IOSR Journal Of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT)
ISSN: 2319-2402, ISBN: 2319-2399. Volume 2, Issue 3 (Nov. - Dec. 2012), PP 25-29
www.Iosrjournals.Org

Effect of different pretreatment protocols on seed germination of *Tetrapleura tetraptera* (Schum and Thonn)

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Abstract: *Tetrapleura tetraptera (Schum and Thonn) is a valued forest species in Nigeria for its medicinal and culinary uses. But the plant is uncultivated, and its seeds are dormant. This poses an obvious challenge in efforts aimed at its conservation. Different pretreatment protocols (scarification) were tested for their efficiency to break the hard seed coat of T. tetraptera. Mature fruits of T. tetraptera were collected from Oban west forest in Cross River State, and the seeds extracted and scarified before germination. Mechanical scarification was done by rubbing the seeds against a rough cement wall; chemical scarification by soaking the seeds in concentrated sulphuric acid, concentrated hydrochloric acid and concentrated nitric acid for 15 minutes; while heat scarification was done by soaking the seeds in hot water. Seed dormancy was successfully broken either by mechanical or chemical scarification. Significant difference ($p<0.001$) was observed in germination and imbibition percentage within the non-scarified and scarified seeds. While the non-scarified seeds had the least germination (18.3%), seed scarified with sulphuric acid had the highest (90%) followed by mechanically scarified seeds (85%) and nitric acid scarified seeds (81.7%). Imbibition percentage increased up to 100% in the scarified seeds in contrast to 66.6% in the non-scarified seeds. This study shows that sulphuric acid scarification is an effective method for breaking seed coat-imposed dormancy in Tetrapleura tetraptera.*

Key words: *germination, seed dormancy, scarification, Tetrapleura tetraptera.*

I. Introduction

In Nigeria, several tree species serve as sources of food, wood, fibre and medicine to indigenous people. The forest trees also have added value of conservation for scenic purposes, stabilization of climate, maintenance of water supply and preservation of erosion. It is unfortunate that man has misused these forest resources due to overexploitation and lack of purposeful management, with a resultant negative effect on the environment [1]. In order to prevent the extinction and derive maximum benefits from these indigenous forest trees, it is necessary to preserve their germplasm, as well as promote their conservation in the environment. However, most of these forest trees are uncultivated and exhibit varying levels and different kinds of seed dormancy. Therefore, overcoming the problem of seed dormancy in these forest species becomes imperative, as seed germination is crucial to the survival of the next generation, and by extension, the environmental conservation of forest species.

Tetrapleura tetraptera (Schum and Thonn), commonly known as aidan tree, is a deciduous tree belonging to the family *Fabaceae*. It reaches a height of 20-35cm with the girth of 1.5-3cm [2]. The fruits and seeds add good aroma and flavor to food, thereby increasing the pleasure of food consumption [3]; [4]. The fruit is used to prepare soup for nursing mothers from the first day of birth to prevent *post partum* contraction [5]. Its fruits are used for the management of convulsions, leprosy, inflammation and rheumatism. Its leaves are essential for the treatment of epilepsy [6] and possess strong molluscicidal activity [7]. The aqueous fruit extract has also been shown to possess hypoglycaemic properties [8]. The bark is active against cough and bronchitis. It is also used as a decoction in drinks. When put into vapour bath, the bark is used against rheumatism and fever. The root is used for the treatment of gastrointestinal clinical problems [4]. *T. tetraptera* is equally valued in timber as fairly hard heartwood [2]. In spite of the economic value of *T. tetraptera*, the population of the plant is declining at an alarming rate due to overexploitation, and the absence of sustained conservation measures. A very important contributing factor is the fact that only a small percentage of the seeds germinate in the field, a lot more are dormant [9]. And this poses an obvious challenge in efforts aimed at its environmental conservation. Seed dormancy is a temporary failure of a mature viable seed to germinate under environmental conditions that would normally favour germination [10][11] . Many flowering plants exhibit some level of primary seed dormancy that can be one or both of the following types: coat imposed or embryo dormancy [11]. Within the *Fabaceae*, it is common to find hard or water-impermeable seeds. The hard seed coat in addition to preventing seed germination helps to protect against fluctuations in temperature, humidity and microbial attack [12]. However, the agricultural and forest industries rely on seeds that exhibit high rates of germination and vigorous synchronous growth after germination; hence dormancy is sometimes considered an undesirable trait.

Coat imposed dormancy can be eliminated by seed pretreatment protocols, which include chemical treatment, heat treatment, abrading or piercing the seed coat, e.t.c, which allow imbibition and germination to proceed[13] . Commercial growers are known to scarify seeds by; soaking in concentrated sulphuric acid for about 10 minutes, rinsing in water before sowing, filing seeds with a metal file, rubbing with sandpaper, nicking with a knife, or cracking gently with a hammer to weaken the seed coat. [14] reported that seed dormancy in *Piliostigma thonningii* can be successfully broken by physical and chemical scarification using concentrated sulphuric acid for 15 minutes with germination rates of 91.7 ± 4.01 and 95.0 ± 2.24%, respectively. Santana *et al.*[15] reported that simulated temperature regimes can break physical seed dormancy in legumes, and that the germination response varies among species. Scarification with heat of 150^{o}C for 5minutes released *Mimosa pudica* seeds from dormancy and stimulated germination [16]. This work is therefore aimed at determining a highly effective pretreatment protocol for breaking the seed coat-imposed dormancy in *T. tetraptera.*

II. Materials and Methods

1.1 Source of plant material

Dried, mature fruits of *Tetrapleura tetraptera* were collected from Oban west forest (latitudes 4^0 54′ and 5^0 45′N and longitudes 8^0 18′ and 8^0 50′E) in Cross River state, Southeast Nigeria.

2.2 Methods

The fruits were cracked open and seeds extracted. The seeds were scarified mechanically, chemically and with high temperature. The mechanical scarification was done by gently rubbing the seeds against a rough cement wall for 3 minutes; chemical scarification involved soaking the seeds in concentrated sulphuric acid (H_2SO_4), hydrochloric acid (HCl), and nitric acid (HNO_3) for 15 minutes, followed by rinsing in water. The heat scarification was done by soaking the seeds in hot water (80^{o}C), the seeds remained soaked in the hot water till the temperature dropped to room temperature. The scarified seeds were then surface decontaminated with 3.5% sodium hypochloride (NaOCl) for 10 minutes followed by rinsing in distilled water. Both the scarified (mechanical and chemical) and non-scarified seeds were then soaked in distilled water for 20 minutes to allow imbibition. 20 seeds each from the control group (non-scarified), mechanically scarified, sulphuric acid, hydrochloric acid, nitric acid, and heat scarified groups were placed in petri dishes lined with filter paper moistened with distilled water. The petri dishes were incubated in the dark at 28^{o}C ±2^{o}C. Each treatment and the control had three replicates and the experiment was laid in Completely Randomized Design (CRD).

2.3 Data collection and analysis

Germinated seeds, based on the protrusion of the radicle, were counted and removed from the petri dish every 24 hours. Based on visual examination (imbibed seeds are easily distinguished from non-imbibed ones) the number of imbibed seeds for scarified and non-scarified seeds were also counted daily. The data generated were subjected to One-way analysis of variance (ANOVA) using SPSS software package version 15.0. Least Significant Difference (LSD) was used to determine significant means.

III. Results

The means and standard errors, and analysis of variance for imbibition and germination percentage are shown on tables 1 & 2. Mean germination percentage for non-scarified seeds was 18.3%, 85% for mechanically scarified seeds, 90% for sulphuric acid, 76.7% for hydrochloric acid, 81.7% for nitric acid and 56.7% for hot water scarified seeds. The ANOVA showed a very high significant difference ($p<0.001$) in the germination percentage of the scarified and non-scarified seeds. The LSD at 5% showed that germination of the non-scarified seeds differed significantly from scarified seeds. It also showed that germination percentage differed significantly among the various seed scarification methods. Figure 1 shows the cumulative percentage germination.

The percentage imbibition for non-scarified seeds was 66.9%, 100% for mechanically and nitric acid scarified seeds, 96.7%, 95% and 98% for sulphuric acid, hydrochloric acid and hot water scarified seeds, respectively. The analysis of variance (table 2) showed a very high significant difference ($p<0.001$) in the imbibition percentage among non-scarified and scarified seeds. The LSD showed significant difference between the non-scarified and scarified seeds but no difference among the seeds scarified with different methods, in imbibition percentage. Figure 2 shows the cumulative percentage imbibition.

Also, a significant difference ($p<0.01$) was obtained for mean days to germination within the scarified and non-scarified seeds. LSD showed that although the non-scarified seeds differ from the scarified seeds, the seeds scarified with different methods did not differ from one another. The mean days to germination for the non-scarified seeds was 9.5, while 5.5, 4.5, 5.0, 5.5 and 7.0 for mechanical, sulphuric acid, hydrochloric acid, nitric acid and hot water scarified seeds, respectively.

IV. Discussion

Proper seed germination and growth are indispensable for the continued existence of any plant. Germination, *sensu stricto* includes those events commencing with imbibition or uptake of water by the quiescent dry seed and culminates with the elongation of the radicle [17](Bewley and Black, 1994). Visible evidence of the completion of germination is usually protrusion of the radicle through the seed structures surrounding the embryo (such as the testa and endosperm, or megagametophyte). However, some seeds fail to complete germination under seemingly favorable conditions, even though they are viable. Such seeds are said to be dormant. Dormancy in seeds has to be broken, irrespective of the type, for effective germination and vigorous growth.

This study indicated that mechanical and chemical scarification methods are effective in rendering seeds of *Tetrapleura tetraptera* permeable, leading to germination up to 90% after 6 days. This confirms the earlier reports of Onyekwelu[18] . A previous study by Lemos-Filho *et al.* [19] showed that mechanical and chemical scarifications are more effective in breaking the hard seed coat of *Senna multijuga*. It was equally observed in this study that within the scarification methods, chemical scarification with sulphuric and nitric acids, as well as mechanical scarification were more effective, confirming earlier reports by Ayisire *et al.* [14] in *Piliostigma thonningii*, Lacerna *et al.*[20] in *Senna multijuga* (*Caesalpinoideae*) and *Plathymenia reticulata* (*Mimosoideae*). Sulphuric acid had the highest germination percentage (90%), followed by mechanical and nitric acid scarified seeds with 85% and 76.7% respectively. Germination in the scarified seeds started on the third day, but in the non-scarified seeds, it started on the sixth day.

Imbibition percentage was lowest in non-scarified seeds (66.7%), but highest in nitric acid and mechanically scarified seeds (100%). This was followed by hot water, sulphuric acid and hydrochloric acid scarified seeds with 98%, 96.7% and 95%, respectively. The fact that all scarified seeds imbibed, in contrast to the non-scarified seeds indicated that mechanical and nitric acid scarification led to complete loss of seed coat impermeability in *Tetrapleura tetraptera.* The subsequent increase in the germination percentage, following increase in imbibition percentage, observed in the scarified seeds is a clear indication that the hard seed coat is responsible for the dormancy in *Tetrapleura tetraptera*. Hard seed coats make seeds impervious to water and gases thereby limiting imbibition and germination [13]. The positive response to scarification observed in *Tetrapleura tetraptera* in this study, is typical of seeds with hard seed coat dormancy [17]. This kind of dormancy has been reported for many legumes [21] [22], including *Tetrapleura tetraptera* [18] [9].

Table 1: Effect of different seed pretreatment protocols (Scarification) on imbibition percentatge, germination percentage and days to germination in *Tetrapluera tetraptera*

Treatment	Imbibition percentage	Germination percentage	Days to germination
Control	13.33±0.66	3.66±0.33	9.50±0.86
Mechanical	20.00±0.00	17.00±1.00	5.50±0.76
Sulphuric acid	19.33±0.66	18.00±0.57	4.50±0.64
Hydrochloric acid	19.00±1.00	15.33±0.66	5.00±0.70
Nitric acid	20.00±0.00	16.33±1.45	5.50±0.76
Hot water	19.96±0.33	11.33±0.66	7.00±0.91
LSD (5%)	1.228	0.824	1.795

Values are mean ±SEM

Table 2: Summary of analysis of variance for imbibitions percentage, germination percentage and mean days to germination

	Source of variation (SOV)	Degree of freedom (df)	Sum of squares (SS)	Mean squares (MS)	F-cal	F-tab 5%	F-tab 1%	F-tab 0.1%
Imbibitions percentage	Total	17	112.444	-	***			
	Treatment	5	100.444	20.089	20.89	3.11	5.06	8.89
	Error	12	12.000	1.000				
Germination percentage	Total	17	462.278	-	***			
	Treatment	5	435.611	87.122	39.205	3.11	5.06	8.89
	Error	12	26.667	2.222				
Mean days to germination	Total	37	265.474	-	**			
	Treatment	5	113.474	22.695	4.778	2.51	3.65	5.43
	Error	32	152.00	4.750				

*** highly significant (p < 0.01).*** Very highly significant (p < 0.001).*

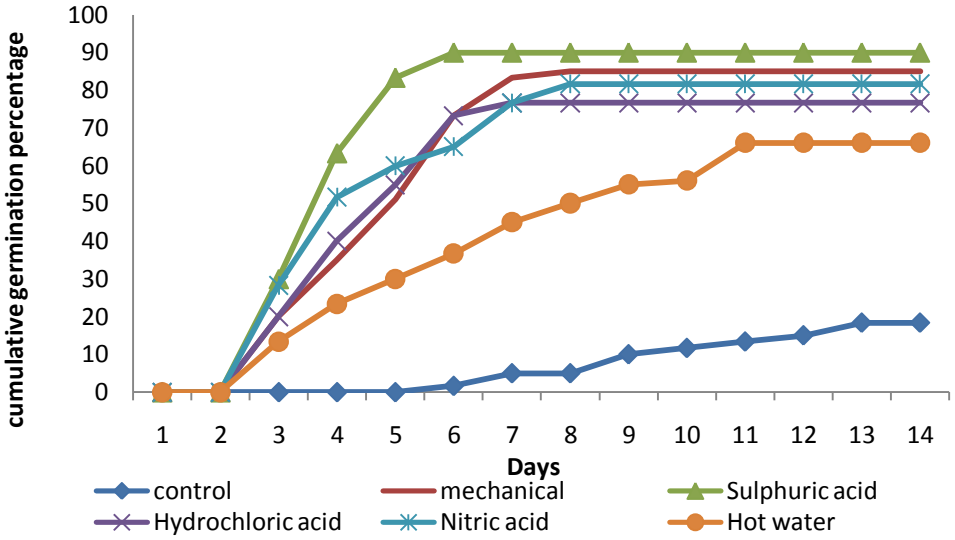

Figure 1: Cumulative germination percentage of *T. tetratptera* seeds after different pretreatment protocols

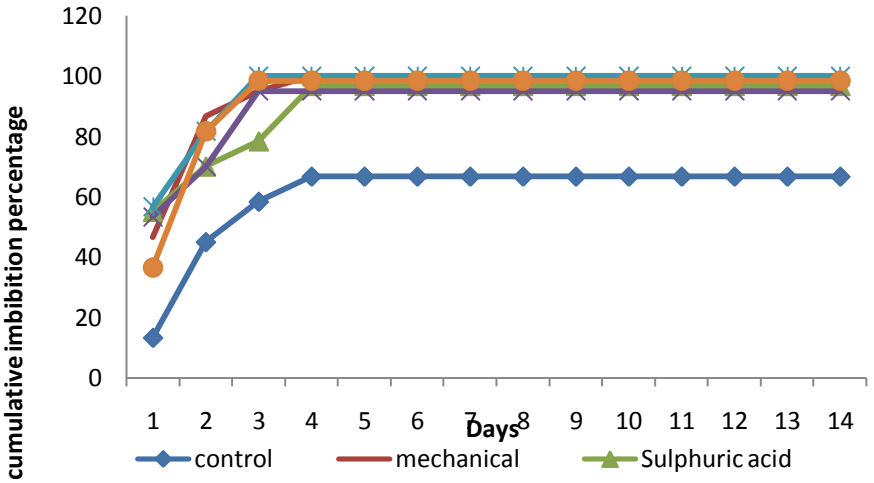

Figure 2: Cumulative imbibition percentage of *T. tetratptera* seeds after different pretreatment protocols

V. Conclusion

Our results revealed that seed dormancy in *Tetrapleura tetraptera* is mainly due to the hard seed coat, which can be broken effectively by either physical or chemical scarification, especially by sulphuric acid preatreatment. It is hoped that the results of this study will provide useful information for domestication and large scale plantation development, and in environmental conservation efforts.

References

[1] Goudie, A. (2006). *The human impact of the natural environment.* Blackwell Publishing: Oxford. p573.
[2] Orwa , C., Mutua, A., Kindt, R., Jamnadass, R., Simons, A. (2009). Agro-forest tree database: a tree reference and selection guide. Version 4.0. (http://www.world agroforestry.org/af/treedb/ Retrieved 2nd August 2012)
[3] Essien, E.U., Izunwane, B.C., Aremu, C.Y., Eka, O.U. (1994). Significance for Humans of the Nutrient contents of the Dry Fruit of *Tetrapleura tetraptera*. *Plant Food and Animal Nutrition*; 45(1):47-51.
[4] Aladesanmi, A.J. (2007). *Tetraplura tetraptera*: molluscicidal activity and chemical constituents. *African Journal of traditional complementary alternative medicines*. 4: 23-36.
[5] Enwere, N.J. (1998). Foods of Plant Origin. *International Journal of Microbiology*. 9 (94):329-334
[6] Akah, P.A., Nwaibie, O.K. (1993). Antibacterial activity of *Tetrapleura tetraptera*. *Journal of Fitoterapia, 1(5): 42-44.*
[7] Adewunmi, C.O., Anderson, H.C., Busk, L. (1991). A potential molluscicide, aridan (Tetrapleura tetraptera), neither induces chromosomal alterations in Chinese Hamster ovary cells, nor mutations in *Salmonella typhimurium*. *Toxicol. Environmental Chemistry*. *30*:69–74.
[8] Ojewole, J. A. and Adewunmi, C. O. (2004). Anti-inflamatory and hypoglycaemic effects of *Tetrapleura tetraptera* (*Fabaceae)* fruit aqueous extract in Rats. *Journal of Ethnopharmacology*. *95:177*-182.

[9] Jimoh, S.O. (2005). Potentials of seedling growth parameters as selection indices in *Tetrapleura tetraptera* from Southwestern Nigeria. *Environtropica*. 2:74-83.
[10] Hilhorst, H.W.M. (1995). A critical update on seed dormancy I; Primary dormancy. *Seed Science Research.* 5:61–73.
[11] Li, B. and Foley, M.E. (1997). Genetic and molecular control of seed dormancy. *Trends in Plant Science.* 2: 384–389.
[12] Mohamed-Yasseen, Y., Barringer, S.A., Splittstoesser, W.E. and Constanza, S. (1994). The role of seed coats in seed viability. *The Botanical Review* 60:426–439.
[13] Jones, H.D. (1999). 'Seeds get a wake-up call'-Dormancy, a survival mechanism for seeds, or a useful tool for agriculture. *The Biologist.* 46:65–69.
[14] Asiyire, B.E., Akinro, L.A., and Amoo, S.O. (2008). Seed germination and *in vitro* propagation of *Piliostigma thonningii*-an important medicinal plant. *African Journal of Biotechnology.* 8(3): 401-404.
[15] Santana, V., Bradstock, R.A., Ooi, M., Denham, A.J., Auld, T.D. and Baeza, M. (2010). Effects of soil temperature regimes after fire on seed dormancy and germination in six Australian Fabaceae species. *Australian Journal of Botany*. 58 (7): 539-545.
[16] Chauhan, B.S. and Johnson, D.E. (2009). Germination, emergence, and dormancy of *Mimosa pudica*. *Weed Biology and Management.* 9: 38–45.
[17] Bewley, J.D. and Black, M. (1994) *Seeds: Physiology of development and germination* (2nd edition). New York, Plenum Press. p445.
[18] Onyekwelu, S. S. (1990). Germination studies in *Tetrapleura tetraptera*. *International Tree Journal*. 6(1):59-66.
[19] Lemos-Filho, J.P., Guerra, S.T.M., Lovato, M.B. and Scotti, M.R.M. (1997). Germinação de sementes de *Senna macranthera*, *Senna multijuga* e *Stryphnodendron polyphyllum*. *Pesquisa Agropecuária Brasileira* 32: 357–361.
[20] Lacerna, D.R.; Lemos-Filho, J.P., Goulat, M.F., Ribeiro, R.A. and Lovato, M.B. (2004). Seed dormancy variation in natural populations of two tropical leguminous tree species: *Senna multijuga* (*Caesalpinoideae*) and *Plathymenia reticulata* (*Mimosoideae*). *Seed Science Research*. 14: 127-135.
[21] Baskin, C.C. and Baskin, J.M. (1998). *Seeds: ecology, biogeography and evolution of dormancy and germination*. Academic press, San Diego.
[22] Cruz, E.D., Martins, F.O. and Carvalho, J.E. (2001). Biometria de frutos e sementes e germinação de jatobácuruba (*Hymenaea intermedia* Ducke, Leguminosae – Caesalpinioideae). *Revista Brasileira de Botânica*. 24: 161–165.