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Full Length Research Paper

Effect of seed pre-treatment and its duration on germination of *Detarium microcarpum* (Guill. and Perr.)

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Detarium microcarpum **Guill and Perr is a multipurpose tree species indigenous to semi-arid regions of Sub-Saharan Africa. It is being exploited to local extinction due to high dependence for fuelwood and other uses. The present study explored different pre-treatment methods for enhancing seed germination and growth of *D. microcarpum* in the Guinea savanna zone of Ghana. The experiment employed a 4 × 4 factorial design with seeds subjected to four pre-treatments (50% sulphuric acid concentration, 98% sulphuric acid concentration, cold water and hot water) at four pre-treatment time durations. Percentage germination varied significantly between pre-treatments ($p < 0.05$) with cold water treatment recording the highest mean germination (73.06%) and the 98% sulphuric acid concentration recording the least (47.72%). Germination rate had a moderate positive relationship with plant height ($r_{xy} = 0.49$) and collar diameter ($r_{xy} = 0.54$). The study recommends seed immersion in cold water for 48 h as the most efficient pre-treatment for *D. microcarpum.***

Key words: *Detarium microcarpum*, germination percentage, plant height, pre-treatment.

INTRODUCTION

Detarium microcarpum is a perennial woody plant indigenous to the semi-arid regions of Sub Saharan Africa which occurs predominantly in Benin, Burkina Faso, Cameroon, Central African Republic, Ghana, Guinea, Guinea Bissau, Niger, Nigeria, Senegal and Togo (Oibiokpa et al., 2014). There are two reported species of the genus with *D. senegalensis* growing in riparian and dry forests areas, whilst *D. microcarpum* grows in dry savannas (Tropical Plant Database, 2019). *D. microcarpum* thrives in a wide variety of soils including degraded and rocky areas with annual rainfall of about 600-1000 mm (Abreu et al., 1999). Although it is commonly found in fallow lands and wild bushes, it is sometimes retained on farmlands for soil improvement, fuelwood, food and medicinal purposes (Oibiokpa et al., 2014).

According to FAO (1995), *D. microcarpum* is a leguminous tree species which improves soil fertility when retained on farmlands through nitrogen fixation and leaf litter decomposition. The edible fruits of *D. microcarpum* are consumed by human and wild animals in regions where the species is found (Akpata and Miachi, 2001).

The fruit flour is reported to contain about 42% carbohydrates, 36% lipids and 11% protein (Anhwange et al., 2004). The fruit pulp is rich in minerals and essential

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vitamins such as vitamin C, E, B_2 and folic acid which which serve as a major food supplement during the dry season (Oibiokpa et al., 2014). These nutritional properties highlight the potential contribution of *D. microcarpum* to food security in Africa. The fruits are equally sold in local markets and contribute to economic empowerment in rural communities (Akpata and Miachi, 2001).

Moreover, *D. microcarpum* is used in traditional medicine for the treatment of various ailments including tuberculosis, meningitis and diarrhea due to its antimicrobial properties (Abreu et al., 1998). The seed coat is also reported to possess antimicrobial activity which could be used in the control of infectious diseases (Ebi and Afieroho, 2011). It also serves as a major fuelwood species with charcoal produced from the wood delivering about 1968 KJ/kg of caloric power (Kabore et al., 2005) and ranked among the most preferred fuelwood species in its naturally growing areas (Sawadogo, 2007).

The multipurpose uses have resulted in overexploitation of the species to local extinction in some areas (Kabore, 2005) mainly due to the high dependence on wild plant sources with little attention on domestication of the species. However, effective domestication will require knowledge on regeneration and other aspects of the plant biology (Bohra et al., 2018). Seed germination is known to be an important step towards raising a successful crop stand (Finch-Savage and Bassel, 2016), but there is a paucity of information on pre-treatment methods for *D. microcarpum* seeds. Kouyate and Van Damme (2006) recommended immersion of seeds in sulphuric acid as a good pre-treatment for *D. microcarpum* seeds but their study was not explicit on the recommended acid concentration and best duration of seed immersion in acid. The present study examined the percentage germination and initial growth performance of *D. microcarpum* using different pre-treatment methods.

MATERIALS AND METHODS

Study area

The experiment was conducted at the tree nursery of the Faculty of Natural Resources and Environment of the University for Development Studies, Ghana. It lies between latitude 9° 25' N to 10° 40' N and longitude 0° 58' N to 1° 12' W (Figure 1). The area records a monomodal rainfall pattern with a mean annual rainfall of 1127 mm. Mean monthly minimum and maximum temperatures are 26.6 and 35.6°C respectively, with a mean annual temperature of 29.7°C (SARI, 2016). The vegetation is dominantly grassland interspersed with indigenous and exotic tree species such as *Parkia biglobosa* (dawadawa), *Vitellaria paradoxa* (shea), *Lannea acida* (lanea), *Azardirachta indica* (neem), *Magnifera indica* (mango*), Tectona grandis* (teak), *Senna siamea* (cassia). Seed boxes were kept in a shade net with average light intensity of 11.19 Lux.

Seed collection and viability test

Fruits of *D. microcarpum* were collected from fallow lands of Nandom community in Upper West region of Ghana. Collected fruits were initially sorted to eliminate diseased and bruised fruits. The fruits were cracked to remove seeds for pre-treatment. Prior to the germination experiment, 50 seeds were sampled for a seed viability test using floating test.

Experimental design

Seeds were subjected to four pre-treatment methods at four pre-treatment time durations with the untreated seeds as control. The pre-treatment methods were;

(i) Seeds soaked in 98% sulphuric acid concentration for 1, 5, 10 and 15 min
(ii) Seeds soaked in 50% sulphuric acid concentration for 1, 5, 10 and 15 min
(iii) Seeds soaked in cold water for 12, 24, 36, and 48 h
(iv) Seeds soaked in boiled water (100°C) for 12, 24, 36, and 48 h
(v) Control (untreated seeds)

Each treatment combination had 45 seeds, making a total of 765 seeds for the experiment. The pre-treated seeds were sown in seed boxes (50 cm × 15 cm × 10 cm) half filled with topsoil. The seed boxes were arranged in a Completely Randomized Design with 15 seeds per box. Each treatment combination was replicated in three seed boxes. Seed boxes were watered twice daily with 1500 ml of water per box. Weeds were controlled by hand to prevent competition.

Data collection

Data was collected on seed emergence, plant height, collar diameter and root length. Number of seeds emerged was recorded daily per seed box from the day of first germination to the end of the germination period (4^{th} week after sowing). Growth parameters were recorded once every two weeks from the 4^{th} week after sowing to the 10^{th} week after sowing. Seedlings were uprooted after the tenth week for root length measurement. Plant height and root length were measured with a measuring tape whilst a caliper was used for collar diameter.

Data analysis

Percentage seed Germination (PG) and Germination Rate (GR) were estimated with following equations:

$$PG = \frac{SG}{TS} \times 100 \quad (1)$$

Where SG is number of seeds germinated and TS is total number of seeds planted.

$$GR = \frac{GP}{T} \quad (2)$$

Where, PG is Percentage Germination and T is Time taken in days.

Data set on seedling emergence proportions were arcsine-square root transformed before each factor was subjected to Analysis of Variance (ANOVA). Treatment means were separated with Bonferroni corrections at 95% confidence level. Pearson's correlation coefficient was used in establishing relationship between germination rate and plant size (plant height, collar diameter and root length). All analysis was done with Genstat 18^{th} edition.

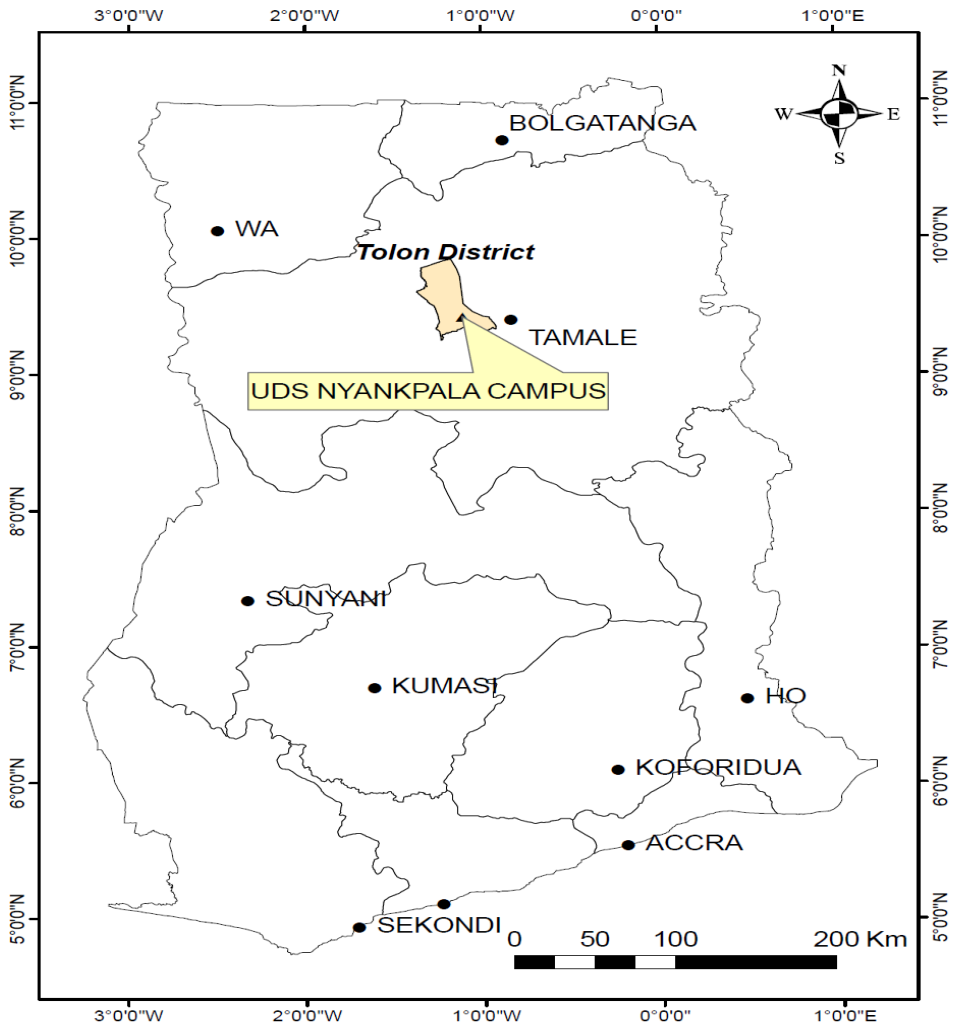

Figure 1. Map of the study area.

RESULTS AND DISCUSSION

Effect of pre-treatment methods on germination of *D. microcarpum*

Percentage seed germination varied significantly between pre-treatment methods ($P < 0.05$) with cold water treatment recording the highest mean percentage germination of 73.06% while seed soaking in 98% sulphuric acid resulted in the lowest germination (46.72%). Amongst sulphuric acid treatments, seed soaking in 50% sulphuric acid recorded higher germination (53.22%) when compared with seed soaking in 98% sulphuric acid (46.72%) (Figure 2).

Aside the general significant effect of pre-treatment on seed germination, the duration of seed immersion also had significant effect within treatments. For instance, percentage seed germination differed significantly between the four time durations of seed immersion in cold water with 48 h recording the highest germination of 86% (Table 1). Again, seeds immersed in 50% sulphuric acid concentration for 15 min had significantly higher germination percentage (68%) when compared with other time durations of immersion in 50% sulphuric acid. Pre-treatment method also influenced number of days to seed emergence. Seeds immersed in cold water for 48 h germinated earlier (8 days), whereas untreated seeds took longest time (12 days) to first seed germination.

The cold water treatment recording the highest percentage as well as shortest time to first seed emergence could be attributed to the ability of cold water to enhance seed coat permeability. This enabled gaseous exchange and enzymatic hydrolysis to transform the embryo into a seedling without negatively affecting the functional organs of the seed (Olatunji et al., 2013). This also agrees with Azad et al. (2011) who identified water as a necessary requirement for seed germination. *D. microcarpum* has a hard seed coat which needs to be

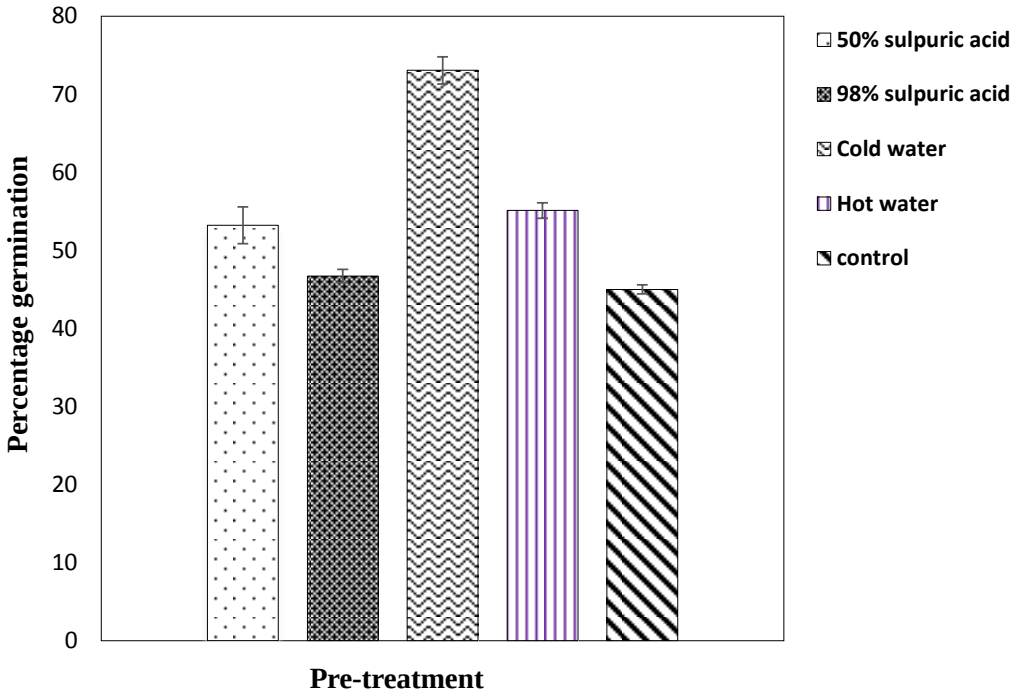

Figure 2. Effect of pre- treatment on percentage of germination of *D. microcarpum* seeds.

Table 1. Effect of different pre-treatment time on percentage germination, first seed emergence and germination rate of *D. microcarpum.*

Pre-treatment	Time	% germination	Days to emergence	Germination rate (seedlings/day)
50% Sulphuric acid	1 min	32.4^{a}	11	0.76
	5 min	62.3^{hi}	11	1.62
	10 min	50.0^{de}	11	1.48
	15 min	68.1^{j}	11	1.19
98% sulphuric acid				
	1 min	50.3^{fg}	11	1.19
	5 min	50.1^{ef}	9	1.18
	10 min	46.3^{cd}	9	1.43
	15 min	40.1^{fg}	9	1.10
Cold water				
	12 h	67.8^{cd}	10	1.62
	24 h	78.4^{gh}	11	1.86
	36 h	60.0^{b}	10	1.43
	48 h	86.0^{ij}	8	2.05
Hot water				
	15 min	58.0^{fg}	11	1.38
	30 min	56.4^{efg}	11	1.33
	45 min	46.0^{bc}	11	1.10
	60 min	60.0^{gh}	9	1.43
Control	Untreated	52.5^{ef}	12	0.92

NB. Means having a common superscript letter within a column are not significantly different at significance threshold of 0.05.

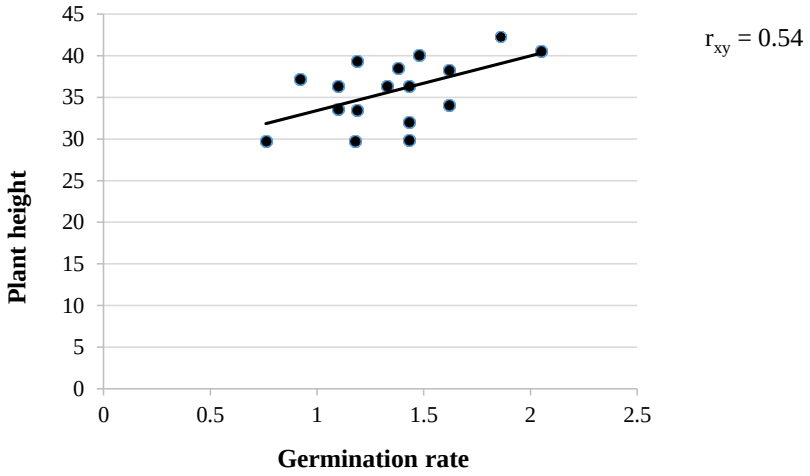

Figure 3. Relationship between germination rate and plant height in *D. microcarpum.*

ruptured before radicle and plumule emergence. Hence seeds that were immersed in cold water for a longer period (48 h) had an early seed coat rupture and permeability which facilitated a faster rate of emergence. This could explain the significantly higher percentage germination and early days to emergence recorded among seeds that were immersed in cold water for 48 h. This phenomenon confirms earlier reports by Missanjo et al. (2013) and Mwase and Mvula (2011), who reported seed coat permeability as one of the determinants of seed germination.

The relatively low percentage germination in 98% sulphuric acid as compared to control (Figure 2) seem to suggest detrimental effect of this chemical to *D. microcarpum* seeds at higher concentrations. This is in accordance with Asl et al. (2011), that sulphuric acid has a detrimental effect on seed embryo. This could be attributed to the fact that some enzymes have specific pH ranges, therefore higher acid concentration above this range tends to provide unfavourable pH conditions for normal enzymatic activity. However, at low concentrations, sulphuric acid could have a positive effect on seed germination; this was evident in the fact that 50% sulphuric acid had a higher percentage germination than the control treatment (Figure 2).

Time of immersion in sulphuric acid also had an effect on seed germination with germination percentage decreasing with increasing time duration of seed immersion in 98% sulphuric acid concentration (Table 1). However, in 50% sulphuric acid concentration percentage germination generally increased with increasing time of immersion. The effect of time duration of treatment on germination is not limited to *D. microcarpum*. This positive effect of time duration of pre-treatment on seed germination was equally reported in *Tamarindus indica* (Abubakar and Muhammad, 2013; Muhammad and Amusa, 2003).

Hot water pre-treatment resulted in a low germination percentage as compared to the cold water treatment probably due to the high temperature the seeds were exposed. This argument is supported by the findings of Singh et al. (2019) who indicated that hot water may tend to be detrimental to enzymatic activities at higher temperatures when used as pre-treatment. The higher percentage germination recorded in hot water treatment as compared to 50% sulphuric acid treatment is dissimilar to the response of *T. indica* to the same pre-treatments. *T. indica* seeds immersed in 50% concentrated sulphuric acid recorded a higher germination percentage than seeds that were immersed in hot water (Abubakar and Muhammad, 2013). Again, *Adonsonia digitata* seeds rather recorded a higher percentage germination in 80% sulphuric acid concentration as compared to cold water treatment for 72 h (Oboho and Ahanon, 2017). This variation in tropical plant species response to pre-treatments points out differences in the inhibitory factors to germination between plants.

The control treatment generally recorded low percentage germination as compared to most pre-treatments (Cold water, 50% sulphuric acid and hot water) which could be an indication of some level of dormancy in *D. microcarpum*. This suggests that pre-treatments have positive influence on germination of *D. microcarpum* which could be a boon to nursery managers and foresters for the domestication of the species.

Relationship between germination rate and growth of *D. microcarpum* seedlings

Germination rate correlated positively with plant growth parameters recording moderate positive relationships with plant height (r_{xy} = 0.52) and collar diameter (r_{xy} = 0.49) (Figures 3 and 4). However, root length had a weak positive relationship with germination rate (r_{xy}=

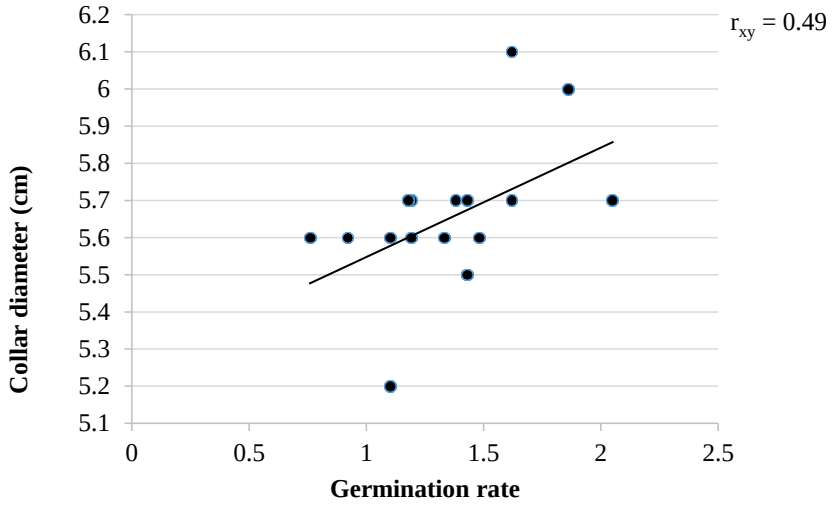

Figure 4. Relationship between germination rate and collar diameter.

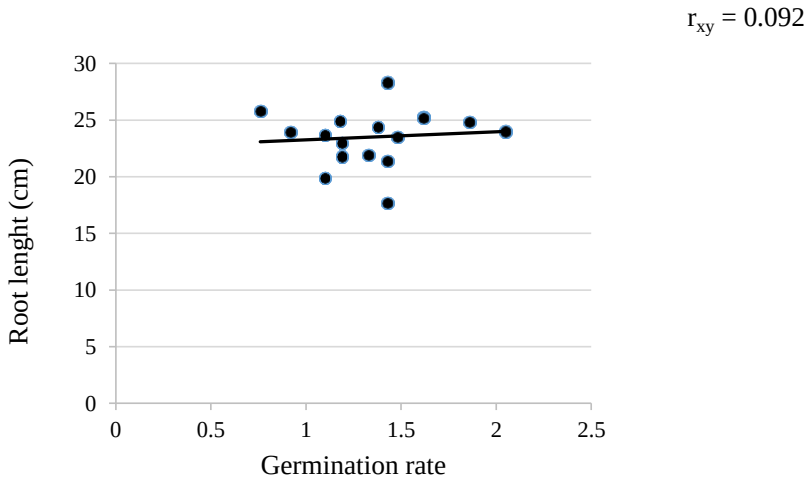

Figure 5. Relationship between germination rate and root length.

0.092). El-Bably and Rashed (2018) have reported positive effects of pre-treatment on growth of *Adansonia digitata* seedlings. The positive relationship between germination rate and seedling growth performance suggests that, effect of pre-treatment on germination of *D. microcarpum* also translates into seedling size. This is similar to the findings of Chubamerenla et al. (2015) who reported a significant variation in growth of *Delonix regia* seedlings that emerged from different pre-treatments. This equally reveals an indirect influence of pre-treatment on growth performance of seedlings. Therefore, the positive relationship between germination rate and growth parameters of *D. microcarpum* seedlings implies that, benefits of pre-treatment does not end in germination but also contribute to the survival and establishment of seedlings in the field (Figure 5).

Conclusion

The study concludes that both pre-treatment method and duration of treatment have significant effects on the germination and growth performance of *D. microcarpum.* Soaking *D. microcarpum* seeds in cold water for 48 h could be recommended for large scale production of seedlings as it resulted into 86% germination. Although acid treatment can equally enhance germination, higher acid concentration could result in detrimental effects. Similarly, cold water treatment resulted in an early germination of *D. microcarpum* seed as compared to hot water pre-treatments. Significant effect of pre-treatment on germination translated into a positive effect on seedling size with plant height, collar diameter and root length all positively correlated with germination. The

study therefore recommends cold water as an effective pre-sowing treatment for *D. microcarpum*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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