# Propagation of Indigenous Species: The effects of various Seed Pre-treatments to Improve Germination in *Strychnos cocculoides (*Monkey orange) and *Guibourtia coleosperma* (False mopanne) from Kavango West Region, Namibia.

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# 1. Abstract

*Strychnos cocculoides* (Monkey orange) and *Guibourtia coleosperma* (False mopane) are socio-economic important indigenous species found in the Northern regions of Namibia. The impact of six seed pre-treatments (cold water, warm water, hot water, scarification and 32% Hydrochloric Acid) on seed germination were investigated for both species. Seed viability was assessed with two treatment methods (Tetrazolium and Ragdoll). Overall average germination results indicated highly significant difference between the six pre-treatments. The germination percentage ranged from 0% for *S. cocculoides* seeds soaked in HCl to 83% for *G. coleosperma* seeds soaked in warm water. High and low average germination percentage in *G. coleosperma* and *S. cocculoides* were observed on seeds treated with warm water (51%), (30%), HCl (37%), and (0%) respectively. Water soaking was the most efficient pre-treatments for all the species while Hydrochloric Acid was less effective pre-treatments to both the species. Pre-treatment of *S. cocculoides* and *G. coleosperma* seed is recommended to improve germination as they help to break dormancy and initiate germination in the species. It is recommended that for future studies, indigenous germination response on different temperature and light intensity need to be investigated.

**Key words:** *Strychnnos cocculoides, Guibortia coleosperma,* pre-treatment, viability, Tetrazolium, Ragdoll, Germination Percentage (GP)

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## 2. Introduction

Namibia is rated to have the driest climate in sub-Saharan Africa, with the mean annual rainfall of approximately 270 mm (Namibia, 2006). Located in the north eastern and western part of the country, the dry woodlands is characterized by *Baikea plurijugia, Strychnos cocculoides, Burkea africana, Guibourtia coleosperma* and *Pterocarpus angolensis* as the key important indigenous species. Rural communities depend heavily on these species for food and wood products. As they use them in parkland agroforestry systems. Parklands are mixture of indigenous trees and shrubs that farmers select for certain functions and cultivate together with other staple food crops (Kalinganire, *et al*. 2008). Trees and shrubs provide many functional benefits to rural people as sources of food (fruits and oils), traditional medicines, fuel wood, construction materials and fodder for livestocks. Indigenous forest species are thus indispensable (not only as a parkland agroforestry system) to the communities in the country (Mohan & Priyadarshan, 2009).

The utilization and high dependence on indigenous species and products are however resulting in biological biodiversity depletion (Frank *et al.* 1995). This have caused rapid decline in the availability and productivity of the land to produce the goods and services adequate for human survival (Akinnifesi *at el.* 2008). Nursery experiment on assisted germination on indigenous woodland trees have been limited. There is a need to establish germination protocols for the indigenous species and provide well documented information on germination techniques that can be applied in future (De Cauwer & Younan 2015).

Re-introduction is essential for indigenous species plant conservation, especially for species with few remaining wild population (Hartmann *et al*. 2011). Namibian government is committed to natural and artificial regeneration (tree propagation methods) of commercially important indigenous species (Forestry, 2011); through research, conservation of natural resources (*in situ* and *ex situ)* and collections of germplasm throughout the country (Namibia, 2006). However, forests counteract depletion in biological biodiversity propagation (seeds and vegetative cuttings) of commercial important indigenous tree species, need to be understood.

This study concentrated on the best practice to improve germination of two such commercial important indigenous species, *Strychnos cocculoides* (Monkey orange) and *Guibourtia coleosperma* (False mopane). They are both woody indigenous tree species in the northern regions of Namibia (Mwamba,

2006). Both species are utilized by local communities for food consumption and income creation (Elago, 2015). Resulting from high utilization, these species might be depleted in the near future.

Fruit of *S. cocculoides* or "Maguni" is globose in shape; with a dark green color when unripe and yellow to orange when mature (Akinnifesi *et al*. 2008). The wood is white in color and used for tool handles and building materials, while roots and leaves are used for traditional medicine (Mwamba, 2006). *Guibourtia coleosperma* or "Usivi" fruit are red bean like and edible when cooked, while roots are used for medicine (Akinnifesi *et al*. 2008). The trees are mostly used for timber and NTFP's such as fruits and medicine.

Efforts to germinate, grow and increase the number of trees of these two species are hampered by poor and erratic seed germination and slow growth (Kwapata, 1990; Maghembe *et al*. 1994). There are also limited studies available on the appropriate methods to propagate these species. The current study focused on six different pre-treatments (control, cold water, warm water, hot water, scarification and chemical) of the seeds for both *S. cocculoides* and *G. coleosperma* to improve germination percentage.

Considering that in practice the propagation of these species is accomplished mainly by seeds, the objectives of this study were to evaluate the impact of pre-treatments on seed germination percentage of *S. cocculoides* and *G. coleosperma;* evaluate the weekly germination rate over a seven week period and test seed viability before sowing with two biological tests (tetrazoliun and ragdoll). Specific research questions included: how will the seeds respond to different pre-treatments?; is there significance difference in germination rate between the treated and untreated seeds?; which treatment will have the highest germination rate?; does time have an effect on seed germination percentage?; how will seeds react to the two different viability test methods?; and what is the seed viability before sowing?

## 3. Materials and Methods

*Guibourtia coleosperma* and *Strychnos cocculoides* seeds were harvested from plus trees in the Kavango West region in Namibia, during 2014. Seeds were processed and sowed within six months after harvesting. *Guibourtia coleosperma*, seeds were dried in direct sun for 48 hours to remove moisture and stored in sealed containers (water and airtight) at 10 °C pending sowing. *Strychnos*

*cocculoides* seeds were washed to remove all the pulp, dried for seven days (open wind and not direct sunlight) and stored in sealed containers (water and airtight) at 10 °C pending sowing.

### 3.1 Seed Viability

Seed viability was tested in a parallel experiment to confirm germination potential of seeds. Two methods were investigated, namely Tetrazolium and ragdoll also known as top of paper. Tetrazolium (2, 3, 5 triphenyl tetrazoliun chloride) is a reliable and widely utilized method as living tissue (for example embryo) will be stained pink (Ellis *et al.* 1985). In the Ragdoll method, seeds are placed between two sheets of moist filter paper inside a glass petri-dish (Bicksler, 2011).

Ten randomly chosen seeds from *S. cocculoides* and *G. coleosperma* were cut in half and placed embryo down into a 1 % tetrazolium solution for four hours. Color change of the embryos was rated as either viable (pink) or non-viable (colorless). For the Ragdoll method, ten untreated seeds were also chosen randomly per species. Petri dishes were kept moist at room temperature (25 $^{o}$C) for 10 days and the number of viable seeds was counted daily. The seeds were considered viable when the radicle was visible (Hartmann *et al.* 2011). Percentage seed viability was documented daily and calculated as follows:

$$SV = \left(\frac{no\ \ of\ viable\ seeds}{no\ of\ tested\ seeds}\right) X\ 100$$

### 3.2 Pre-sowing seed treatments

In total, 360 seeds (180 for both *S. cocculoides* and *G. coleosperma*) were pre-treated to evaluate the impact on germination percentage. The pre-treatments were divided into four groups: mechanical (rubbing the seeds between two sheets of medium grained sand paper to remove the outer coat or testa without injuring the embryo); physical (soaking seeds in distilled water (45 and 100 $^{o}$C) for 24 hours); chemical (immersing seeds in 32 % Hydrochloric Acid (HCl) for five, 10 and 20 minutes respectively); and control. After each treatment, the seeds were rinsed thoroughly with distilled water.

### 3.3 Seedling Growth

The seeds were germinated under controlled laboratory conditions (*in vitro*) in an incubator (LTIS). Temperature inside the incubator was kept at a constant 25 °C as seeds can germinate between 20 and 30

°C (Rasheed *et al.* 2015). Growth lights were kept under photoperiod of 12 hours lights and 12 hours darkness. Water was applied daily to keep seeds moist. The number of germinated seed was collected weekly for seven weeks. The seeds were considered germinated when the embryonic plant begins to grow and the seed coat breaks open above the substrate (Hartmann *et al.* 2011).

### 3.4 Experimental design and data collection

The experiment employed a completely randomized block design with a factorial treatment structure two species and seven time periods (weeks) with three replications each and six pre-treatments. An experimental unit (species x replication) consisted of 30 seeds per pre-treatment. An analysis of variance (ANOVA) for each species was performed using SAS/STAT® software (version 9.2, 64 bit, System for Windows 7). A Shapiro Wilk test for normality was conducted before the results could be assumed reliable. A Fischer Least Significant Difference (LSD) with $p$ = 0.05 (5 %) was used to compare treatment means per species (Shapiro & Wilk 1965, Ott & Longnecker 2001, SAS 2008).

The sources of variation were partitioned into species, replications within pre-treatments, species an time, as well as the interactions of pre-treatments, species and time. The statistical model is given by:

$$X_{ijkl} = \mu + Y_i + L_j + YL_{ij} + G_k + GY_{ik} + GL_{jk} + GYL_{jik} + \in_{ijkl}$$

with observed germination percentage ($X_{ijkl}$); general mean ($\mu$); pre-treatment ($Y_i$); species ($L_j$); interaction of pre-treatment and species ($YL_{ij}$); time ($G_k$); interaction of time and pre-treatment ($GY_{ik}$); interaction of time and species ($GL_{jk}$); interaction of pre-treatment, time and species ($GYL_{jik}$) and error ($\in_{ijkl}$).

## 4. Results

### 4.1 Seed Viability (SV) of *Strychnos cocculoides* and *Guibourtia coleosperma*

Seeds viability was tested by using Tetrazolium and Ragdoll test methods. In Tetrazolium the seeds were tested after pre-treatments whereas Ragdoll test seeds were tested without pre-treatments.

#### 4.1.1 Tetrazolium test method

Results indicated that *G. coleosperma* seeds had a higher viability with average of 8.7%, compared to *S. cocculoides* (8%). Moreover the chemical treatment had the lowest viability for both the species

(**Fig 1**). **Figure 2** indicated stained embryo of *G. coleosperma* and *S. cocculoides* after four hours in Tetrazolium solution. The pink color indicates the viability of the seeds.

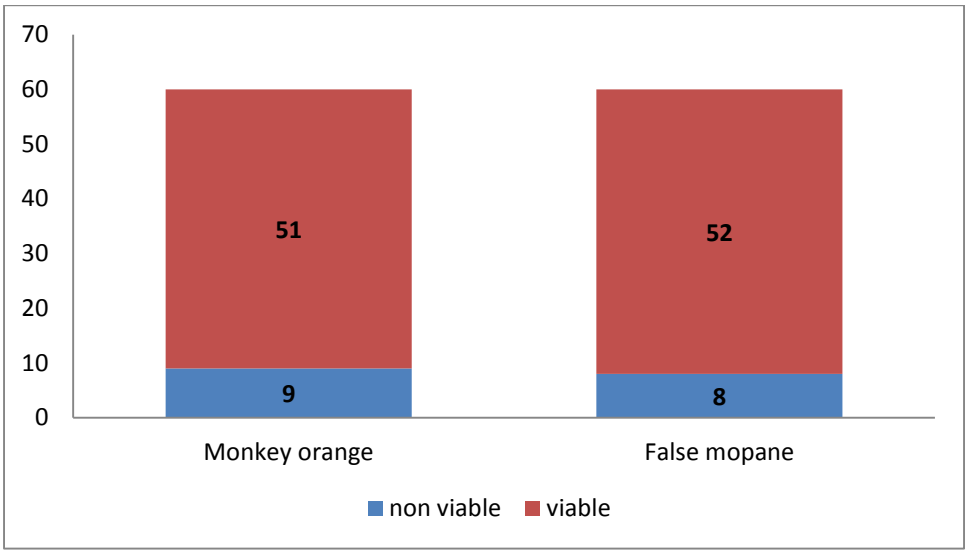


***Figure 1:*** *The effect of the Tetrazolium test on seeds viability from a total of 60 seeds.*

***Figure 2****: Pink stained embryos of* G.coleosperma *and* S.cocculoides *after four hours in the Tetrazolium solution (pink color indicating viability).*

### 4.2.1 Ragdoll test method

Test results observed over a period of 10 days indicated that *G. coleosperma* seeds soaked in the petri dish had germinated radicles compared to *S. cocculoides* with none (**Figure 3**). This demonstrates that only *G. coleosperma* seeds were viable in the ragdoll test. **Figure 4** indicates radicle from *G. coleosperma* seeds after 10 days in ragdoll experiment and non-germinating *S. cocculoides* seeds.

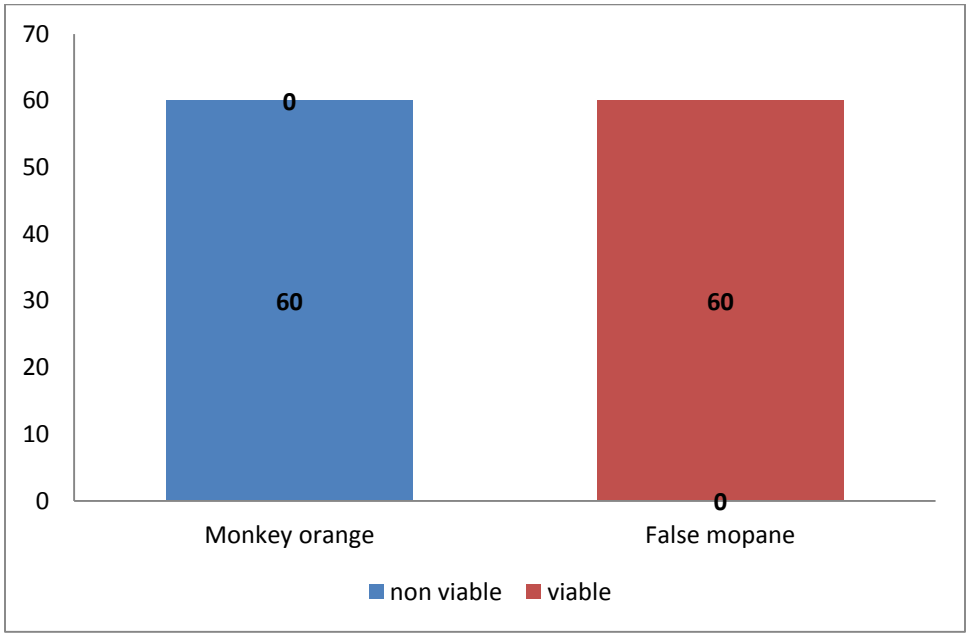


***Figure 3:*** *The effect of Ragdoll test method on seed viability from a total of 60 seeds.*

***Figure 4****: Seeds of* G. coleosperma *and* S. cocculoides *germinating during the Ragdoll test method*

### 4.2 Effect of pre-treatments on Germination Percentages

There was a difference in Germination Percentages (GP) between the six pre-treatments in *S. cocculoides* **(Table 1)** and *G. coleosperma* **(Table 2)**. The GP ranged from 0% for *S. cocculoides* seeds soaked in HCL to 83% for *G. coleosperma* seeds soaked in warm water. Overall GP in *S. cocculoides* indicated poor germination in seeds treated with chemical (0%) and scarification (3.33%). However, the seeds have responded well to cold (70%) and warm (80%) water treatments. GP in *G. coleosperma* on the other hand, indicated low GP in seeds treated with chemical and scarification with an average of 67% for both treatments. The highest GP germination percentages were observed in cold (80%) and warm (83%) water treatments.

**Table 1**: Effect of six pre-treatments on final germination percentage of *S. cocculoides* over a seven week period

| | | **Treatment** | | | | | |
|---|---|---|---|---|---|---|---|
| | **Weeks** | **Control** | **Cold water** | **Warm water** | **Hot water** | **Chemical** | **Scarification** |
| *Strychnnos cocculoides* | **1** | 0 | 0 | 0 | 0 | 0 | 0 |
| | **2** | 0 | 0 | 0 | 0 | 0 | 0 |
| | **3** | 10 | 3.33 | 10 | 0 | 0 | 0 |
| | **4** | 26.67 | 30 | 26.66 | 16.67 | 0 | 0 |
| | **5** | 46.67 | 43.33 | 33.33 | 20 | 0 | 3.33 |
| | **6** | 56.67 | 46.67 | 60 | 23.33 | 0 | 3.33 |
| | **7** | 63.33 | 70 | 80 | 40 | 0 | 3.33 |

**Table 2:** Effect of six pre-treatments on final germination percentage of *G. coleosperma* over seven week period

| | | **Treatment** | | | | | |
|---|---|---|---|---|---|---|---|
| | **Weeks** | **Control** | **Cold water** | **Warm water** | **Hot water** | **Chemical** | **Scarification** |
| *Guibortia coleosperma* | **1** | 0 | 0 | 0 | 0 | 0 | 0 |
| | **2** | 0 | 6.67 | 3.33 | | 0 | 0 |
| | **3** | 20 | 15.28 | 36.67 | 36.67 | 20 | 43.33 |
| | **4** | 63.33 | 73.33 | 70 | 60 | 50 | 56.67 |
| | **5** | 70 | 80 | 83.33 | 63.33 | 60 | 60 |
| | **6** | 80 | 80 | 83.33 | 66.67 | 63.33 | 63.33 |
| | **7** | 80 | 80 | 83.33 | 70 | 66.67 | 66.67 |

Results indicated a highly significant ($p < 0.001$) difference between the six pre-treatments, species by pre-treatment interaction as well as pre-treatment by time interaction. Germination percentage of *S. cocculoides* seed can be divided into three groups **(Table 3)** according to significant differences: control, cold water and warm water; hot water; as well as chemical and scarification.

**Table 3:** Comparison of *S. cocculoides* average germination percentage for six pre-treatments over seven weeks period (n=21 and standard error of 7.02)

| Pre-treatments | Mean | *t*-grouping |
|---|---|---|
| Control | 29.05 | A |
| Cold water | 27.62 | A |
| Warm water | 30 | A |
| Hot water | 14.29 | B |
| HCL 32% | 0 | C |
| Scarification | 1.43 | C |

Means with the same letter are not significantly different

*Guibourtia coleosperma* showed no significant difference in average germination percentages **(Table 4)** between seeds treated with chemical (37.143%) and the rest of the treatments: control (44.762%); cold water treatment (50.952%); warm treatment (51.429%); hot treatment (42.381%) and scarification (41.429%).

**Table 4:** Comparison of *G. coleosperma* average germination percentage for six pre- treatments over seven weeks period (n= 21 and standard error is equal to 7.66)

| Treatments | Mean | *t*-grouping |
|---|---|---|
| Control | 44.76 | A |
| Cold water | 50.95 | A |
| Warm water | 51.43 | A |
| Hot water | 42.38 | A |
| HCl 32% | 37.14 | ABC |
| Scarification | 41.43 | AB |

Means with the same letter are not significantly different

Time (weeks) also had an effect on average germination percentage of the *S. cocculoides* and *G. coleosperma*. Week 1 had 0% germination for all the pre-treatments in both species, while in week seven germination percentage was 83% for warm water pre-treatment in *G. coleosperma* seeds. In addition, cold and warm water pre-treatments had the shortest germination period in all the species **(Table 3 and 4).**

## 5. Discussions

Seeds germination is the process of the seed beginning to grow after a period of dormancy (Ofori *et al.* 2015) but may vary as seeds are produced under different environment conditions (Baskin & Baskin, 2001). For instance *G. coleosperma* seed can germinate well from different pre-treatments such as soaking in hot and cold water overnight, or immersing in chemicals (Moses, 2012). Previous studies reported on propagation of *S. cocculoides* by seeds been successful, with 80% germination for seeds sown in the propagation box during summer (Taylor *et al*.1996; Akinnifesi *et al.* 2008).

Seeds were stored at 10°C for approximately six months before sowing in the study. However, in practice seeds can be stored at the same temperature for up to 12 months before sowing (Hartmann *et al*. 2011). Viability test was therefore carried out to determine the germination potential and likely number of seeds to be sown per replicate during the experiments. The Tetrazolium test was carried out after the six pre-treatments but before sowing and was in contrast with a previous study by Moses (2012). According to the study, the viability was tested on the seeds that failed to germinate (Moses, 2012). Current study, Ragdoll test was carried out before sowing but with untreated seeds.

Highly significant ($p< 0.001$) germination differences between the six pre-treatments was observed. The water soaked seeds (cold 50.95%, warm 51.42% and hot water 42.38% pre-treatments) had an average germination percentage higher in both species than the chemical (0%) and scarification (1.42%) pre-treatments. Time had an effect on germination percentage, for example water pre-treatments germinated earlier (week 2) than scarification and chemical pre-treatments (week 4). This indicates that the impermeability of the pericarp to water and possibly oxygen was a major constrained on germination success. It was also observed, many seeds in chemical and scarification pre-treatments died, indicating that the seed embryo might be damaged. In this case, the two treatments (chemical and scarification) were not conducive for both species germination. In contrast, *G. coleosperma* had the

highest germination percentage (more than 80%) compared to *S. cocculoides* (70 %). This correlates with previous studies by De Cauwer & Younan, (2015) and Moses, (2012).

Our results demonstrate how species respond to different pre-treatments. This indicated different ecological characteristics and requirements for germination (Mapongmetsem, *et al*. 1999). The germination percentage chemical pre-treatment in *G. coleosperma* (67%) was higher than for *S. cocculoides* (0%); implying poor germination of *S. cocculoides* seeds in chemical pre-treatment. In general, chemical pre-treatment had the lowest germination percentage for both species. This correlates with a study by Sy *et al*. (2001), indicating that immersion of seeds in chemicals could be insufficient and lead to damaged seeds.

During this study, seeds were immersed at three different time periods (five, ten and 20 minutes) in 32% HCl to determine the optimum amount of time needed for successful germination. Five minutes deemed successful as the seeds were not damaged and the colour did not change. In depth studies to determine chemical requirements of the species seeds are needed in the future.

## 6. Conclusions and recommendations:

In conclusion, the results demonstrated *Strychnos cocculoides* and *Guibourtia coleosperma* responded well to some of the pre–treatments with the highest germination percentage for warm and cold water treatments. Furthermore, the two species need two weeks or longer to sufficiently break dormancy. The viability tests (Tetrazolium and Ragdoll) confirmed that seeds were viable even after six months of storage and before sowing. It is recommended that for future studies, the germination response on different temperature and light intensity need to be investigated. In addition, pre-treatments can help to break dormancy and initiate germination in indigenous species by softening the hard seed coat with water. Pre-treatment of seed is recommended to improve and multiply germination on *S. cocculoides* and *G. coleosperma.*

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