

# Effects of substrate and pre-sowing treatment on Fagara (*Zanthoxylum zanthoxyloides* (Lam.) Watermann) seeds germination

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## Abstract

*Zanthoxylum zanthoxyloides* (Lam.) Watermann seeds excised from ripe and fresh fruits were evaluated for their germination capacity under different pre-sowing and substrates conditions. Initial moisture content (iMC) of seed lots was assessed using 4x10 seeds while initial germination was evaluated on three different substrates: agar 1% concentration, river sand and a composite substrate of soil, sand and manure, using 4x25 seeds per assay. In a second experiment, seeds were subjected to three pre-treatments prior to sowing. Seeds iMC varied between 7 and 8% suggesting that they are likely orthodox. Seed started to germinate between 24 and 45 days after sowing. This minimum incubation period is rather long if one considers that the usual duration for tree seeds germination testing is 28 days. An improvement of germination was observed when combining the best substrate with the best pre-sowing treatments. Highest percentage, recorded on the composite substrate, rose from 42±0.71% when seeds were sown intact, to 59±0.70% when rubbed with sand and to 61±1.41% when washed with soap prior to sowing. We concluded that *Z. zanthoxyloides* seeds exhibit dormancy due to impermeable and/or oily seed coat. For seedlings production, seeds should be previously washed with soap or polished with sand and sown on a mixture of soil, sand and manure in a ratio of 3v/1v/1v.

**Keywords:** *Zanthoxylum zanthoxyloides*, medicinal plant, tropical tree seeds, dormancy, Burkina Faso.

## Effets du substrat et du traitement avant semis sur la germination des semences du Fagara (*Zanthoxylum zanthoxyloides* (Lam.) Watermann)

### Résumé

Des semences de *Zanthoxylum zanthoxyloides* (Lam.) Watermann extraites de fruits frais matures ont été étudiées pour les effets du substrat et du prétraitement sur leur germination. La teneur en eau initiale (TEi) des lots de semences a été évaluée en utilisant 4 x 10 graines, tandis que la germination initiale a été évaluée sur trois substrats différents : Agar 1%, sable et un mélange de terre, sable et fumier, en utilisant 4 x 25 graines par essai. Dans une seconde expérience, les graines ont été soumises à trois prétraitements avant le semis. La TEi des semences variait entre 7 et 8%, ce qui suggère qu'elles sont probablement orthodoxes. Les graines ont commencé à germer entre 24 et 45 jours après le semis. Cette période minimale d'incubation est assez longue si l'on considère que la durée habituelle des tests de germination des graines d'espèces tropicales est de 28 jours. Une amélioration de la germination a été observée en combinant le meilleur substrat avec les meilleurs prétraitements. Le pourcentage le plus élevé, obtenu sur le substrat composite, est passé de 42±0.71% lorsque les graines ont été semées intactes, à 59±0.70% lorsqu'elles sont scarifiées au sable et à 61±1.41% lorsqu'elles sont lavées avec du savon avant le semis. Nous avons conclu que les graines de *Z. zanthoxyloides* présentent une dormance due à un tégument imperméable et / ou huileux. Pour la production de plants, les graines doivent être préalablement lavées avec du savon ou polies avec du sable et semées sur un mélange de terre, de sable et de fumier dans les proportions de 3v / 1v / 1v.

**Mots clés :** *Zanthoxylum zanthoxyloides*, plante médicinale, semences d'espèces tropicales, dormance, Burkina Faso.

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## Introduction

*Z. zanthoxyloides* (Lam.) Zepernick & Timler (syn. *Fagara zanthoxyloides* L.) (Rutaceae) is a woody plant species found in West African savannah and the inshore areas from Senegal to Nigeria (ARBONNIER, 2004). The tree can reach up to 18 m high and 0.5 m diameter and the whole plant is of medicinal importance, highly appreciated by the populations. Indeed, most species of the *Zanthoxylum* genus are recognized for their medicinal qualities and are source of essential oils and good ornamental trees/shrubs (OUATTARA *et al.*, 2016). Several ethno pharmacological surveys (VAN ANDEL *et al.*, 2012; BOROKINI *et al.*, 2013) have highlighted the use of *Z. zanthoxyloides* in traditional medicine. Particularly, the anti-sickling properties of the stem bark and roots extracts have been studied (OUATTARA *et al.*, 2009; OUEDRAOGO *et al.*, 2011; OGUNBOLUDE *et al.*, 2014; OUEDRAOGO *et al.*, 2015;) and have resulted in the development of phytomedicines such as “*FACA*” used to treat sickle cell anaemia. Furthermore, roots bark is also known for its insecticidal activities (BUXTON *et al.*, 2017). Despite its high medicinal value, *Z. zanthoxyloides* is not a protected tree species in Burkina Faso and uncontrolled and destructive harvesting methods of the species by pulling up roots provoke a significant decline of *Z. zanthoxyloides* populations in their native locations.

Literature on the medicinal values of the species can qualified as been rich, while it is difficult to find a tangible research results on the biology of the species making data on its germination rather sparse. The few studies on *Z. zanthoxyloides* seeds biology (SANON *et al.*, 2004 and NEYA *et al.*, 2017) reported that the species experiences regeneration difficulties.

The first group of authors investigated on the species seeds desiccation tolerance and concluded that they exhibit physiological dormancy and should therefore be stored at 4°C during 9 months before they can achieve a maximum germination percentage of 37%. Regarding, the second study, NEYA *et al.* (2017) have tried to improve the germination percentage of fresh and dried seeds using different pre-sowing treatments and concluded that *Z. zanthoxyloides* seeds exhibit physical dormancy that could be overcome by chemical scarification.

Following these two pioneer studies, which concluded differently regarding the type of dormancy involved in the species seeds germination, the current study was initiated to get more insight in *Z. zanthoxyloides* seeds by focusing on the double impact of pre-treatments and substrates as means to improve this multipurpose species seeds germination. The final aim being to facilitate the production of its seedlings in nurseries and build the foundation for its domestication. It is anticipated that improved germination would lead to increased use of the species in reforestation programs, degraded land restoration, and communities’ medicinal trees plantation projects.

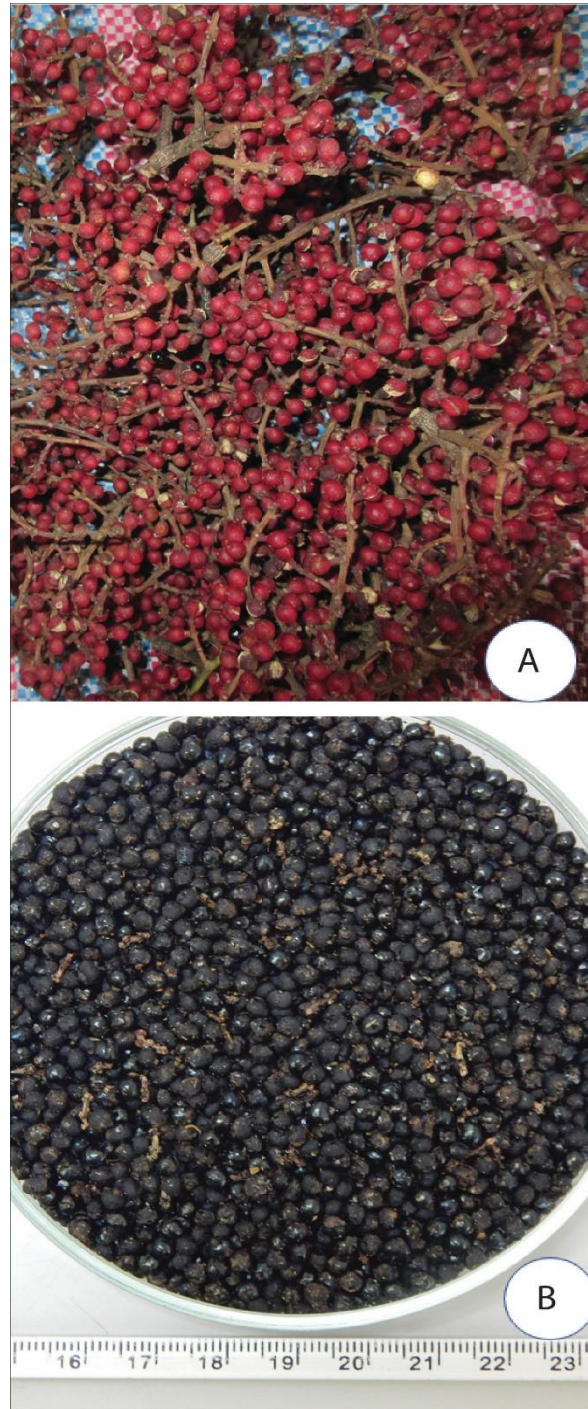
## 1-Material and methods

### 1.1. The site of study

Fruits of *Z. zanthoxyloides* were collected in December 2016 from natural stands in four different sites: Bandougou, Orodara, Toussiana and Toussiamasso. All the collecting sites are located in the South-Sudanian zone due to the fact that the species is already missing in the other phytogeographic zones of Burkina Faso. The South Sudanian zone is located between the latitudes 10° and 12°30' North and between the longitudes 2° East and 5° West. The climate is of sudanian type and the rain season lasts 4 to 6 months. The annual mean rainfall vary between 900 and 1200 mm (FONTES & GUINKO, 1995). The average annual temperature of the last ten years varied between 25 and 30°C.

## 1.2-Fruits collection and seed processing

For each site, red mature fruits (Figure 1A) were harvested from a minimum of 15 individual trees. Freshly harvested fruits were spread on cotton bags in the laboratory where the ambient temperature is between 30 and 35°C during daytime and between 20 and 25°C during the night and the relative humidity (RH) ranged between 40 and 45%. This treatment was intended to cause the dehiscence of the follicles and facilitate seeds extraction. The next day, seeds were processed by scrubbing the follicles between the two hands to extract the seeds (Figure 1B).



**Figure 1:** *Z. zanthoxyloides* mature fresh fruits (A) and seeds extracted from mature fresh fruits (B).

## 1.2-Seed initial moisture content assessment

For each site, seeds moisture content at harvest was assessed according to ISTA (2015) rules using four replicates of ten seeds. Replicates were weighted before and after drying in an oven (Binder E28, German) at 103°C for 17 hours. Moisture contents were expressed on fresh weights basis.

## 1.3-Comparative substrates test

In this experiment, seeds were sown without any pre-treatment. Germination assays were performed in the laboratory using three different substrates: river sand previously sterilized at 200°C in the previously described oven for two hours, agar at 1% concentration and a mixture of 3 volumes of soil, 1 volume of sand and 1 volume of organic manure. For each substrate germination tests were performed using 4 replicates of 25 seeds sown in Petri dishes. After sowing, the Petri dishes were placed at 35°C in a Sanyo brand incubator; model MIR 553, made in Japan. Distilled water was used for watering the sand and the mix substrate when necessary. Germination based on the emergence of the radicle from the tissues enclosing it (REDONDO-GÓMEZ *et al.*, 2007; BEWLEY *et al.*, 2013) was recorded every two days. Final germination percentage was calculated as recommended for germination tests of tropical forest seeds (ISTA, 2015).

## 1.4-Comparative pre-sowing test

In this experiment, seeds were subject to three distinct treatments (see table) prior to sowing on the above described substrates in laboratory. Germination speed provides valuable information about the degree of dormancy loss and the favourability of germination conditions (BASKIN & BASKIN, 2014). Therefore, germination speed,  $V$ , defined as the number of germinated seeds per day, was calculated according to MAGUIRE (1962) as follow:

$$V = \sum_{i=1}^n \frac{G_i}{D_i} \quad \text{Eq. (1)}$$

where  $G_i$  is the number of germinated seeds on day  $i$  and  $D_i$  is the number of days after sowing. For each assay, minimum incubation period defined as the number of days from sowing to beginning of germination (HOSSAIN *et al.*, 2005) was recorded.

**Table I:** Pre-sowing treatments tested on *Z. zanthoxyloides* seeds.

Pre-sowing Treatment	Description
Water soaking 24h	Seeds are soaked in tape water 24h prior to sowing.
Soap washing	Seeds are placed in a plastic cup and liquid soap is poured until they are covered. They are then scrubbed 20mn at least, washed and soaked in tape water 24h before being sown.
Sand washing	Seeds are mixed with humid river sand (three times the amount of seeds) and scrubbed 20mn at least, then washed with tape water. They are then spread on a cotton bag at ambient temperature in the lab and sown the next day.

## 1.5-Data analysis

Univariate descriptive statistics were calculated for each variable measured using the software package R. Boxplots were designed to appreciate variable's distribution compare to the mean.

Normal distribution and homogeneity of variance were tested for residues using statistics provided by the univariate procedure.

All the data collected were subjected to an analysis of variance (ANOVA). The data were organized into factor (substrate, pre-sowing treatment, replicate) in order to follow the conditions of the ANOVA. An arcsin transformation of the germination frequencies whose values were between 0 and 1 was performed prior to ANOVA. All the frequencies equal to 0 and 1, have been improved by using respectively the following formulas:

$$f = \frac{1}{4n} \quad \text{and} \quad f = 1 - \frac{1}{4n} \quad \text{Eq. (2)}$$

where  $n$  is the total number of observations.

Angular transformation was then performed with the following formula:

$$\theta = \sin^{-1} \sqrt{p} \quad \text{Eq. (3)}$$

where  $\theta$  is the improved value and  $p$ , the gross germination frequency.

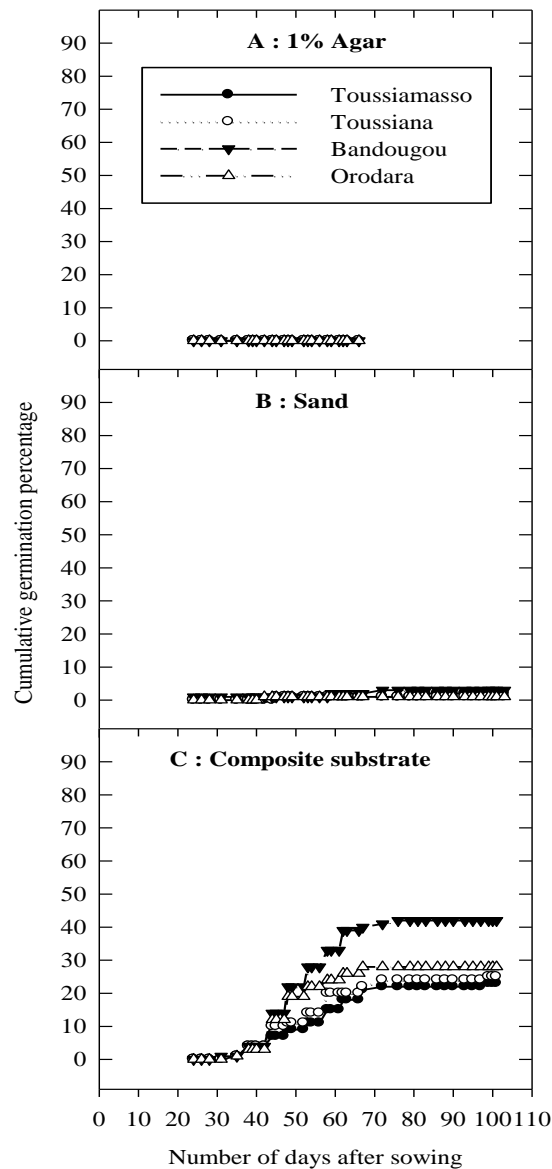
Mean comparison for germination characteristics (percentage and speed) was performed with Tukey High Significant Difference (HSD) test at  $P < 0.05$ , when the ANOVA test was significant. Illustration graphs were generated from Sigma Plot 11.0.

## 2-Results

### 2.1-Seeds initial quality

Moisture content at harvest was 8.4% for seed lot collected in Toussiamasso, 8.0% for that of Toussiana, 8.8% for Bandougou, and 7.5% for the seed lot collected in Orodara.

The total duration of germination monitoring was 103 days. Descriptive statistics of all seed lots gathered revealed that the average germination percentage at harvest of *Z. zanthoxyloides* seeds was  $12.7 \pm 15.3\%$ . ANOVA revealed that the substrate affected very significantly both seeds initial germination and speed ( $P = 0.000$ ), the composite substrate being the best with a mean germination percentage of  $32.4 \pm 8.2\%$  and a germination speed of 0,589 germinated seeds per day. Indeed, seeds did not germinate on agar 1% (figure 2A) regardless the seed lot, while they germinated between 1 and  $3 \pm 0.70\%$  on river sand (figure 2B). Germination percentages ranging from  $23 \pm 0.70$  to  $42 \pm 0.70\%$  were obtained with seeds sown on the composite substrate (3v soil + 1v sand + 1v manure) (figure 2C). On this substrate, seeds started germinating 31 to 35 days after sowing.



**Figure 2:** Initial cumulative germination curves of *Z. zanthoxyloides* seeds excised from **mature fresh fruits and sown on 1% agar, river sand and a composite substrate (60% soil + 20% sand + 20% cattle manure).**

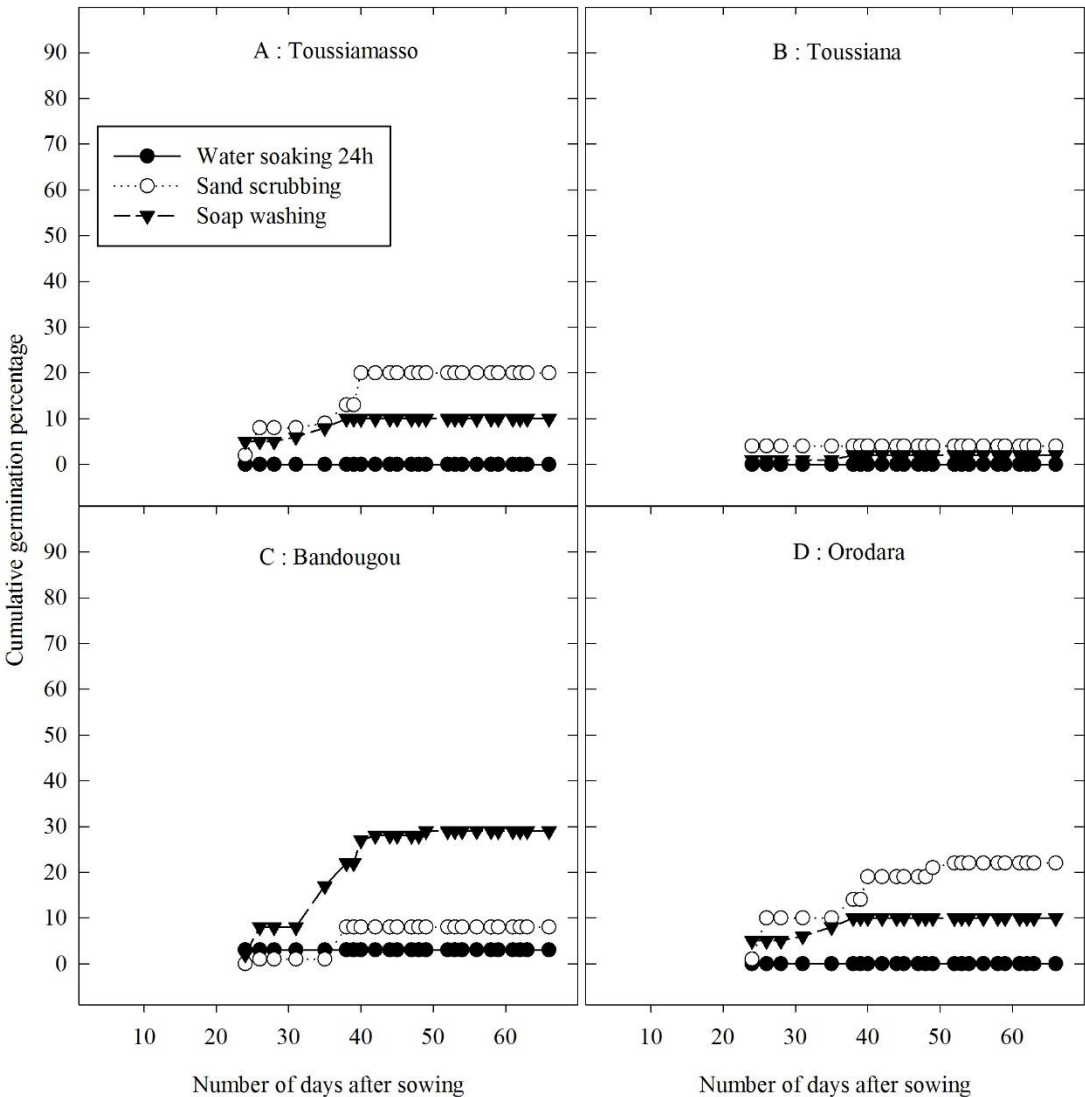
## 2.2-Effect of pre-sowing treatment and substrate on seed germination

The results showed that pre-sowing treatment had a very significant effect on seeds germination percentage and speed ( $P = 0.000$ ). Pairwise comparison revealed that soap washing, and sand scrubbing were the best with respective mean germination percentages of  $31.38 \pm 16.9\%$  and  $29.82 \pm 13.29\%$ , all seed lots and substrates gathered.

Our results further confirmed that substrate affected very significantly seeds (control and pre-treated) germination percentage as well as their speed ( $P = 0.000$ ). The composite substrate was again revealed as the best with a mean germination percentage of  $38.8 \pm 10.62\%$  all treatments gathered. On this substrate, seeds germinated at 0.823 seedlings per day. Agar 1% was once

revealed as the worst among the three substrates tested with a mean germination percentage of  $12.7 \pm 12\%$ , all seed lots and pre-treatments considered.

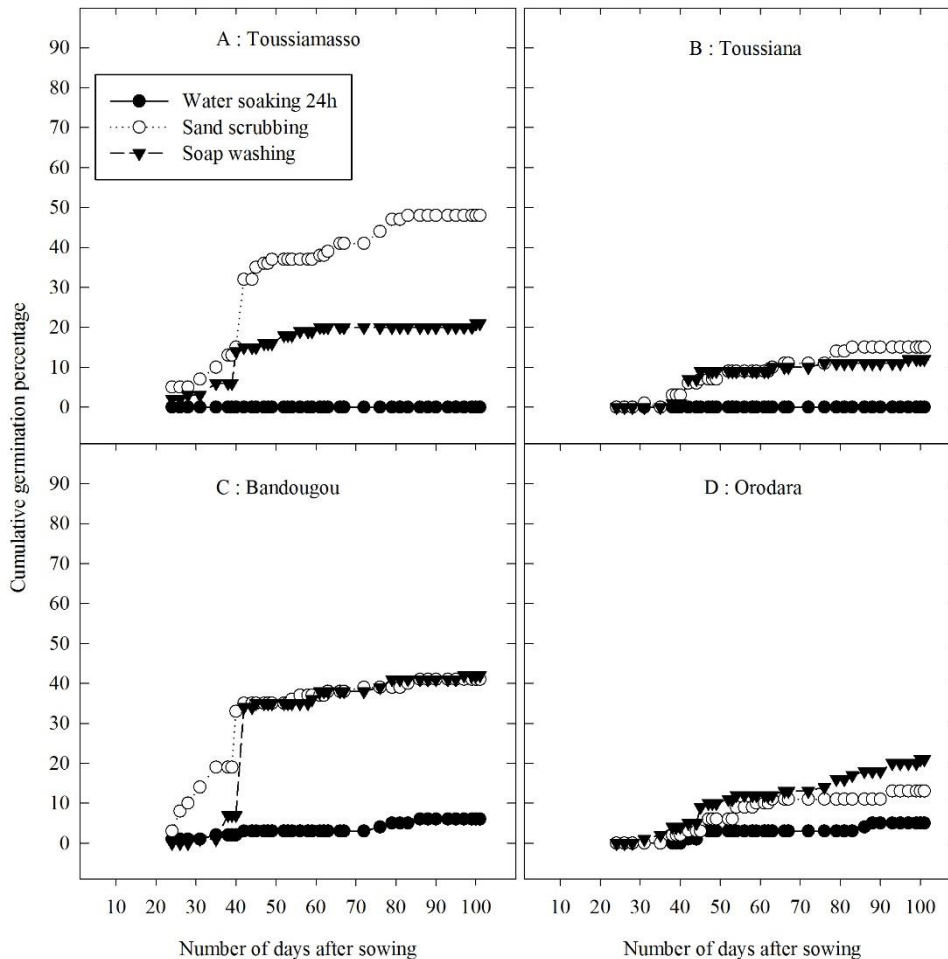
When seeds were sown on agar, those soaked in tap water 24h did not germinate except those collected in Bandougou which germinated at 3% 24 days after sowing (figure 3C). Conversely, when they were washed with soap prior to sowing on the same substrate, all seed lots germinated although at a very disparate percentages going from  $2 \pm 0.70\%$  (figure 3B) to  $29 \pm 0.70\%$  (figure 3C). Minimum incubation period was 26 days for seeds collected in Bandougou (figure 3C) and 24 days for the other seed lots. When scrubbed with river sand prior to sowing on agar 1%, all seed lots start germinating 24 days after sowing with percentages going from 4% (figure 3B) to  $22 \pm 1.4\%$  (figure 3D).



**Figure 3:** Cumulative germination curves of *Z. zanthoxyloides* seeds excised from mature fresh fruits and subject to different pre-sowing treatments before sowing on 1% agar.

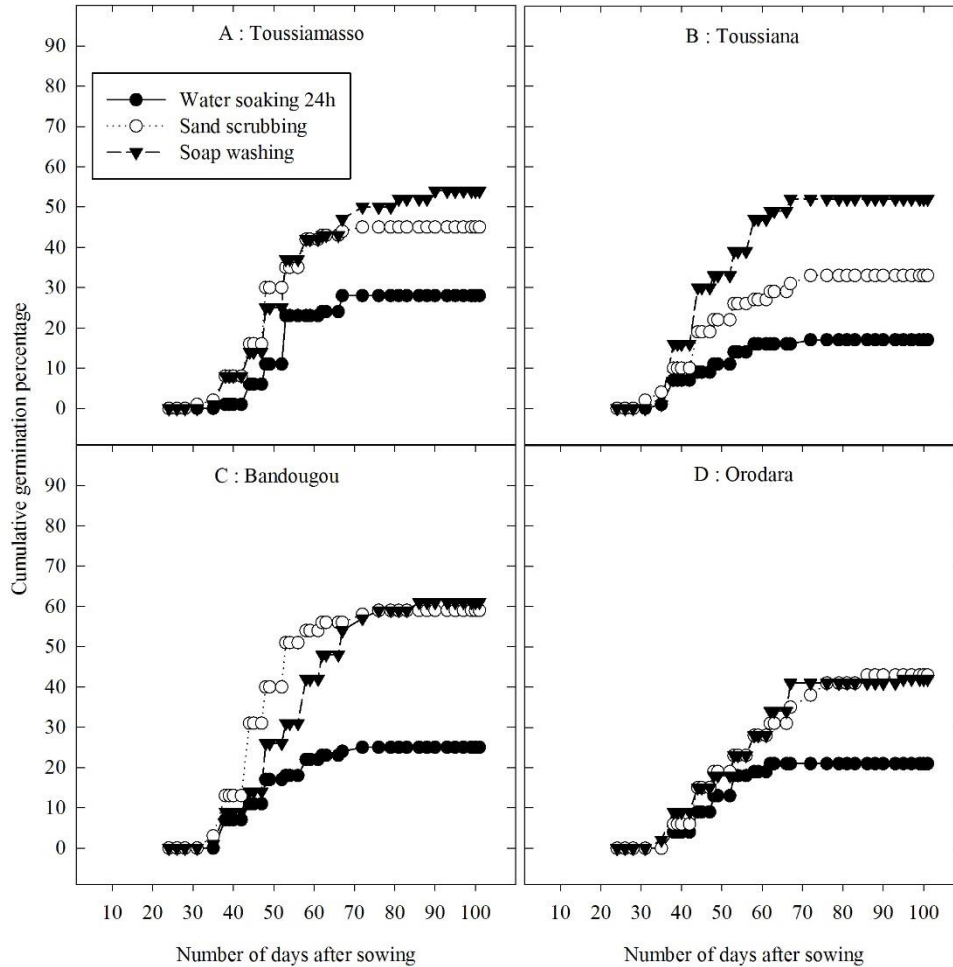
When sown on river sand, germination percentages varied between 0 and  $7 \pm 0.71\%$  for seeds soaked in tap water 24h prior to sowing, while those washed with soap and sown on the same substrate had germination percentages between  $21 \pm 1.41\%$  (figure 4A & D) and  $42 \pm 0.71\%$  (figure 4C), excepted for those of Toussiana (figure 4B) which germinated at  $12 \pm 0.70\%$ . When

rubbed with river sand prior to sowing on sand, two trend of seeds germination were observed:  $41\pm 0.70$  and  $48\pm 2.12\%$  for seeds lots from Toussiamasso (figure 4A) and Bandougou (figure 4C), and  $13\pm 1.41$  and  $15\pm 0.70\%$ , for those collected in Toussiana (Figure 4B) and Orodara (figure 4D) respectively. On this substrate, minimum incubation period varied from 24 to 45 days all seed lots gathered.



**Figure 4:** Cumulative germination curves of *Z. zanthoxyloides* seeds excised from mature fresh fruits and subject to different pre-sowing treatments before sowing on **river sand**.

When sown on the composite substrate, all seed lots germinated, regardless of the pre-sowing treatment applied. Seeds soaked in tap water before sowing presented germination percentages between  $17\pm 0.70\%$  (figure 5B) and  $28\pm 2.82\%$  (figure 5A). For all seed lots scrubbed with sand prior to sowing, germination varied from  $33\pm 1.41\%$  (figure 5B) to  $59\pm 0.70\%$  (figure 5C). Higher germination percentages ranging from  $42\pm 0.70\%$  (figure 5D) to  $61\pm 1.41\%$  (figure 5C) were obtained when seeds were washed with soap prior to sowing. On this last substrate, seeds started germination 31 to 39 days after sowing, all seed lots gathered. Germination speed varied between 0.37 and 0.55 seedlings per day when seeds were soaked in tape water prior to sowing, between 0.83 and 1.16 seedlings per day when they were washed with soap. These values were respectively 0.82 and 1.27 seedlings per day when seeds were scrubbed with sand prior to sowing.



**Figure 5:** Cumulative germination curves of *Z. zanthoxyloides* seeds excised from mature fresh fruits and subject to different pre-sowing treatments before sowing on a **composite substrate (60% soil + 20% sand + 20% cattle manure)**.

### 3-Discussion

All studied seed lots, had their initial moisture content between 7% and 8%, meaning that seeds were quite dry at harvest. These results suggested that *Z. zanthoxyloides* seeds were either orthodox or intermediate.

Seedling's emergence was very heterogeneous over time and the results of this study clearly demonstrated that pre-sowing treatments can significantly improve both the germination speed and the final germination percentage of *Z. zanthoxyloides* seeds. These results suggested the existence of dormancy in *Z. zanthoxyloides* seeds, but whether this dormancy is of physical or physiological type is still unknown. Recent investigation done by NEYA *et al.*, (2017), showed that seeds of *Z. zanthoxyloides* germinate poorly due to seed dormancy apparently caused by the integument. A previous study of SANON *et al.*, (2004), concluded that the low germination percentage of *Z. zanthoxyloides* seeds (2%) was linked to a physiological dormancy, and that seeds should first be stored at 4°C during 9 months before they can achieve their highest germination percentage (37%). In this current study, the highest germination percentages were obtained with seeds washed with soap or scrubbed with river sand prior to sowing. Soap is a product composed of amphiphilic molecules obtained during chemical reaction of a fatty substance with a strong base, specifically sodium hydroxide (NaOH) or potassium hydroxide

(KOH). It has a detergent power and dissolves fat compound thanks to the lipophilic part of its molecules. In the current study, the soap cleans the oil on the integument but it can also weaken it and lead to rapid germination. Indeed, HOU and SIMPSON (1994), when investigating the effect of NaOH and KOH solutions on *Hordeum vulgare* L. (Barley) seed germination, found that promotion of germination up to 100% was achieved by alkaline treatments with a wide range of solution concentrations and immersion time. Modification of the seed coat and enhanced water uptake were observed in the treated caryopses. It has been suggested that the cuticular layers in the seed coat may form permeability barriers, and the cuticle can be depolymerized by alkaline hydrolysis. In 2006, similar investigations carried out by SUN *et al.* revealed that NaOH treatment for 20 hours effectively removed the herbaceous *Iris lacta* var. *chinensis* seedcoat and improved germination percentage from 0% to 56%. Later on, AL ZOUBI (2020) found also, when working on *Phoenix dactylifera* L. seeds, that germination percentages of 86.70%, and 80.0% was exhibited for 30 min with NaOH and KNO<sub>3</sub> solutions, respectively.

In our investigations, untreated seeds regardless the seed lot, achieved germination percentages between 23 and 42 % when sown on the mixture substrate. The improved germination obtained with seeds washed with soap or rubbed with river sand can be attributed to removal of some substances (starting by oil) from seeds integument as well as to softening of seeds integument. This makes it difficult to classify as physiological or physical the type of dormancy encountered in *Z. zanthoxyloides* seeds. Indeed, seeds of *Z. zanthoxyloides* are black coloured to blue shaded with a shiny testa (ARBONNIER, 2004). This shiny testa is in fact surrounded with a layer of fat pulp, which, when stripped, leaves a hard and dull seed. Thus, apart from hardness of the integument, the non-permeability of the seed may be due to the presence of this fatty layer, which would contribute to further slowing the penetration of water, and thus the imbibition. Washing the seeds with soap and rubbing them with sand helped, on one hand, to scratch the seed coat and thus weaken it, and on the other hand strip off the fatty layer allowing a better imbibition of the seed and thus facilitate its germination. These findings are comparable to those of OKEYO *et al.*, (2011), who found that washing *Zanthoxylum gillettii* seeds with a soap solution improved overall imbibition compared to unwashed seed, as well as their germination, reaching 23% for washed seeds and only 8% for the unwashed ones. These authors further stated that unwashed seeds presented 29.1% of oil content while washed ones had 16.3%, indicating that washing removed 12.8% of oil and fruit appendages originally on the seedcoat. It was concluded that soap solution removed the oil from the seedcoat and possibly leached out inhibitors thus improving imbibition and overall germination. Similar results were also obtained with *Zanthoxylum capense* seeds, even though in the case of this species, germination was increased by hot water pre-treatment to 71% and by mechanical scarification to 55%, while control seeds only germinated at 17% (BODEDE *et al.*, 2015). It seems that hot water promotes germination by influencing factors such as permeability of the seedcoat to water and gases, and the release of germination inhibitors as argued by SHARMA *et al.* (2008).

The current findings on *Z. zanthoxyloides* suggest that the hard seedcoat and oily testa influence seed germination and possibly contribute to dormancy exhibited by *Z. zanthoxyloides* likewise *Z. gillettii* seeds. Indeed, most of the essential oil in *Zanthoxylum* species is unsaturated (ADESINA, 2005; SETZER *et al.*, 2005); this oil becomes rancid during seed processing and storage, possibly contributing to maintain dormancy and poor germination of *Zanthoxylum* species. It is suggested that to faster and optimize *Z. zanthoxyloides* seeds germination, they should be previously washed with soap or rubbed with river sand.

This study also revealed that substrate affected significantly *Z. zanthoxyloides* germination. Indeed, the analysis of variance revealed that the substrate used affected germination percentage, seeds being treated or not before sowing. Effect of substrate on seed germination

has been demonstrated by several studies, including those of RODRÍGUEZ *et al.* (2014) on *Jatropha curcas*, LUZ *et al.* (2015) on *Dypsis onilahensis* commonly known as sibara palm, BARMAN *et al.* (2015) on *Syzygium cumini*, and MANOLOVA *et al.* (2015) when investigating the germination of four wild species of the genus *Goniolimon*.

The shortest incubation period to germination (24 days) was recorded with agar substrate but paradoxically, the lowest germination percentage and speed were recorded with this substrate too. Similarly, poor germination (3%) of seeds sown on agar 1% was reported for *Z. gillettii* regardless of the pre-treatment applied (OKEYO *et al.*, 2011). They concluded that this was probably due to the presence of chemicals which inhibited the germination. However, poor germination on agar solution may be explained by the length of germination assays (at least 24 days), on a substrate that cannot absorb water. It seems that the agar dries before the seeds are fully imbibed and ready to germinate. Based on this experiment, it could be argued that agar is not suitable for germination assays of seeds with long incubation period such as those of *Z. Zanthoxyloides*.

The relatively good germination performances (speed and percentage) obtained with river sand and the composite substrate could be explained by the consistency and the texture of these substrates. Sand is light and very porous; this makes it adapted for seeds germination testing. Indeed, with a 0.5 to 1cm layer of sand or mixture in the Petri dish, there is a larger stock of water available for seeds to fully imbibe and initiate germination. Consequently, seeds on sand or on the mixture do not have to be watered often. According to BASKIN & BASKIN (2014) if materials such as sugars, amino acids or germination inhibitors leach from seeds, they will be more diluted in sand or soil-filled dishes (because of the greater volume of water) and dilution of leachates helps to reduce the chances of seeds being attacked by fungi or germination being reduced by inhibitors. Regarding the mixture, this substrate is heavy and compact because of the soil and the manure. These two components contain fine particles which, when moistened, can make a kind of cement around the sand grains. Such texture may delay the emergence of the seed radicle compared to those sown in the sand or agar. This could explain the long incubation period of seeds sown on the mix substrate. Nevertheless, the best germination performances, in terms of germination speed and percentage were obtained with seeds sown on this substrate. These results are comparable to those obtained by YERIMA *et al.*, (2015) with sunflower seeds who recorded the highest germination rate on soil substrate in a similar study. Similar investigations by KANMEGNE *et al.* (2017), found also that *Xylopia aethiopica* seeds germinated better on a substrate composed of a mixture of forest topsoil and river sand in a 1:1 (v/v) ratio. The current results suggest that a mixture of soil, sand and manure in a 3:1:1 (v/v/v) proportion be used as substrate for the propagation of *Z. zanthoxyloides* from seeds.

Regardless of seed lots and pre-treatments, the shortest minimum incubation period is 24 days and the longest 45 days. If we consider the substrate on which the germination was higher, the last germinated seeds were recorded between the 62<sup>th</sup> and the 76<sup>th</sup> day after sowing, all seed lots and pre-sowing treatments considered. Comparably, OKEYO *et al.* (2011) found that *Z. gillettii* seeds started to germinate on the fifth week (37 days) and continued up to the 17<sup>th</sup> week (119 days) after incubation. These observations raise a concern about the generalisation of the usual duration of 28 days recommended for tree seeds germination assays (ISTA, 2015). It is possible that several germination assays of tree seeds were stopped before seeds initiate germination; leading to wrong statements and conclusions about their germination and storage behaviour. Therefore, this study recommends that investigations be deepened to get a better insight into the duration of tropical tree seeds germination.

## Conclusion

This study clearly established that *Z. zanthoxyloides* seeds germination can be significantly improved by applying the proper pre-treatments (seeds washing with soap or rubbing with sand) and by sowing them on the appropriate substrate (mixture of soil/sand/manure). Although more investigations may be needed to determine the nature of the species seeds dormancy; specifically seeds storage behaviour, the results achieved in this study regarding the improvement of this multipurpose species seeds germination is of huge importance for its seedlings production. It is recommended that for seedlings production, *Z. zanthoxyloides* seeds, freshly harvested, be washed with soap or polished with sand as pre-sowing treatment and sown on a composite substrate which should be a mixture of soil, sand and manure in a ratio of 3v/1v/1v. It is anticipated that seedlings production in nurseries will promote the utilization of the species in reforestation programs, in agroforestry parklands and in community's medicinal species plantation.

The identified pre-treatments are certainly effective, less expensive and easy to apply. However, for large quantities of seeds, these operations may be somewhat difficult to apply. Investigations should then be deepened to improve the practicability of these pre-treatments.

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