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Efficiency of Sulphuric Acid, Mechanical Scarification and Heat Treatment on Breaking Dormancy of *Tetrapleura tetraptera* Seeds

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ABSTRACT

An *in-vitro* trial was conducted to determine the effect of different scarification methods on seed germination of *Tetrapleura tetraptera.* The trial was carried out using a completely randomized design in three replications. Treatments compared were different concentrations of sulphuric acid (absolute, 90, 70 and 50%), mechanical scarification using sand paper and heat treatments using boiling water at different durations (1, 5 and 7 min). Absolute sulphuric acid used for 7 min resulted in 100% germination of *T. tetraptera* seeds. This was followed by 90% germination with 90% sulphuric acid, also for 7 min. The use of 50 and 70% sulphuric acid at 7 min resulted in 30 and 10% seed germination, respectively. Heat treatment at 100°C resulted in 16% germination when applied for 7 min. Mechanical scarification using sand paper also resulted in 90% germination and is therefore recommended as an alternative for efficient seed germination in the absence of sulphuric acid.

Keywords: germination, hardseededness, seeds treatment, under-exploited spice
Abbreviations: H_2SO_4, sulphuric acid; **DMRT**, Duncan's multiple range test

INTRODUCTION

Tetrapleura tetraptera (Schumach and Thonn) Taub, locally known as 'Aidan' in South Western Nigeria, is a medicinal plant. It belongs to the family Mimosaceae, and is generally found in lowland forests of tropical Africa (Aladesanmi 2007). It is a single stemmed robust, perennial tree about 30 m in height. The fruit consists of a fleshy pulp with small, brownish-black seeds with a characteristically pungent aromatic odour which contributes to its use as a spice (Odesanmi *et al.* 2010). It also has therapeutic use in the management of convulsion, leprosy, inflammation and rheumatoid pains (Dalziel 1948; Adelaja 2005).

Antimicrobial activity exploited in the formulation of dried powdered fruit of the plant improved the foaming ability of soaps (Adebayo *et al*. 2000).

Fruits are used to prepare soups for mothers in Eastern Nigeria from the first day of delivery to prevent post partum contraction (Nwawu and Akah 1996). The fruit also contains cinamic acid, caffeic acid and carbohydrates, the latter two being common in most spices (Adesina 1982). The major critical factor in productivity and expansion of large-scale cultivation of this medicinal plant is the development of hard seeds which is responsible for the seed dormancy probably caused by the seed coat's impermeability to water resulting in the inability of the embryo to penetrate the seed coat during the process of germination (Carvalho 1994). Seed coats that are impermeable to water or oxygen, mechanical restrictions or a combination of these factors, together with the presence of chemical inhibitors, often typify tropical tree seeds (Malavasi 1988). Similar seed inhibition has been reported in other leguminous species such as *Parkia biglobosa*, *Tetrapleura tetraptera*, *Acacia* sp., and *Caesalpinia pulcherima* (Ayisire *et al.* 2009).

One of the factors that hinders the spread of *T. tetraptera* is the high degree of seed dormancy, preventing germination. Numbness is the phenomenon by which seeds of certain species, even if feasible and with all environmental conditions favourable for germination, fail to germinate. In nature, numbness is a resource used by plants that produce seeds to perpetuate the species, since dormancy prevents all seeds from germinating at the same time, increasing their chance of survival and reducing the risk of extinction of that species (Carvalho and Nakagawa 2000; Nascimento *et al.* 2009). Yap and Wong (1983) regarded numbness as the phenomenon responsible for dormancy in legumes and some species of the Malvaceae, Chenopodiaceae, Convolvulaceae, Solanaceae and Liliaceae families. They attributed this to the palisade layer of cells whose cell walls are thick and covered externally by a cuticular waxy layer. Bewley and Black (1994) described numbness as substances that promote and inhibit germination as a result of impermeable seed coat and embryo immaturity. The seed coat impermeability can be determined by the deposition of substances such as suberin, lignin, cutin and gums, forehead, pericarp or nuclear membrane, which is the most common mechanism of dormancy between species of the Leguminosae family.

Considering the fact that only a small percentage of seeds germinate under natural conditions, dormancy of various cutaneous forest species can be broken through scarification (mechanical and chemical) in addition to soaking the seeds in hot water. The application and effectiveness of these treatments depends on the degree of dormancy, which varies between different species, origin and year of collection (Oliveira *et al.* 2003; Nascimento *et al*. 2009).

The presence of a hard seed coat is an indication of the existence of a long period of seed dormancy. An increase in awareness of the utilization of *T. tetraptera* as a spice in Nigeria requires improved seed germination through pretreatments before sowing in order to remove the hard seeds from a long period of dormancy. The objective of this investigation was thus to identify the best pre-sowing treatments for the seeds of this plant.

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Short Communication

Table 1 Effect of pre-sowing treatments on the germination of *Tetrapleura tetraptera* seeds.

Treatments	Duration (min)	Germination percentage
100% H_2SO_4	1	90.0 bc
100% H_2SO_4	5	96.7 ab
100% H_2SO_4	7	100 a
90% H_2SO_4	1	83.3 c
90% H_2SO_4	5	86.6 c
90% H_2SO_4	7	90.0 bc
70% H_2SO_4	1	20.0 e
70% H_2SO_4	5	23.3 e
70% H_2SO_4	7	30.0 d
50% H_2SO_4	1	3.3 f
50% H_2SO_4	5	6.7 f
50% H_2SO_4	7	10.0 e
100^0C H_2O	1	3.3 f
100^0C H_2O	5	10.0 e
100^0C H_20	7	16.7 e
Sand paper		90.0 bc
Control*		0 f

Means followed by the same letters are not significantly different at $P = 0.05$ using Duncan multiple range test (DMRT)

*Each separate treatment had a separate control but the control is clustered since germination was 0% in all controls.

MATERIALS AND METHODS

The experiment was carried out in a laboratory using a completely randomized design with three replications. Three methods of seed scarification (acid, heat treatment, mechanical) were used, with 10 seeds per treatment. Sulphuric acid (H_2SO_4) was tested at four concentrations (50, 70, 90, 100%) while heat treatment involved soaking the seeds in boiling water (100°C) for 1, 5 or 7 min; mechanical scarification was possible by grinding the seeds with No 80 sand paper (Rodex Abrasives and Chemical Co., China), ensuring no damage to the embryo. Seeds soaked in distilled water served as the control. Thereafter the treated seeds were maintained in Petri dishes in moist cotton wool and incubated at room temperature. Percentage germination was recorded for each treatment in three replications, these were subjected to analysis of variance and the means were separated using Duncan multiple range test (DMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

The effects of pre-sowing treatment on the germination of *T. tetraptera* seeds is shown in **Table 1**.

The use of acid scarification resulted in the highest percentage germination compared to other treatments. Seeds soaked in concentrated H_2SO_4 for 7 min gave the highest germination (100%), closely followed by 96.7 and 90% germination after 5 and 1 min exposure to concentrated H_2SO_4, respectively. Onyekwelu (1990) also reported 100% germination of *T. tetraptera* seeds by dipping in H_2SO_4. Treatment with H_2SO_4 has been used successfully to overcome dormancy of seeds of *Ormosia arborea* (Lopez *et al*. 2004). *Leuceana leucocephala* treated for 15-20 min resulted in a germination percentage of 90% while *Dimorphandra mollis* seeds scarified with H_2SO_4 for 45-90 min showed 90% germination (Nascimento *et al*. 2009).

The percentage germination reduced significantly as the concentration of H_2SO_4 decreased. Germination percentage was 90, 30 and 10% when seeds were treated (all for 7 min) with 90, 70 and 50% H_2SO_4; no control seeds germinated irrespective of durations (**Table 1**).

Seeds immersed in boiling water for 7 min gave the highest germination (16%); no germination was observed in the control (**Table 1**). A sudden dip of dry seeds in boiling water may rupture the seed coat, allowing physiological changes and subsequent germination of the embryo (Agboola and Etegere 1991; Agboola and Adedire 1998). Fernández *et al.* (2000) and Nascimento *et al.* (2009) also reported that the rupture of the seed coat allows for immediate imbibition and germination.

Mechanically scarified seeds using sand paper also resulted in 90% seed germination irrespective of the durations (**Table 1**). Seed dormancy resulting from an impermeable seed coat may be overcome by peeling off the coat (Nikoleave 1977; Adelaja 2005). Ezumah (1986) and Onyekwelu (1990) reported 73% germination of *T. tetraptera* seeds treated by rubbing them with sand paper and rough coarse surfaces, lower than the 90% we recorded (**Table 1**). The mechanical scarification of seeds by friction against abrasive surfaces is recommended for small seed lots of *Ormosia arborea* (Vell.) Harms (López *et al*. 2004), *Operculina macrocarpa* (L.) Farwel. (Medeiros Filho *et al.* 2002) and *Parkia platycephala* Benth. (Nascimento *et al*. 2009).

Medeiros Filho *et al.* (2002) reported that mechanical scarification causes cracks in the seed coat, increasing permeability and allowing the absorption of water and therefore, the initiation of germination. In legumes and other families such as the Mimosoideae, Malvaceae, Solanaceae, Liliaceae and Convolvulaceae, when seed coats are treated with H_2SO_4 and manual scarification there is usually a high level of germination (Nascimento *et al*. 2009).

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