtained for seeds immersed in hot water at 50°C for 35mins. Treating seeds with concentrated sulphuric acid gave the highest percentage germination of seeds (90 -100) compared to hot water (15-100), cattle (34-49) and untreated (30).

Key words: germinability, viability, seeds, scarification, cattle

INTRODUCTION

Albizia saman (Jacquin) F. Mueller is sometimes referred to as *Samanea saman* (Jacquin) Merrill, *Enterolobium saman* (Jacquin) Prain ex king, *Pithecelobium saman* (Jacquin) Benth, *Mimosa saman* (Jacquin), but *Samanea saman* is the most preferred (George and Craig, 2005) with these common names: rain tree, monkey pods and saman. The name rain tree has been attributed to the leaflets which are light-sensitive and closes up on cloudy days (as well as from dusk to dawn) allowing rain to fall through the canopy to

the ground like rain. In addition, nectarines on the leaf petioles excrete sugary juice that sometimes drop from the tree like rain (SPPIA, 2005).

Potential of rain tree as source of feeds for livestock lies in its pods and leaves. The seeds are indehiscent and remain on the tree until removed by harvesters, browsers, natural senescence or mechanical action such as rain and wind. Moreover, rain tree is known to produce and shed its pod at peak of dry season in November-March (Aribido, 1988). Ruminant animals ingest

large quantity of seeds with their feeds. The digestion or survival of seeds during passage through the digestive tract of grazing animals has implication on the population dynamics of plants species and for the nutrition of grazing animals (Gardener *et al.*, 1993). When animal consume the pods, the seeds are either passed out with faeces or are digested by the animals. The survival of ingested seeds and recovery from ruminant faeces vary with plant species, seeds characteristics (shape, size and hardness), animal species and the proportion of the seeds in the diet (John *et al.*, 1992; Jolaosho *et al.*, 2006a).

Bradbeer (1988) reported that seed viability is a measure of how many seeds are alive and could develop into plants which will reproduce themselves, given the appropriate conditions. Germination potential, is perhaps the most important quality measurement in seed testing, it is used to determine sowing rates as well as whether seed must be sown immediately or can be stored. Forest Nursery Manual (1984) stated that seeds of different species have different requirements for optimum germination. The potential can be evaluated directly by germinating seeds under predetermined positions or estimated indirectly with biochemical staining, embryo excision, cutting tests, x-ray radiography or hydrogen peroxide tests.

Previous research by Jolaosho *et al.* (2006b) had shown that the highest proportion (60%) of rain tree seeds was dispersed by grazing animals after 2 days of ingestion and the same proportion of the seeds will germinate after 96 hours of ingestion. The aim of this study is to determine the effect of longer time of ingestion

(5days) on the total number of the recovered seeds from penned animals and the viability after 50days of germination test. This study also examined closer regimes of temperature of hot water (30-75°C) to improve the germination percentage reported for *A. saman* (Jolaosho *et al.*, 2006b).

MATERIALS AND METHODS

Test Trials

Three trials were carried out to determine the percentage germination and viability of rain tree seeds which were: (i) recovered at different intervals after ingestion by cattle; (ii) treated with hot water and (iii) concentrated sulphuric acid for varying periods. The trials were carried out at the Teaching and Research Farms of the College of Animal Science and Livestock Production and the laboratory of the Department of Pasture and Range Management, University of Agriculture Abeokuta, Ogun State, Nigeria (6°20'N, 2°41'E).

Seed Collection, Weight and Proportion of Seeds in Pods

The pods of *Albizia saman* were collected from the University of Agriculture, Abeokuta main campus from December, 2005 to March, 2006. Twenty (20) samples of the whole pods were weighed. The pods were opened to obtain the seeds to determine the average number of pods that will give an average of 1000seeds.

Trial 1: Intake and recovery of seeds ingested by cattle.

The experiment involved three breeds of cattle namely White Fulani, N'dama and Muturu with three animals per breed with an average live weight of 230, 118 and 130kg, respectively. The experimental animals were housed in individual pens where

feed and water were provided *ad libitum*. This allows easy collection of the faeces and to avoid any loss of seed through grazing. They were fed individually once early in the morning with pods of rain tree containing an average of 1000 seeds. The faeces from each animal were collected for five (5) consecutive periods after ingestion (24, 48, 72, 96 and 120 hours). The faeces collected were dispersed in water and the seeds were separated using 2mm mesh size sieve. The seeds collected were air dried and the intact and broken/partially chewed seeds were counted. Intact seeds were those which were not deformed or broken and which had no visible holes.

Trial 2: Hot water treatment

Intact seeds obtained directly from pods of rain tree were soaked in hot water at 10 regimes of temperature (30, 35, 40, 45, 50, 55, 60, 65, 70 and 75°C) for 8 periods (5, 10, 15, 20, 25, 30, 35 and 40 minutes) in water bath with regulated temperature. The untreated seeds served as the control.

Trial 3: Acid treatment

Seeds obtained directly from the pods of rain tree were soaked in sulphuric acid (H_2SO_4) of two concentrations (undiluted acid and diluted acid at 5 moles concentration) for eight periods (5, 10, 15, 20, 25, 30, 35 and 40 minutes) while the untreated seeds served as control. The seeds were then removed from the acid and washed thoroughly under running tap water immediately before they were planted.

Planting procedure, germination and viability testing.

For trial 1, all the seeds recovered from the faeces of each of the animal at differ-

ent periods were planted in Petri dishes on moist cotton wool which were watered daily. The seeds were considered to have germinated when the cotyledons had emerged from the testa or seeds had radicle more than 5mm long. The germinated seeds were counted daily and recorded. The seeds that did not germinate after 50 days were tested in tetrazolium solution to determine their viability. A total of 45 petri dishes were used for this experiment comprising of three (3breeds) X three (3 animals as replicates) X 5 periods of ingestion.

For trial 2, a total of 243 petri dishes were used comprising of ten (10) temperature regimes X eight (8) periods with each treatment replicated 3 times. Each Petri dish contains 10 intact rain tree seeds. Same number of seeds were selected for the untreated control.

For trial 3, ten (10) intact seeds for the acid treated and untreated/control treatments were selected for the germination test. The treatments were replicated 3 times thus a total of 51 petri dishes were used for the experiment.

The number of seeds that germinated (NGS) was recorded daily until no further seeds germinated after 50, 45 and 5 days in Trials 1, 2 and 3, respectively.

Cumulative germination percentage was calculated with the formula:

Cumulative germination =

$$\frac{\text{Total number of seeds that germinated} \times 100}{\text{Number of seeds sown}}$$

Mean germination time was calculated with the formula:

$$[\sum (nxt)]/N$$

where, n is the number of new germinants; t is the time when half of the total germinant was recorded; and N is the final grand total of germinants.

Viability testing

The remaining seeds that did not germinate from the animal trial and hot water treatments were soaked differently in 2, 3, 5-triphenyl tetrazolium chloride solution until the colourless solution changed to red. The seeds were then opened and the seeds with red embryo were considered viable. The seeds from acid treatments were not treated with tetrazolium solution because high percentage germination was recorded within the five days.

Data collection

The following data were collected: number of seeds recovered (NSR) from animal, number of germinated seeds from recovered seeds (NGS), germinated seeds from hot water treatment for each temperature at different levels of immersion period and number of germinated seeds from sulphuric acid treatment.

The above data were used to obtain the following parameters:

%Seed recovered =

$$\frac{\text{number of recovered seeds} \times 100}{1000}$$

$$1000$$

%Seed germination =

$$\frac{\text{Germinated seeds} \times 100}{\text{Number of recovered seeds}}$$

For hot water and acid treatments,

$$\% \text{ Germination} = \frac{\text{Germinated seeds} \times 100}{10}$$

% Daily germination =

$$\frac{\text{Number of seeds germinated per day} \times 100}{\text{Number of seeds planted}}$$

Statistical Analysis

The effect of animal breeds and times of seed retention in the rumen were determined using 3 x 5 factorial experimental design. The effect of hot water on viability was determined using 8 x 10 factorial design with 8 periods of immersion and 10 levels of temperature. The effect of acid on viability was determined using 2 x 9 factorial design.

Data collected were subjected to analysis of variance using General Linear Model of Minitab (1998) statistical package. Tukey's pairwise comparison was used to compare differences among individual means

RESULTS

Seed weight

Mean pod weight and thousand seed weights were 14 and 185g, respectively. There were 4500 – 5990 seeds/kg with an average of 5405 seeds (Table 1).

Table 1: Weight of pods and seeds of *Albizia saman* used in the study

Parameter	Average	Range
Pod weight (g)	13.78	7.76-20.25
Number of seeds/pod	18	12-25
Weight of seeds/pod (g)	3.57	2.72-4.48
Weight of seeds + pod (g)	17.38	10.82-25.11
Seed weight (g)	0.20	0.17-0.27
1000seed weight	185	170-220
Number of seeds/kg	5405	4545-5882

Effects of Breeds and retention time on the recovered seeds and seeds germination

Breed of cattle appeared to have no significant effect ($p>0.05$) on the numbers of recovered (NRS), intact (IS), germinated

(NGS), ungerminated seeds (NUG) and percentage germination (PG) (Table 2). The highest and least numbers of germinated seeds (NGS) and viable seeds (NV) were recorded for seeds that passed through N'dama and Muturu, respectively. The percentage viability of seeds from White Fulani was least.

Table 2: Effect of breed on number of recovered (NRS), broken (BS), Intact (IS), germinated seeds (GS), Ungerminated Seeds (UG), Viable seeds (NV) Percentage Germination (PG), and viability (PV)

Breeds	NRS	BS	IS	NGS	PG	NUG	NV	PV
WF	130 ¹	26	104	40	38.5	64	28b	43.8b
Muturu	81	22	59	27	45.8	32	19c	59.4a
N'dama	147	29	118	55	46.6	63	40a	63.5a
Total	358	77	281	122		159	87	
Mean	119	26	94	41	44	53	29	55.6
SD	5.3	1.3	4.4	1.6	6.0	3.1	1.5	6.9
P=	0.198	0.730	0.154	0.078	0.089	0.193	0.152	0.052

¹ Means in the same column with different subscripts are significantly different at $P\leq 0.05$

The time that seeds spent in the rumen had a great influence ($p < 0.05$) only on the number of intact (IS), germinated seeds (NGS) and viable seeds (NV) as shown in Table 3. Seed excretion increased with the length of retention in the rumen up to 96 hours and similar trend was observed in the number of intact seeds. The highest and least number of intact seeds was recovered at 96 and 24 hours after ingestion, respectively. There was no difference ($p < 0.05$) in the number of germinated seeds recovered at 72 hours (111 seeds) and 96 hours (108 seeds).

From Table 4, the differences in total number of seeds recovered from the breeds were not significant ($p \leq 0.05$). The average number of seeds recovered in 5 days from the 3 breeds represents 60% of the number fed. The seeds recovered from the faeces of animal gave higher germination percentage than the untreated seeds. The percentage germination values of the recovered seeds after 24 hours and that of the untreated seeds were 45.3 and 30.0%, respectively. The highest germination values were obtained through N'dama cattle and seeds voided after 120 hours had the highest percentage germination.

Table 3: Effect of period of ingestion on number of recovered (NRS), broken (BS), Intact (IS), germinated seeds (NGS), ungerminated Seeds (NUG), viable seeds (NV) Percentage Germination (PG), and viability (PV)

Period of ingestion (hrs)	NRS	BS	IS	NGS	PG	NUG	NV	PV
24	501	16	34c	21d	45.3	22	6d	27.3
48	118	42	76b	57c	48.0	45	14c	31.1
72	165	27	138a	111a	68.0	77	31a	40.3
96	175	29	146a	108a	64.2	87	24b	27.6
120	94	17	77b	73b	77	37	12c	32.4
Total	602	131	471	370		268	87	
Mean	120	26	94	74	60.5	54	17	31.7
SD	6.8	1.7	5.6	2.0	7.8	4.0	2.0	8.9
P=	0.079	0.289	0.041	0.020	0.559	0.080	0.035	0.981

¹ Means in the same column with different subscripts are significantly different at $P \leq 0.05$

Table 4: Number of seeds recovered and germinated after ingestion by cattle.

Period of ingestion (hrs)	Recovered Seeds				Germinated Seeds			
	WF	Muturu	N'dama	Mean	WF	Muturu	N'dama	Mean
24	60.0	51.7	38.5	50.0	23.3 (38.8) ¹	10.0(19.3)	30.0(77.9)	21.1(45.3)
48	125.0	101.6	127.6	118.1	50.0(40)	50.0(49.2)	70.0(54.9)	56.7(48.0)
72	158.6	55.0	283.3	165.6	100(63.1)	40(72.7)	193.3(68.2)	111.1(68.0)
96	206.4	125.0	193.4	174.9	96.7(46.9)	96.7(77.4)	133.3(68.7)	108.9(64.2)
120	98.5	70.0	114.9	94.5	93.3(94.7)	46.7(66.7)	80.0(69.6)	73.3(77.0)
Untreated					9.0(30)	9.0(30)	9.0(30)	9.0(30.0)
Total	647	404	757	602	363.3	243.4	506.6	
Mean	129.7	80.67	147.0		72.66(56.7)	48.68(57)	101.32(67.9)	
SD	11.756	11.756	11.756		3.532(13.48)			

¹Values in parentheses are percentages of the number of seeds recovered and germinated.

Effects of temperature and time of immersion in hot water on seeds of rain tree

Seed germination improvement was 100% with hot water treatment up to 50°C at 35min (Fig. 1). A threshold appeared to exist between 50 and 60°C for maximum germination before declining. The effect of temperature of scarification was significant ($p < 0.05$) on the number of germinated rain tree seeds, number of ungerminated seeds, number of viable seeds and percentage viability (Table 5). Generally,

water heated to 55 and 60°C gave the highest germination percentages and least numbers of ungerminated but viable seeds. Temperatures above 70°C gave the least germination percentages which were even less than values recorded for the untreated seeds. The highest number of ungerminated seeds was at 0-35°C and 70-75°C. The highest percentage viability of ungerminated seeds was between 0 and 45°C. The influence of immersion time of seeds in hot water was not consistent.

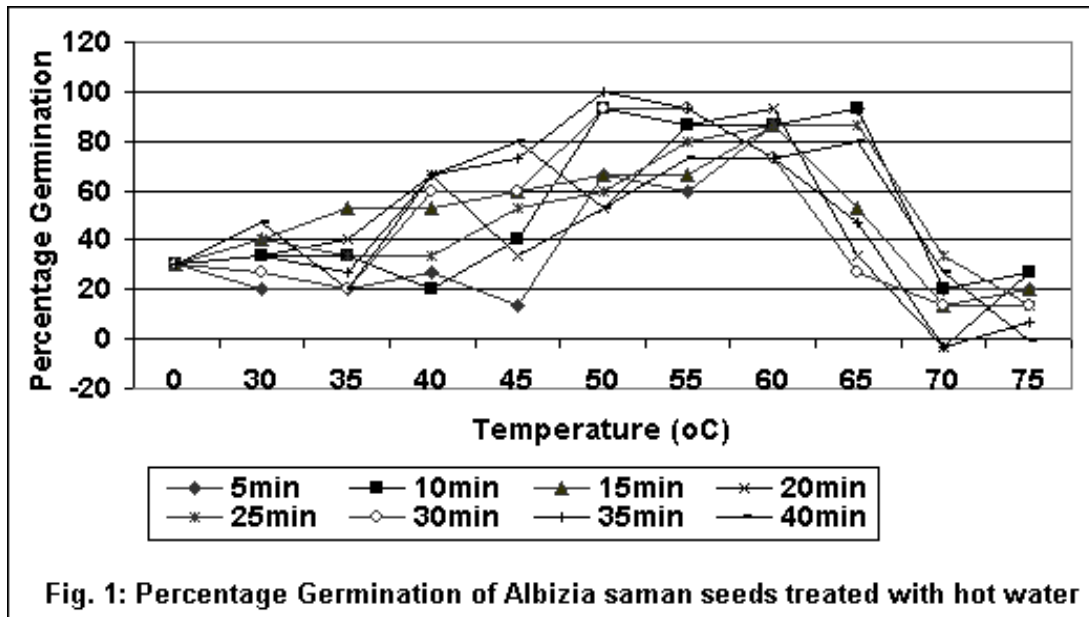


Table 5: Effects of Temperature and Time of immersion in hot water on number of germinated seeds (NGS), ungerminated seeds (NUG), viable seeds (NV) and percentage viability (PV).

oC	Temperature				Mins	Time			
	NGS	NUG	NV	PV		NGS	NUG	NV	PV
0	3e	7.0a	3.7a	56.2a	5	4.3b	5.7a	2.6ab	42.5a
30	3.4e	6.5a	3.8a	55.0a	10	5.3a	6.7b	2.0bc	36.9ab
35	3.1e	6.9a	2.5b	37.6b	15	5.1a	4.9ab	2.2bc	43.3a
40	4.9cd	5.1c	2.3b	50.3a	20	4.7ab	4.3bc	1.9bc	30.4c
45	5.2cd	4.8c	2.7b	52.5a	25	5.2a	4.8ab	2.1bc	38.6ab
50	7.3ab	2.7d	1.2c	32.5b	30	4.8ab	4.6bc	1.9bc	32.7bc
55	8.0a	2.0de	0.8c	29.9c	35	5.2a	3.5d	1.4c	29.4c
60	8.3a	1.8de	0.7c	27.4c	40	5.2a	3.7cd	1.8bc	44.3a
65	6.4c	3.6cd	1.3c	29.3c					
70	1.6f	5.5ab	2.4b	31.2c					
75	1.6f	6.3a	2.1b	26.9c					
SD	0.263	0.325	0.207	4.636		0.235	0.291	0.185	4.147
P	0.000	0.000	0.000	0.000		0.022	0.000	0.001	0.05

¹ Means in the same column with different subscripts are significantly different at P≤0.05

Effects of treatment and duration with concentrated sulphuric acid

Scarification of *A. saman* seeds with concentrated sulphuric acid gave percentage germination of 93.0 to 100 (Table 6). There were no significant differences in the germination percentage values among the times (5-40mins) of soaking the seeds in undiluted acid. The percentage germi-

nation was significantly ($p < 0.05$) higher when rain tree seeds were treated with undiluted sulphuric acid than at 5% diluted level (46.7 – 80%) than the untreated seeds. Use of concentrated sulphuric acid to scarify the seed is more effective and consistent with 100% germination from 30 to 40 minutes of immersion than at 5% dilution level.

Table 6: Effect of time of immersion in sulphuric acid on germination of *A. saman* seeds.

Immersion period (Minutes)	Undiluted	5% Dilution	Means
0	30.0	30.0	30.0e
5	93.3	53.3	73.3d
10	90.0	73.3	91.7a
15	93.3	80.0	96.7a
20	96.6	53.3	75.0c
25	96.6	66.7	81.7b
30	100	73.3	96.7a
35	100	46.7	73.3d
40	100	53.3	76.7c
SED	3.58	3.58	
Average	88.7A	58.9B	

a,b Means in the same column with different subscript are significantly different at $P < 0.05$

A, B. Means in the same row with different subscript are significantly different at $P < 0.05$

Effect of period of ingestion on the germination time

Half of the seeds recovered from White Fulani germinated within 6 days while those from N'dama and Muturu were faster and similar (4 days) as shown in

Table 7. Seeds recovered after 48 hours of ingestion had the highest mean germination time of 6 days while others are between 4 and 5 days. Seeds recovered from cattle germinated over 30-48 days with half of the seeds germinating within 3-7 days.

Table 7: Mean germination time and time of final germination for seeds recovered from ruminant animals.

Period of ingestion (hours)	Mean germination time (days)				Time of final germination (days)			
	Muturu	N'dama	White Fulani	Mean	Muturu	N'dama	White Fulani	Mean
24	3	4	6	4	10	48	39	32
48	6	6	7	6	34	48	48	43
72	3	4	5	4	43	48	48	46
96	4	4	6	5	46	49	49	48
120	6	3	4	4	48	33	47	43

Effects of water temperature, acid and duration of immersion on the germination time

The seeds treated with hot water germinated within 6 to 40 days with half germi-

nating within 1-9 days, those treated with concentrated sulphuric acid germinated fully within 4-5days with mean germination within 1-2days (Table 8).

Table 8: Mean germination time and time of final germination for seeds treated with hot water and acid.

Temperature (°C)	Mean Germination Time (days)										Time of Final Germination (days)							
	Immersion period (min)																	
Hot Water	5	10	15	20	25	30	35	40	Ave	5	10	15	20	25	30	35	40	Ave
30	9	7	9	6	7	7	7	7	7	34	38	41	32	41	41	41	41	39
35	8	8	8	5	3	9	2	8	6	36	41	36	33	29	41	7	34	37
40	9	6	8	6	9	3	4	4	6	41	36	35	41	41	26	26	26	34
45	4	5	6	6	3	3	3	2	4	18	45	35	31	23	18	35	18	28
50	3	4	4	4	2	4	5	2	4	26	23	23	26	20	31	35	18	25
55	5	2	3	5	6	3	4	4	4	45	12	10	35	29	33	18	29	26
60	3	5	2	3	2	2	1	2	3	18	34	12	23	21	9	7	18	18
65	4	2	1	2	2	2	3	1	2	36	10	7	10	12	10	12	12	14
70	1	2	1	0	2	2	0	1	1	5	10	5	0	12	9	0	3	6
75	1	2	1	2	2	4	3	0	2	7	10	4	12	10	18	9	0	9
Acid	2	1	2	2	2	2	2	2	2	4	5	5	4	5	4	4	4	4

DISCUSSION

Since the pods were fed directly to the animals, some seeds were recovered broken, as a result of their exposure to chewing during ingestion. This contradicts the report of Lamprey (1967), that most tree seeds ingested by ruminants are voided without being damaged. Furthermore, the number of seeds recovered was lower than when seeds are placed directly in the rumen. The non significant increase in number of recovered seeds with increase in time up to the fourth day (96h) could be due to the hardness of rain tree seeds which conforms to the report of Gardener *et al.* (1993) that hard seeds are retained longer in the digestive tract than soft seeds which disintegrate after 2 days in the tract. In this study, the recovery rates were lower than that reported by Jolaosho *et al.* (2006b), this is because the animals used for this study were younger. Halevy, (1974) and Lamprey *et al.* (1974) reported that passing of seeds through ungulates accelerates germination by scarification of the seed coat. This study also affirmed our earlier study that feeding of *A. saman* seeds to small sized cattle especially the N'dama results in higher germination and viability (Jolaosho *et al.*, 2006b). That about half of the ungerminated seeds were still viable showed that at harvest, the seeds were physiologically immature (George and Craig, 2005).

Germination percentage with hot water declined after 60°C. This is in conformity with the report of Erasmus and Pieterse (2001) that higher temperature regimes resulted in a rapid decrease in germination percentage. This could probably be due to a loss in seed viability at higher temperatures.

The high percentage germination of *A. saman* seeds treated with concentrated sulphuric acid may be attributed to the corrosive characteristics of the acid on the seed testa which facilitated imbibitions of water by seeds and germination ability. This supports the findings of Zodape (1991) who observed that soaking of seeds of forest species in concentrated acid for 5-75 minutes enhanced germination. Although, treating seeds with concentrated sulphuric acid increased germination rates to 90-100% and all seeds germinated within 5 days, which is often undesirable, since establishment can fail where rains are unreliable especially germination.

CONCLUSION

This study showed that passing seeds of *A. saman* through the digestive system of cattle enhanced germination but at lesser rate and frequency compared with treatments using concentrated acid and hot water. More viable seeds were recovered from cattle after 72hours of ingestion. The utilization of *Albizia saman* pods by cattle is limited by a high proportion of seeds passing through the digestive system undigested. To reduce the high costs involved in bush clearing, dung voided by animals fed rain tree seeds could be collected and distributed along the boundaries to form live fences. Based on the result of this study, it is recommended that cattle be confined for at least 3 days after feeding with rain tree seeds to allow the passage of viable seed. Rain tree pod inclusion as non-conventional feedstuff in compounded feed for cattle should be encouraged. Further studies should be extended into effects of anti nutritional substances contained in the rain tree pods and leaves on the animals.

Although, concentrated sulphuric acid treatment gave the best result, the hot water treatment could be recommended for use because it gives almost the same result with that of acid and that it is easier to handle and less costly. In the case of acid treatment all the seeds germinated within 5 days compared with hot water treatment that spreads up to 40 days. So, if there is any dry period after planting the seeds treated with concentrated acid will be low. Further studies are needed with hot water treatment at lower periods of immersion than the level used in this study.

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