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Effect of Fresh Cow Milk and Coconut Milk on the Germination of *Tamarindus indica* Seeds

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ABSTRACT

To meet the current demand for the forest products through domestication, there is need to embrace cheap, fast, natural, accessible and adoptable physiological techniques as soaking of seeds in fresh cow milk and coconut milk. Fresh cow milk and coconut milk contain hormones that relieve seeds of photo, thermo, physiological and mechanical dormancy. There is dearth of information on the effects of natural sources of hormones such as fresh cow milk and coconut milk on the seeds of agro-forestry tree species. In light of this, this study was conducted to assess the effects of fresh cow milk and coconut milk on the germination percentage and mean germination time of Tamarindus indica seeds. The study involved two experiments, which were laid in split-plot experimental design with four replicates to assess the effect of concentrations of fresh cow milk (25, 50, 75 and 100%) and treatment times (0, 6, 8, 12 and 14hours) and concentrations of coconut milk (25, 50, 75 and 100%) and treatment times (0, 6, 8, 12 and 14hours) on the germination of seeds. Result revealed that the percentage germination value of seeds soaked in all concentrations of fresh cow milk for all time of treatments ranged from 70% to 100%. The percentage germination ranged from 65% to 100% for all concentrations and treatment times for seeds soaked in coconut milk. A significant germination percentage value of 100% was recorded for seeds treated for 14 hours in 50% and 100% concentrations of coconut milk and fresh cow milk respectively compared to control. Soaking of T. indica seeds for 14hours in 50% of coconut milk and 100% of fresh cow milk are recommended for mass production of its seedlings for agro-forestry programmes.

Key words: Fresh cow milk, Coconut milk, Hormones, Germination, Physiology

INTRODUCTION

Tamarindus indica is indigenous to tropical Africa, particularly in the Sudan and cultivated in Cameroon, Nigeria and Tanzania (Morton, 1987). It belongs to the dicotyledonous family leguminosae, sub family Caesalpiniaceae which is the third largest family of flowering plants with a total of 727 genera and 19,327 species (Lewis *et al.,* 2005). The common names for the species are Tsamiya, Ichekuoyibo, Ajagbon, Tamarind in Hausa, Ibo, Yoruba and English languages, respectively. The young seedlings, leaves and flowers of mature trees are eaten as vegetables and are used in curries, salads and soup. Its sour prods are cooked as seasoning with rice, fish and meats.

Tamarindus indica fruit pulp is used for the preparation of beverages in different parts of the world, including the Northern parts of Nigeria and other West African countries (Atawodi *et al.,* 2014). *Tamarindus indica* fruit contains high levels of carbohydrate, which provides energy and has good content of protein with many essential amino acids that help to build strong and efficient muscles (Atawodi *et al.,* 2014). It is also rich in the minerals: potassium, phosphorus, calcium, magnesium and can provide small amounts of iron as well as vitamin A (Samina *et al.,* 2008). Phytochemical investigations of the aerial parts of this plant have demonstrated the presence of tartaric, acetic, citric and succinic acids, gum, pectin, sugar, tannins, alkaloids, sesquiterpenes and glycosides (Aida *et al.,* 2001). *Tamarindus indica* is a plant that is widely used in traditional medicine in Africa for the treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastro intestinal disorders (Ferrara, 2005). Pharmacological investigation on *T. indica* extracts revealed that the plant has antibacterial, antifungal, hypoglycaemic, cholesterolemic, cytotoxic, anti-inflammatory, gastrointestinal (Coutino-Rodriguez *et al.,* 2001), hypolipomic and antioxidant activities (Ferrara, 2005; Martinello *et al.,* 2006). *Tamarindus indica* extracts were found to have anti-miracidial and anti-cercarial activities, anti-diabetic

potency and anti-burkholderia pseudomallei (*Pseudomonas pseudomallei*) activity (Rajkumar *et al.,* 2005).

In spite of the enormous potentials of *T.indica*, it is still faced with low rate of domestication as a result of dormancy of its seeds (Ajiboye *et al.,* 2009). Ajiboye (2010) reported that the *T. indica* seeds do not germinate when placed under conditions which are normally regarded as favourable for germination and therefore, it is dormant. Inadequacy of simple, cheap, fast, natural, accessible and adoptable modern physiological methods such as the use of fresh cow milk and coconut milk to break dormancy of the *T. indica* reduces the domestication potential of the species. Most of the methods of pre-sowing treatment such as physical, chemical and mechanical scarification only degrade the seed coat for germination (Aliero, 2004; Abubakar and Muhammad, 2013); without, always, rapidly and uniformly influencing the physiology of the seeds (Dewir *et al.,* 2011) and seedlings (Gehlot and Kasera, 2012) as well as not overcoming physiological dormancy of seeds (Habib *et al.,* 2015).

There is dearth of information on the effect of natural sources of hormones such as fresh cow milk and coconut milk on the germination of the seeds of agro-forestry tree species compared to synthetic sources. In light of this, this experiment was conducted to assess the effect of fresh cow milk and coconut milk on the germination and mean germination time of *T. indica*. Hormones help to relieve photo, thermo and physiological dormancy in seeds (Schmidt, 2000) as well as encourage mass production of seedlings for agro-forestry programmes (Adelani *et al.,* 2014a).

MATERIALS AND METHODS

Experimental Site

The pot experiment was carried out at the nursery site of Federal College of Forestry Mechanization, Afaka, Kaduna state. The college is located in the Northern Guinea Savannah ecological zone of Nigeria. It is situated along Kaduna – Lagos road, Igabi Local Government Area of Kaduna state, Nigeria. It is located between latitude 10^{o} 34′ and 10^{o} 35′ and longitude 7^{o} 20′ and 7^{o} 21′ (Adelani, 2015). Mean annual rainfall and humidity are approximately 1000 mm and 29% respectively. The vegetation is open woodland with tall trees, usually small boles and broad leaves (Otegbeye *et al.,* 2001).

Fruit Collection and Materials

Tamarindus indica fruits were harvested from its mother tree at the Federal College of Forestry Mechanization, Afaka, Kaduna state. The sowing media (river sand), which was collected from the College dam, was sieved with 2mm sieve and sterilized at 160^{o}C for 24 hours (Adelani and Joseph, 2014). The viability of the randomly selected seed samples was assessed by cutting method (Schmidt, 2000). The poly pots (size: 20x25x25cm^3) used for the experiment were filled with the sterilized sand in the nursery. The preparation of coconut milk involved the equal mixture of coconut water and the liquid extract from crushed white fleshy coconut.

Experimental Procedure

Experiment1: Effect of fresh cow milk on the germination of the T. indica seeds

To investigate the effect of fresh cow milk on the germination of the *T. indica* seeds, a split-plot experimental design with four replications was involved (Nuga *et al.,* 2014; Aduradola, 2000; Aduradola, 2003; Goos and Vandebroek, 2003; Aduradola, 2004; Aduradola *et al*., 2005; Gomez and Gomez, 1984, Sananse and Maidapwad, 2014). Four concentrations of fresh cow milk (25, 50, 75 and 100%) and five treatment times (0, 6, 8, 12 and 14 hours) constituted main and sub plot treatments, respectively. Eight hundred (800) *T. indica* seeds were extracted from the fruits. Forty (40) seeds were soaked in four concentrations of fresh cow milk (25, 50, 75 and 100%) for 0, 6, 8, 12 and 14 hours. The concentration of fresh cow milk was prepared in the laboratory. After treatment, seeds were washed with distilled water and air dried for 30 minutes and treated with fungicides (vinclozolin) to prevent seed from fungi contamination which can negatively affect germination. Treated seeds were sown in 4cm depth of sterilized sand and 80 ml of water per seed was applied regularly at two days interval following the recommendation of Adelani (2009). Seeds that were not soaked in fresh cow milk served as control. A seed was considered germinated when the radicle was able to break open the seed coat at the sight of plumule emergence.

Experimental 2: Effect of Coconut Milk on the Germination of T. indica seeds

The effect of coconut milk on the germination of *T. indica* seeds was assessed using a split- plot experimental design with four replications (Casler, 2013; David and Adehi, 2014; Jones and Nachtscheim, 2009, Hinkedlmann and Kempthorne, 2008; Ramirez and Tobias, 2007; Cohram and Cox, 1956). Four concentrations of coconut milk (25, 50, 75 and 100%) and five treatment times (0, 6, 8, 12 and 14hours) constituted main and sub- plot treatments, respectively. Eight hundred (800) seeds were extracted from the fruits. Forty seeds were soaked in the four concentrations of coconut milk (25, 50, 75 and 100%) for five treatment times (0, 6, 8, 12 and 14 hours). The

concentration of coconut milk was prepared in the laboratory. After treatment, seeds were washed with distilled water, air dried for 30 minutes and treated with fungicides (vinclozolin) to prevent seeds from fungi contamination which stop germination. Treated seeds were sown in 4cm depth of sterilized sand and 70ml of water per seed was applied regularly at two days interval following the recommendations of Adelani (2009). Seeds that were not soaked in coconut milk served as control. A seed was considered germinated when the radicle was able to break open the seed coat at the sight of plumule emergence.

For both experiments, germination percentage and mean germination time were calculated using the following formula (1 and 2) suggested by Schelin *et al*. (2003)

$$\text{Germination } (\%) = \frac{Total\ seed\ germinated}{Total\ seed\ sown}\ x\ 100 \ldots.(1)$$

Mean germination time was calculated using the relation

$$\text{MGT} = \frac{\sum(fx)}{\sum X} \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where: x is the number of newly germinated seeds on each day; f is the numbers of days after seeds were set to germinate; X is the Total number of seeds that germinated at the end of the experiment.

Data Analysis

The data collected on the effect fresh cow milk and coconut milk on seed germination and mean germination time were subjected to two way analysis of variance (ANOVA) using SAS (2003) software. Separation of significant means was carried out using Least Significant Difference (LSD). All percent germination data were arcsine percentage transformation prior to analyses because it is appropriate for percentage data covering a wide range (Akindele, 2004; Gomez and Gomez, 2010; Sananse and Maidapwad, 2014).

RESULTS AND DISCUSSIONS

Hormonal composition of coconut milk and fresh cow milk

The results of hormonal analysis of coconut milk and fresh cow milk are represented in Table 1. Coconut milk had 1.52 µg/ml, 0.023 µg/ml and 0.092 µg/ml of (IAA) Indole Acetic Acid, (GA3) Gibberellic acid and (ABA) Abscissic acid, respectively. Fresh cow milk had 0.012 µg/ml and 0.006 µg/ml for IAA and ABA, respectively. The excellent performance of fresh cow milk in breaking the dormancy in *T. indica* seeds could be attributed the presence of hormones such as (IAA) Indole Acetic Acid and (ABA) Abscissic acid (Adelani and Maisamari, 2016). Cow milk (organic or otherwise) has been shown to contain 35 different hormones and 11 growth factors (Djamgoz and Jane, 2015). Hormones are known to speed up the rate of germination of plant seeds. Major plant growth regulators (PGRs) significantly enhanced seed germination rate in black gram and horse gram (Chauhan *et al.,* 2009), floral buds in Jojoba (Prat *et al.,* 2008) and other growth parameters in different plants. Growth regulators are organic substances besides nutrients, synthesized in plants, causing alteration in their cellular metabolism (Rastogi *et al.,* 2013). These hormones break the dormancy in plant seeds. Various studies of hormonal treatments for different crops, viz. *Albizia lebbeck*, *Senna siamea*, *Prosopis africana* and *Parkia biglobosa* seeds (Ebofin *et al.,* 2003) and in *Lagenaria siceraria* seeds (Vwioko and Longe 2009) revealed that hormones break dormancy in seeds. For example, smoke dried seeds resulted in 73% germination while the mechanical scarified seeds and the seed soaked in 1AA for 24 hours after cracking the testa had 70 and 90% seed germination, respectively (Ehiagbonare and Onyibe , 2007).

Main Effect of Concentrations of fresh cow milk on the germination of T. indica seeds

The results of the main effect of different concentrations of fresh cow milk on the germination of *T. indica* seeds are presented on Table 2. Irrespective of treatment time, germination percentage values of seeds soaked in 25% and 75% concentrations of fresh cow milk ranged from 91.25% to 93.50%. The highest germination percentage value of 93.50% was recorded for seeds treated in 75% concentration of fresh cow milk. Germination percentages of seeds soaked among different concentration of fresh cow milk were not significantly different. It can be inferred that high or low concentration of fresh cow milk did not influence the germination percentages of seeds.

Table 1: Hormonal composition of coco nut milk and fresh cow milk

Sample	IAA (µg/mL)	GA3 (µg/mL)	ABA (µg/mL)
Coconut milk	1.52	0.023	0.092
Fresh cow milk	0.012	ND	0.006

Where: ND= Not detected. IAA: Indole acetic acid; GA3: Gibberelic acid; ABA: Abscisic acid.

Table 2: Effect of concentrations and treatment times of fresh cow milk on the germination of *T. indica* seeds

Effect of fresh cow milk concentrations			Effect of treatment times		
Concentrations of fresh cow milk (%)	Seed germination (%)	MGT (days)	Treatment times (hr)	Seed germination (%)	MGT (days)
			0	72.50[b]	13.63[b]
25	91.50[a]	18.70[a]	6	95.63[a]	20.00[a]
50	92.00[a]	18.60[a]	8	96.88[a]	20.00[a]
75	93.50[a]	18.80[a]	12	98.13[a]	20.00[a]
100	92.50[a]	18.80[a]	24	98.75[a]	20.00[a]
SE (±)	1.59	0.21	SE (±)	1.78	0.24

Means on the same column having different superscript are significantly different (P<0.05) vertically. MGT; Mean germination time

Table 3: Interactive effect of concentrations and treatment times of fresh cow milk on the germination of *T. indica* seeds

Concentrations of fresh cow milk (%)	Treatment times (hr)				
	0	6	8	12	14
25	72.50[b]	95.00[a]	95.00[a]	95.00[a]	99.8[a]
50	70.00[b]	95.00[a]	99.50[a]	97.50[a]	97.50[a]
75	77.50[b]	95.00[a]	97.50[a]	99.60[a]	97.50[a]
100	70.00[b]	97.50[a]	95.00[a]	99.80[a]	100.00[a]
SE (±)	2.25	2.52	2.52	2.52	2.52

Means on the same rows having different superscript are significantly different (P<0.05) horizontally

Table 4: Interactive effect of mean germination time of concentrations and treatment times of fresh cow milk on the germination of *T. indica* seeds

Concentrations of fresh cow milk (%)	Treatment times (hr)				
	0	6	8	12	14
25	13.50[b]	20.00[a]	20.00[a]	20.00[a]	20.00[a]
50	13.00[b]	20.00[a]	20.00[a]	20.00[a]	20.00[a]
75	14.00[b]	20.00[a]	20.00[a]	20.00[a]	20.00[a]
100	14.00[b]	20.00[a]	20.00[a]	20.00[a]	20.00[a]
SE (±)	0.24	0.24	0.24	0.24	0.24

Means on the same rows having different superscript are significantly different (P<0.05) horizontally

Table 5: Effect of concentrations and treatment times of coconut milk on the germination of *T. indica* seeds

Effect of coconut milk concentrations			Effect of treatment times		
Concentrations of coconut milk (%)	Seed germination (%)	MGT (days)	Treatment times (days)	Seed germination (%)	MGT (days)
			0	66.88[b]	9.63[b]
25	91.00[a]	17.70[a]	6	95.00[a]	19.75[a]
50	91.50[a]	17.50[a]	8	98.13[a]	19.50[a]
75	91.50[a]	17.30[a]	12	98.13[a]	19.00[a]
100	90.50[a]	17.20[a]	14	97.50[a]	19.25[a]
SE (±)	1.15	0.38	SE (±)	1.28	0.43

Means on the same column having different superscript are significantly different (P<0.05) vertically. MGT; mean germination times

Table 6: Interactive effect of concentrations and treatment times of coconut milk on the germination of *T. indica* seeds

Concentrations of coconut milk (%)	Treatment times (hr)				
	0	6	8	12	14
25	67.50[b]	92.50[a]	97.50[a]	99.80[a]	97.50[a]
50	65.00[b]	95.00[a]	99.7	97.50[a]	100.00[a]
75	67.50[b]	97.50[a]	97.50[a]	97.50[a]	97.50[a]
100	67.50[b]	95.00[a]	97.50[a]	97.50[a]	95.00[a]
SE (±)	2.56	2.56	2.56	2.56	2.56

Means on the same rows having different superscript are significantly different (P<0.05) horizontally

Table 7: Interactive effect of mean germination times of concentrations and treatment times of coconut on the germination of *T. indica* seeds

Concentrations of coconut milk (%)	Treatment times (hr)				
	0	6	8	12	14
25	10.00[b]	20.00[a]	19.50[a]	19.50[a]	19.50[a]
50	9.00[b]	20.00[a]	20.00[a]	19.50[a]	19.00[a]
75	9.50[b]	19.50[a]	19.00[a]	19.00[a]	19.50[a]
100	10.00[b]	19.50[a]	19.50[a]	18.00[a]	19.00[a]
SE (±)	0.43	0.43	0.43	0.43	0.43

Means on the same rows having different superscript are significantly different (P<0.05) horizontally

Contrary to this result are reports of Naeem *et al.* (2004) and Gulluoglu, (2004) who stated that only hormones in low concentrations regulate growth, differentiation and development, either by promotion or inhibition and also allow physiological processes to occur at their normal rate. Similarly, auxins may regulate cell elongation, tissue swelling, cell division, formation of adventitious roots, callus initiation and growth, induction of embryogenesis and promote cell wall loosening at very low concentration (Azad *et al.,* 2004; Woodward and Bartel, 2005; Muthukumar *et al.,* 2007; Abel and Theologis, 2010).

Main Effect of Treatment Times on the germination of T. indica seeds

The result of main effect of treatment times on the germination of seeds soaked in fresh cow milk is presented in Table 2. The percentage germination value was lowest (72.50%) for control treatment and highest (98.75%) for seeds soaked in fresh cow milk for 14 hours (Table 2). Significant percentage germination was recorded for seeds soaked for all treatment times compared to control. It can be deduced that variation among treatment time did not influence germination percentage of *T. indica* seeds. Increase in treatment time did not significantly increase the germination percentages of seeds. This result is contrary to the documentation of Adelani *et al*. (2014b) who reported that germination percentage value of *Balanites aegyptiaca* seeds increased with increasing hydro-priming hours.

Interactive Effect of Concentrations and Treatment times of Fresh cow milk on the Germination of *T. indica* seeds

A significant germination percentage was recorded for seeds treated in fresh cow milk for treatment times compared to control (Table 3). This is an indication that component of fresh cow milk as hormone influence the seed germination percentage for treatment times. There is evidence that different natural growth regulators improve seed germination and seedling vigor of many crops (Renugadevi and Vijayageetha, 2009).

Interactive Effect of Mean Germination Time of Concentrations and Treatment times of Fresh cow milk on the Germination of T. indica seeds

The least mean germination time of 13days was recorded for seeds not treated in 50% concentration of fresh cow milk (Table 4).

Main Effect of Concentrations of Coconut milk on the Germination of T. indica seeds

The results of main effect of different concentrations of coconut milk on the germination of *T. indica* seeds are presented in Table 5. There was no significant germination percentage recorded among seeds soaked in different concentrations of coconut milk.

Main Effect of Treatment times of Coconut milk on the Germination of T. indica seeds

Significant percentage germination was recorded for all treatment times of seeds soaked in concentrations of coconut milk compared to control (Table 5).

Results of Interactive Effect of Concentrations and Treatment times of Coconut Milk on the Germination of T. indica Seeds

The percentage germination value of seeds treated in all concentrations of coconut milk, for all treatment times ranged from 65% to 100% (Table 6). Seeds soaked in all concentrations of coconut milk for all treatment time were statistically significant compared to control. This is an indication that component of coconut milk as hormone (Adelani and Maisamari, 2016) influenced the seed germination percentage. This is in consonance with reports of Mng'omba *et al*. (2007) who stated that seeds or fruits contain plant hormones which can either promote or inhibit seed germination. The hormonal components of seeds sometimes enhance seed germination of the same or different plants species. The efficacy of hormones in promoting seed germination have been reported by Bello *et al.* (2013) (*Acacia senegalensis*)., Agboola (2002) (*Ceiba pentandra* and *Terminalia superba*)., Idu *et al.* (2007) (*Hura crepitans*). Plant hormones are therefore extremely important for the regulators of seed dormancy and germination (Koornneef *et al.,* 2002; Finkelstein, 2004). Most works in plant hormones clearly indicate that dormancy and germination are under hormonal control (Idu *et al.,* 2007). It has long been ascertained that plant hormones including auxins, gibberellins, cytokinin and ethylene etc., are involved in controlling developmental events such as cell division, cell elongation and protein synthesis (Tiwari *et al.,* 2011) which led to seed germination.

A significant germination percentage was recorded for seeds soaked for treatment times compared to control. Germination percentage value of 100% was recorded for seeds soaked in 50% concentration of coconut milk for 14hours. Appropriate time duration for pre-sowing treatment influences the number of plant seeds that will germinate. Appropriate time duration for pre-sowing that influences germination percentage is varies with seed species. *Acacia auriculiformis* seeds soaked in H_2SO_4 for 10 minutes, recorded the highest germination percentage of 96% followed by those treated with H_2SO_4 for 5 minutes (92%) while 2 minutes H_2SO_4 treatment and control treatment gave 76% and 42% percentage germination, respectively (Olatunji *et al.,* 2012). Similarly, Aduradola and Shinkafi (2003) reported enhanced seed germination with increasing treatment time for *Tamarindus indica*. Moreover, germination percentages of *Adansonia digitata* seeds improved with increased period of soaking in the acid up to 3 hours (Adio *et al.,* 2006).

Furthermore, Al-Menaie *et al*. (2010) reported increase in seed germination rate for *Cassia siamea* and *Cassia roxburghii* as a result of increased period of soaking the seeds in H_2SO_4. Olmez (2011) recommended that the pretreatment of *Hippohae rhamnoides* seeds by soaking in sulphuric acid for 1 minute should be used to overcome dormancy of the seed species. Similarly, treatment of *Adansonia digitata* seeds in 98% acid concentration for 1 hour had significant effect on germination of the seeds (Falemara *et al.,* 2013). The least value of 9days was recorded for mean germination time of control of 50% concentration of coconut milk (Table 7). Seeds that were not pretreated (control) germinated fast. Pre-sowing treatment did not speed the germination of *T. indica* seeds. Increasing period of pre-sowing decreases speed at which seed germinate. This result is in consonance with the documentation of Afrasyab and Reza (2007) that reported a reduction in seed vigor index, germination rate and increased mean germination time by increasing immersion time in H_2SO_4.

CONCLUSION

This investigation conducted on the effects of fresh cow milk and coconut milk on the germination of *T. indica* seeds revealed that the percentage germination values of seeds soaked in all concentrations of fresh cow milk for all times of treatments ranged from 70% to 100%. Percentage germination ranged from 65% to 100% for all concentrations and treatment times for seeds treated in coconut milk. To obtain high germination percentage value for agro-forestry programmes, it is recommended that *T. indica* seeds should be treated for 14 hours in 50% and 100% concentration of coconut milk and fresh cow milk, respectively.

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