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Seed Germination Enhancement of *Ziziphus spina-christi* (L.) Willd. and *Ziziphus mucronata* Willd. (Rhamnaceae) Using Different Pre-sowing Treatments in Ethiopia

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Abstract: The stony endocarp and impermeable seed coat of *Ziziphus*, including fleshy mesocarp inhibit seed germination in both genera. In present study effects of different pre-sowing treatments on seed germination of *Z. spina-christi* and *Z. mucronata* was studied. Seeds of each species were collected from the Central and Southern Rift Valley and Northeast Ethiopia. Seeds of each *Ziziphus* species, after being divided into cracked and uncracked seed-stone groups were subjected to eight different pre-sowing treatments and planted in pots. The mean germination percentage (GP), mean daily germination (GD), mean germination time (GT), and mean germination index (GI), as well as two-way ANOVA and Tukey's Honestly Significant Difference (THSD), were computed. The two-way ANOVA showed that there exists a significant mean difference among treatment groups under all germination parameters at $p<0.05$ for both species. The THSD for *Z. spina-christi* showed that the highest GP was achieved by cracked seed stones soaked in cold water for 48 hours at a room temperature of 25°C (52.7±6), control (48.5±3), soaked in cold water for 24 hours at a room temperature of 25°C (46.5±5.7), and rubbed with sandpaper (41±12.1), which are significantly different at $p<0.05$, while the highest GI was recorded by cracked seed stones soaked in cold water for 48 hours at a room temperature of 25°C (1.3±0.2), which is significantly different at $p<0.05$. The THSD for *Z. mucronata* indicated that the highest GP, along with the highest GI, was achieved by cracked seed stones soaked in 98% H_2SO_4 for 20 minutes and subsequently rinsed with water (28.5±6), which is significantly different at $p<0.05$. Overall, the findings of this study will contribute to potential seed enhancement technologies for *Ziziphus*, with greater implications for the propagation, conservation, and sustainable utilization of the species in the agricultural and pastoral communities of Ethiopia.

Key words: Dormancy, germination enhancement, *Ziziphus spp.*

Most tree species including the study plants of this research propagate with seeds. Yet, knowledge about information that hinders or accelerates the rate of seed germination is scanty. Tree seeds often exhibit dormancy, which causes delays and irregularities of germination in nurseries and on forest floors (Maiden *et al*., 1990; Oyewole and Adedamola, 2015). Understanding the seed physiology of plants can enhance conservation efforts through afforestation. Thus, knowledge of seed germination behavior is crucial for successful utilization and conservation planning (Zobel and Talbert, 2003).

Ziziphus is a multipurpose shrub or tree with orthodox seed storage behavior, distributed in arid and semi-arid ecosystems of Ethiopia (Bekele-Tesemma, 1993 and 2007; Orwa *et al*., 2009). Members of the genus *Ziziphus* are usually cross-pollinated and are propagated by seed (Vashishtha and Pareek, 1979; Sudhersan and Hussain, 2003; Saied *et al*., 2008; El Maaiden *et al*., 2020). Similar to many other tropical and subtropical species, *Ziziphus* species has been reported to have issues with seed dormancy, which inhibits uniform seed germination in nurseries (Schmidt, 2000; Gebretsadik, 2013). The establishment of *Ziziphus* plantations is generally hindered by poor germination and seedling emergence due to a stony endocarp and seed dormancy related to the impermeability of the seed coat. This can be addressed through the gradual softening of the seed coat or by scarification or acid treatment to free the seeds from the pericarp (El-Siddig *et al*., 2001; Schmidt, 2000; Saied *et al*., 2008). Additionally, mechanical removal of the endocarp and soaking of kernels in water has been found to significantly improve seed germination for several *Ziziphus* species, including *Z. mauritania* and *Z. abyssinica* (Krishnan and Kulasekaran, 1984; Murthy and Reddy, 1989; Sheoran *et al*., 2018). However, there is no documented information on the seed germination behavior of Ethiopian species of *Ziziphus* using different germination treatments. Effective propagation and seedling establishment are the basic requirements for sustainable management for species with notable seed dormancy profiles. In this regard, understanding the seed germination biology of *Ziziphus* species is essential for better forestry practices. The study was aimed at investigating the seed germination responses of *Z. spina-christ* and *Z. mucronata* under different pre-sowing treatments and identifying the best seed treatment techniques suitable to maximize the seed germinations of the species. Therefore, it was hypothesized that pre-sowing seed treatments could enhance seed germination.

Materials and Methods

Seed collection and processing

Seed collection was done between February 2022 and February 2023. Seed samples were collected from the Northern, Central and Southern Rift valley and northeast Ethiopia (Fig. 1). Hence, the seed for *Z. spina-christi* from Asayta district in Afar region (i.e., 41.470555°E,

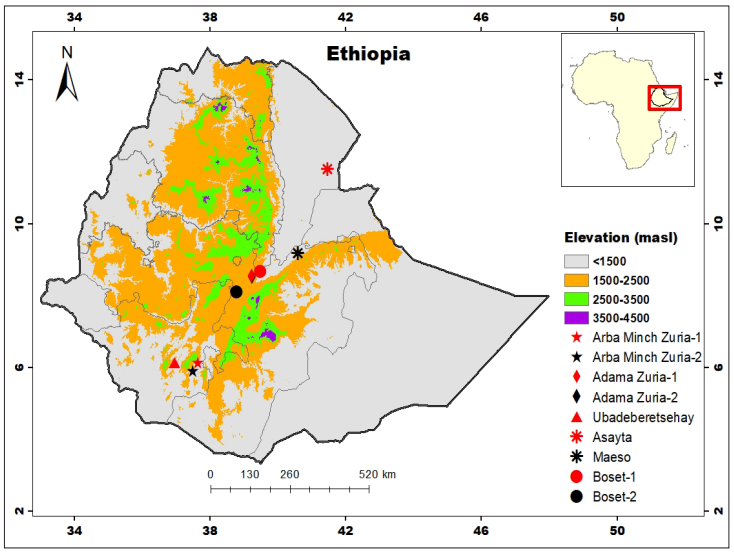

Fig. 1. Map of Ethiopia showing seed collection sites of Z. spina-christi and Z. mucronata.

Table 1. Pre-sowing treatments employed study the seed germination Ziziphus species

No.	Seed treatment types	
1	Control, i.e., received no prior seed treatment	Ctrl
2	Rubbed with sandpaper	RuSP
3	Soaked with cold normal water for 24 hours at room temperature of 25°C	CW24h
4	Soaked with cold normal water for 48 hours at room temperature of 25°C	CW48h
5	Soaking with hot normal water at 65°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C	HW65d
6	Soaked with hot normal water at 75°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C	HW75d
7	Soaked with 98% H_2SO_4 for 10 minutes and subsequent rinsed with water	98HSO10m
8	Soaked with 98% H_2SO_4 for 20 minutes and subsequent rinsed with water	98HSO20m

11.526388°N), Adama Zuria district-1 and 2 (i.e., 39.20774°E, 8.54799°N; 39.2293°E, 8.53156°N) and Boset district (i.e., 39.489722°E, 8.6775) in Oromia region; and *Z. mucronata* from Arba Minch Zuria district-1 and 2 (i.e., 37.62743°E, 6.12204°N; 37.492368°E, 5.894401°N) and Ubadeberetsehay district (i.e., 36.9503°E, 6.11977°N) in SNNPR, Boset district-2 (i.e., 38.77472°E, 8.105833°N) and Maeso district (i.e., 40.58287°E, 9.17381°N) in Oromia. Seeds were collected from conspicuous superior (mother) trees of *Z. spina-christi* and *Z. mucronata*.

About 30-40 seeds per mother tree (about 200 seeds per population) were collected and mixed up to represent the population. The seeds were collected from different populations of each species and thoroughly mixed up and homogenized to represent the species. The homogenized seeds were further processed, cleaned, and purified from inert and unwanted materials. The seeds were dried and the moisture content (MC) of *Z. mucronata* = 4.65%, *Z. spina-christi* = 5.1% was adjusted and checked using the moisture meter (KERN, DBS60-3 German-made moisture tester). All experimental activities were carried out in the nursery house of the Ethiopian Forestry Development (EFD), Addis Ababa, Ethiopia.

Preparations of seed treatments and experimental layout

For this study, seed treatments were prepared based on literature and ISTA protocols (Schmidt, 2000, Hassen *et al*, 2005; ISTA, 2005; Orwa *et al*, 2009; Gebretsadik, 2013). Accordingly, including the control, eight pre-sowing treatments were prepared (Table 1).

Each experiment was laid out in a Completely Randomized Design-CRD following the random tables of Gomez and Gomez (1984) with four replications each. Hence, 36 seed stones per replication (i.e., 144 seed stones per treatment) were tested following the protocol suggested by the International Seed Testing Association (ISTA) (ISTA, 1976 and 2018).

Each *Ziziphus* species was subjected to 16 treatments: seed coat nature (2 levels, i.e., cracked and uncracked) and pre-sowing treatments (8 levels) with 4 replications each having a total of 64 treatment units. The germinations were done in the germination substrate of topsoil, manure, and sand with a ratio of 3:2:1, respectively. The mixture was placed in polyethylene plastic pots with 16 cm diameter. The seeds were then sown on each pot and covered with a transparent plastic polythene sheet to optimize and regulate the moisture and temperature. Germination counting was taken starting from the first week of germination observation after sowing, every two days for two to three months, based on the species, until no new seedlings emerged for two successive weeks' laps. In this study, a seed was considered germinated when the radicle emerged (Bewley and Black, 1994; Zietsman and Botha, 1987).

Statistical data analysis

The data analysis was based on untransformed seed germination counts. The dataset was checked with histogram and has a normal distribution. The nineteen germination measurements (or parameters) were generated of which four parameters, namely the mean germination percentage (GP), mean daily germination percentage (GD), mean germination time (GT), and mean germination index (GI) were analysed and presented for this study. The GP, which is an estimate of the

germinability of the population of seeds, was calculated as follows:

$$GP = \frac{\sum_{i=1}^{k} ni}{N} \times 100 \quad ...1$$

where; n_i = number of seeds germinated in the i^{th} time, and N= total number of seeds used.

The GD, which is the mean number of seeds germinated per day (i.e., the number of seeds germinated daily relative to the maximum number of germinated seeds), was also calculated following Adams and Farrish (1992):

$$GD = \frac{CP}{Tn} \quad ...2$$

where CP = final cumulative germination percentage and T_n = total number of intervals required for final germination.

Following Ellis and Roberts (1981), the **GT**, a measure of the rate and time spread of the germination, of each treatment was calculated using the formula:

$$GT = \frac{\sum(n \times d)}{N} \quad ...3$$

where "n" = number of seeds germinated on each day, "d" = number of days from the beginning of the test, and "N" = total number of seeds germinated at the termination of the experiment.

Furthermore, the GI, which is a measure of the percentage and speed of germination, was calculated following the following formula (AOSA, 1983):

$$GI = \frac{\text{No. of germinating seeds}}{\text{days of first count}} + \ldots + \frac{\text{No. of germinating seeds}}{\text{days of final count}} \quad ...4$$

The higher values for this measure indicate a greater rate of germination (Benech-Arnold *et al.*, 1991).

Moreover, two-way ANOVA was computed to see the level of significance difference among the treatments means for the germination parameters at p≤ 0.05. Moreover, Tukey's Honestly Significant Difference (THSD) was computed to assess the significance of difference between pairs of treatments at p≤0.05 (Steel *et al.*, 1997), allowing all possible pairwise comparisons while keeping the family-wise error rate low.

Results and Discussion

Seed germination tests of Z. spina-christi using different treatments

Germination tests of *Z. spina-christi* for cracked and uncracked seed stones showed that cracked seed stones remarkably germinated much faster than the uncracked seed stones (Fig. 2 and Fig. 3).

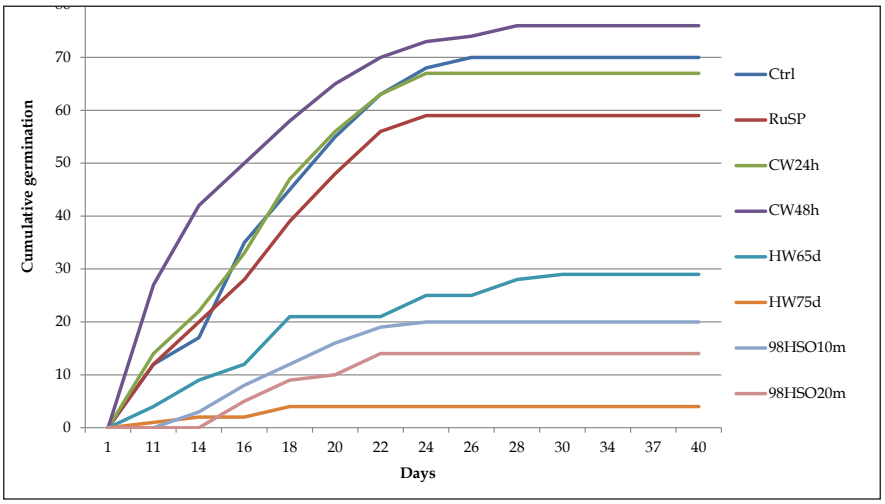

Fig. 2. The cumulative germination and patterns of seed germination of cracked seed stones Z. spina-christi; Ctrl = control, RuSP = Rubbed with sandpaper, CW24h = Soaked in cold water for 24 hours at room temperature of 25°C, CW48h = Soaked in cold water for 48 hours at room temperature of 25°C, HW65d = Soaked in hot water at 65°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C , HW75d = Soaked in hot water at 75°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C, 98HSO10m = Soaked in 98% H_2SO_4 for 10 minutes and subsequent rinsed with water, and 98HSO20m = Soaked in 98% H_2SO_4 for 20 minutes and subsequent rinsed with water.

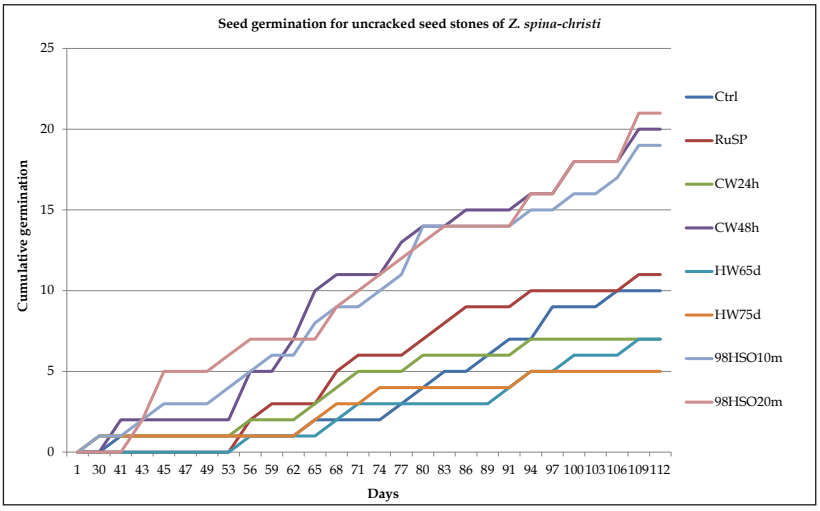

Fig. 3. The cumulative germination and patterns of seed germination of uncracked seed stones Z. spina-christi.

The shorter germination duration (<30 days) was recorded for cracked seed stones regardless of treatment types compared to uncracked seed stones (>3 months). No new germination was observed in all treatment types for cracked seed stones of *Z. spina-christi* while new germinations were observed up to 3 months for uncracked seed stones of *Z. spina-christi* (Fig. 3).

For cracked seed stones of *Z. spina-christi*, the highest cumulative germination (i.e., 76) was recorded by CW48h, followed by Ctrl (70), and CW24h (67) (Fig. 2). However, for uncracked seed stones of *Z. spina-christi*, the highest cumulative germination (i.e., 21) was achieved by 98HSO20m, followed by CW48h (20), and 98HSO10m (19) (Fig. 3).

Table 2. Two-way ANOVA of the mean significance difference among treatment groups for their effect on germination percentage (GP), daily germination percentage (GD), germination time (GT), and germination index (GI) of Z. spina-christi, involving two factors, i.e., the pre-sowing treatments with eight levels, and cracking treatments with two levels (i.e., cracked and uncracked seed stones)

	Df	Sum sq.	Mean sq.	F-value	Pr(>F)
		Mean germination percentage (GP)			
Pre-sowing	7	12163	1738	38.95	<2e-16***
Cracking	1	17424	17424	390.55	<2e-16***
Pre-sowing: Cracking	7	11957	1708	38.29	<2e-16***
Residuals	48	2141	45		
		Mean daily germination percentage (GD)			
Pre-sowing	7	7.385	1.055	50.38	<2e-16***
Cracking	1	15.035	15.035	717.91	<2e-16***
Pre-sowing: Cracking	7	7.383	1.055	50.36	<2e-16***
Residuals	48	1.005	0.021		
		Mean germination time (GT)			
Pre-sowing	7	2668	381	1.127	0.36
Cracking	1	43436	43436	128.407	3.6e-15***
Pre-sowing: Cracking	7	1520	217	0.642	0.719
Residuals	48	16237	338		
		Mean germination index (GI)			
Pre-sowing	7	3.115	0.445	49.13	<2e-16***
Cracking	1	6.126	6.126	676.32	<2e-16***
Pre-sowing: Cracking	7	3.132	0.447	49.40	<2e-16***
Residuals	48	0.435	0.009		

Table 3. Summary of GP, GD, standard deviation (SD) and standard error (SE) of the germination parameters for Z. spina-christi. The significance difference between pairs of treatments at p≤0.05 is calculated using Tukey's HSD test

Pre-sowing treatments	Mean germination percentage (GP)		Mean daily germination percentage (GD)	
	Cracked	*Uncra*cked	*Cracked*	*Uncra*cked
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Ctrl	48.5±3^{a}	7±8.0^{bc}	1.75±0.1^{ab}	0.06±0.08^{d}
RuSP	41±12.1^{a}	7.8±4.6^{bc}	1.48±0.43^{b}	0.07±0.04^{d}
CW24h	46.5±5.7^{a}	5±4.69^{bc}	1.68±0.21^{ab}	0.04±0.04^{d}
CW48h	52.7±6^{a}	14±2.24^{bc}	1.90±0.22^{a}	0.12±0.03^{d}
HW65d	17±0.0^{b}	5±4.69^{bc}	0.60±0.0^{c}	0.04±0.04^{d}
HW75d	2.7±3.7^{c}	3.5±3.3^{bc}	0.10±0.14^{d}	0.03±0.03^{d}
98HSO10m	14±0.0^{bc}	13.3±2.87^{bc}	0.50±0.0^{c}	0.12±0.02^{d}
98HSO20m	9.5±3.0^{bc}	14.5±6.14^{bc}	0.35±0.1^{cd}	0.13±0.06^{d}

The two-way ANOVA for the germination of *Z. spina-christi* showed that, except among pre-sowing treatments and the interactions factor (presowing treatment and cracking of seed stones) of the germination time (GT), there exist a significant mean difference among treatment groups under all other germination parameters (i.e., GP, GD, and GI) at $p<0.05$ (Table 2).

The THSD test for *Z. spina-christi* showed that the highest GP was achieved by cracked seed stones treated with CW48h (52.7±6), followed by Ctrl (48.5±3), CW24h (46.5±5.7), and RuSP (41±12.1) that were significantly different at p<0.05 compared to the other treatment groups under both cracked and uncracked seed stones (Table 3). But no significant GP difference was observed among CW48h, Ctrl, CW24h and RuSP at $p\leq0.05$. The highest GD was recorded by cracked seed stones treated with CW48h (1.90±0.22), except with cracked seed stones of the Ctrl (1.75±0.1) and CW24h (1.68±0.21), that is significantly difference at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (Table 3). The shorter GT under the germination test of cracked seed stones was observed by cracked seed stones treated with HW75d (8.1±9.5), followed by CW48h (15.6±0.7), CW24h (16.8±0.6), and RuSP (16.9±0.7) that were significantly different at p<0.05 compared to the other treatment groups under both cracked and uncracked seed stones. But no significant GT difference was observed among HW75d, CW48h, Ctrl, CW24h and RuSP at p≤0.05. Furthermore, the highest GI was recorded by cracked seed stones treated with CW48h (1.3±0.2) that is significantly different at p<0.05 compared to the other treatment groups under both cracked and uncracked seed stones (Table 4).

Seed germination tests of Z. mucronata using different treatments

The seed germination tests for *Z. mucronata* under different treatments showed no new

Table 4. Summary of GT, GI, standard deviation (SD) and standard error (SE) of the germination parameters for Z. spina-christi. The significance difference between pairs of treatments at p≤0.05 is calculated using Tukey's HSD test

Pre-sowing treatments	Mean germination time (GT)		Mean germination index (GI)	
	Cracked	*Uncra*cked	*Cracked*	*Uncra*cked
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Ctrl	17.4±0.6^{bc}	80.8±19.3^{a}	1.1±0.1^{b}	0.0±0.0^{e}
RuSP	16.9±0.7^{c}	74.9±6.6^{a}	0.9±0.3^{b}	0.0±0.0^{de}
CW24h	16.8±0.6^{c}	48.1±32.8^{abc}	1.1±0.1^{b}	0.0±0.0^{de}
CW48h	15.6±0.7^{c}	73.8±7.4^{a}	1.3±0.2^{a}	0.1±0.0^{de}
HW65d	18.5±0.3^{bc}	64.3±44.6^{ab}	0.4±0.0^{c}	0.0±0.0^{e}
HW75d	8.1±9.5^{c}	54.1±39.4^{abc}	0.1±0.1^{cde}	0.0±0.0^{e}
98HSO10m	18.2±1.3^{bc}	74.5±7.9^{a}	0.3±0.0^{cd}	0.1±0.0^{de}
98HSO20m	18.6±0.1^{bc}	76.6±13.0^{a}	0.2±0.1^{cde}	0.1±0.0^{de}

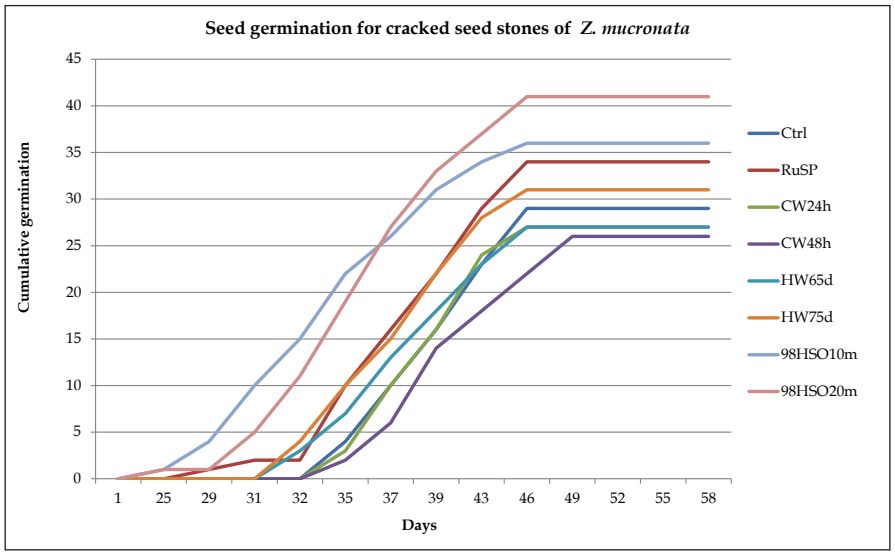

Fig. 4. The cumulative germination and patterns of seed germination of cracked seed stones Z. mucronata.

germinations after six weeks (45 days) for most cracked seed stones (Fig. 4). However, the uncracked seed stones of *Z. mucronata* required longer germination duration (>3 months) yet with lower germination percentages (Fig. 5). The highest cumulative germination record (i.e. 41) was attained by cracked seed stones treated with 98HSO20m (Fig. 4), followed by 98HSO10m (36), and RuSP (34). Generally, however, the germination of uncracked seed stones of *Z. mucronata* was very low (i.e. <20) regardless of treatment types (Fig. 5) even if the highest possible total germination record (i.e. 13) was scored by RuSP, followed by HW65d and HW75d.

The two-way ANOVA for the germination of *Z. mucronata* showed that there exists a significant mean difference among treatment groups, having interaction effects, under all germination parameters (i.e., GP, GD, GT and GI) at $p<0.05$ (Table 5).

The THSD test indicated that the highest GP was achieved by cracked seed stones of *Z. mucronata* treated with 98HSO20m (28.5±6) that is significantly different at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (Table 6), except with cracked seed stones treated by 98HSO10m (25±4.9), RuSP (23.5±3), and HW75d (21.2±2.8). Similarly, the highest GD was achieved by cracked seed stones of *Z.*

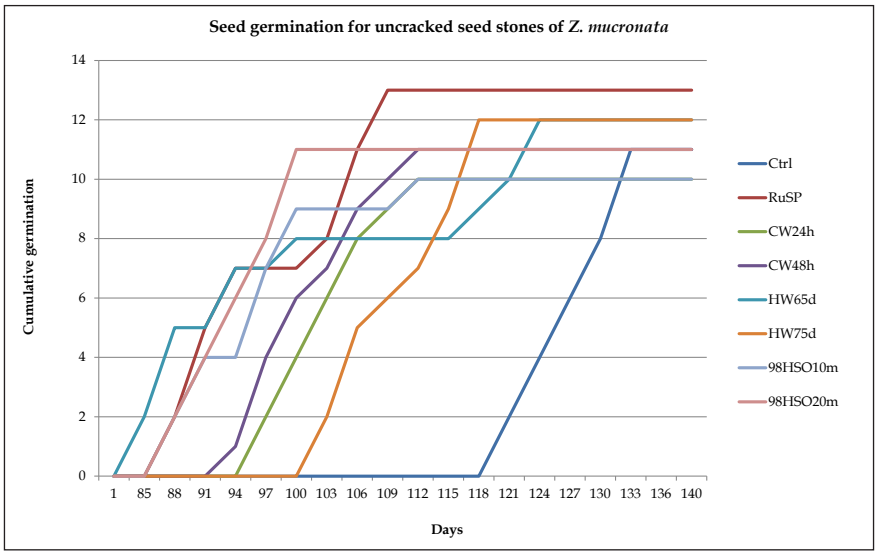

Fig. 5. The cumulative germination and patterns of seed germination of uncracked seed stones Z. mucronata.

Table 5. Two-way ANOVA of the mean significance difference among treatment group for their effect on germination percentage (GP), daily germination percentage (GD), germination time (GT), and germination index (GI) of Z. mucronata, involving two factors, i.e., the pre-sowing treatments with eight levels, and cracking treatments with two levels (i.e., cracked and uncracked seed stones)

	Df	*Sum sq.*	*Mean sq.*	*F-value*	*Pr(>F)*
		Mean germination percentage (GP)			
Pre-sowing	7	397	57	3.856	0.00210**
Cracking	1	8860	8860	602.560	< 2e-16***
Pre-sowing: Cracking	7	391	56	3.800	0.00234**
Residuals	48	706	15		
		Mean daily germination percentage (GD)			
Pre-sowing	7	0.119	0.017	4.890	0.00032***
Cracking	1	3.730	3.730	1070.895	< 2e-16***
Pre-sowing: Cracking	7	0.119	0.017	4.883	0.0003***
Residuals	48	0.167	0.003		
		Mean germination time (GT)			
Pre-sowing	7	1950	279	22.07	5.27e-13***
Cracking	1	68572	68572	5432.85	< 2e-16***
Pre-sowing: Cracking	7	1334	191	15.10	3.08e-10***
Residuals	48	606	13		
		Mean germination index (GI)			
Pre-sowing	7	0.0278	0.0040	8.045	1.92e-06***
Cracking	1	0.5166	0.5166	1045.179	< 2e-16***
Pre-sowing: Cracking	7	0.0275	0.0039	7.944	2.22e-06***
Residuals	48	0.0237	0.0005		

mucronata treated with 98HSO20m (0.7±0.1) that is significantly different at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (Table 6), except with cracked seed stones treated by 98HSO10m (0.6±0.1) and RuSP (0.6±0.1). The shortest GT was scored by cracked seed stones treated with 98HSO10m (35.1±1) and 98HSO20m (36.7±1.7) that is significantly different at $p<0.05$ compared to the other treatment groups under uncracked seed stones, but not significant at $p\leq0.05$ compared to the other treatment groups under cracked seed stones (Table 7). Furthermore, the highest GI was achieved by

Table 6. Summary of germination percentage (GP), Mean daily germination percentage (GD), standard deviation (SD) and standard error (SE) of the germination parameters for Z. mucronata. The significance difference between pairs of treatments at $p\leq0.05$ is calculated using Tukey's HSD test. The values denoted with the same alphabet (s) along the vertical column ($\bar{x}$±SD) of each germination parameter are not significantly different at $p\leq0.05$, and vice versa

Pre-sowing treatments	Mean germination percentage (GP)		Mean daily germination percentage (GD)	
	Cracked	*Uncracked*	*Cracked*	*Uncracked*
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Ctrl	20.0 ± 2.4^{b}	7.8 ± 2.4^{c}	0.5 ± 0.1^{c}	0.1 ± 0.0^{d}
RuSP	23.5 ± 3.0^{ab}	9.0 ± 3.5^{c}	0.6 ± 0.1^{ab}	0.1 ± 0.0^{d}
CW24h	18.7 ± 2.3^{b}	7.0 ± 1.2^{c}	0.5 ± 0.1^{c}	0.1 ± 0.0^{d}
CW48h	18.0 ± 1.1^{b}	7.8 ± 3.9^{c}	0.4 ± 0.0^{c}	0.1 ± 0.0^{d}
HW65d	18.5 ± 3.3^{b}	8.3 ± 4.5^{c}	0.5 ± 0.1^{c}	0.1 ± 0.0^{d}
HW75d	21.2 ± 2.8^{ab}	8.3 ± 2.1^{c}	0.5 ± 0.1^{c}	0.1 ± 0.0^{d}
98HSO10m	25 ± 4.9^{ab}	7.0 ± 1.2^{c}	0.6 ± 0.1^{ab}	0.1 ± 0.0^{d}
98HSO20m	28.5 ± 6.0^{a}	7.8 ± 2.4^{c}	0.7 ± 0.1^{a}	0.1 ± 0.0^{d}

Table 7. Summary of germination time (GT), germination index (GI), standard deviation (SD) and standard error (SE) of the germination parameters for Z. mucronata. The significance difference between pairs of treatments at p≤0.05 is calculated using Tukey's HSD test. The values denoted with the same alphabet (s) along the vertical column ($\bar{x}$±SD) of each germination parameter are not significantly different at p≤0.05, and vice versa

Pre-sowing treatments	Mean germination time (GT))		Mean germination index (GI)	
	Cracked	*Uncra*cked	*Cracked*	*Uncra*cked
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Ctrl	40.4±0.4^{d}	127.3±1.3^{a}	0.2±0.0^{c}	0.0±0.0^{d}
RuSP	39.1±0.8^{d}	98.0±1.2^{c}	0.2±0.0^{c}	0.0±0.0^{d}
CW24h	40.0±0.6^{d}	103.1±3.2^{bc}	0.2±0.0^{c}	0.0±0.0^{d}
CW48h	39.0±0.6^{d}	102.3±3.6^{bc}	0.2±0.0^{c}	0.0±0.0^{d}
HW65d	38.8±1.5^{d}	98.1±11.4^{c}	0.2±0.0^{c}	0.0±0.0^{d}
HW75d	38.4±1.4^{d}	110.9±2.1^{b}	0.2±0.0^{c}	0.0±0.0^{d}
98HSO10m	35.1±1.0^{d}	96.6±5.3^{c}	0.3±0.0^{ab}	0.0±0.0^{d}
98HSO20m	36.7±1.2^{d}	95.0±2.4^{c}	0.3±0.0^{ab}	0.0±0.0^{d}

cracked seed stones treated with 98HSO20m (0.3±0.0) and 98HSO10m (0.3±0.0) that is significantly different at p<0.05 compared to the other treatment groups under both cracked and uncracked seed stones (Table 7).

The findings of this study revealed that seed germination of *Ziziphus* species (*Z. spina-christi* and *Z. mucronata*) is significantly affected by the method of pre-sowing seed treatment employed. In this regard, cracked seed stones showed a higher germination percentage and faster germination time in comparison to batches with uncracked seed stones, even when both batches were given a similar pre-sowing treatment. *Ziziphus* species generally possess hard seed stones (endocarp), a harder inner layer of fruits covering the seed germplasms, which can make it difficult for the embryo to receive the oxygen and moisture necessary for germination. Hence, it is important to apply mechanical and biochemical treatments to the seed (or fruit) in order to improve its ability to germinate in a shorter period of time. In other words, cracking (or breaking), nicking, and opening the harder seed stones are effective ways to improve the rate of germination. It is likely that the natural dormancy of *Ziziphus* seeds is reduced when the fleshy fruit part (mesocarp) is often eaten mainly by humans, birds, and primates. This exposes it to mechanical injuries, which in turn creates an environment conducive to germination by allowing oxygen and moisture to enter the seed's endosperm.

The application of extended soaking to seeds (or fruits) has been proven to facilitate the permeability of moisture and oxygen necessary for germination. In this study, the cracking of seeds stones along with soaking with chemical mainly water (cold and hot) significantly improved the mean germination percentage of *Z. spina-christi*. Saied *et al*. (2008) reported that cracking seed coats, scarification with sandpaper, and soaking in acid generally improve the seed emergence of *Z. spina-christi*. A study report by Gebretsadik (2013) also showed that higher germination percentage was achieved from seeds treated with hot water at 85°C left to cool at room temperature (25°C) for 12 hours, and soaked with 98% H_2SO_4 for 30 minutes.

Similarly, batches with cracked seed stones of *Z. mucronata* tend to have about 6 weeks of germination duration whereas uncracked seed stones result in greater than 3 months of duration. In relation to this, though a very low germination percentage (<20%) was observed, soaking of cracked seed stones with 98% H_2SO_4 as well as rubbing with sandpaper improved the germination capacity of *Z. mucronata*. These findings agree with the report by Hassen *et al*. (2005) that scarification with sandpaper and soaking with Sulphuric acid for up to 20 minutes were the most effective techniques in breaking the seed dormancy of *Z. mucronata*. Moreover, rubbing with sandpaper and soaking in cold water for 48 hours are also suggested to improve the germination capacity of batches with uncracked stones of *Z. mucronata*.

Similarly, Zietsman and Botha (1987) reported that only about l0% germination was observed for uncracked seed stones (endocarp) while more than 70% (<80%) germination for cracked seed stones and partly removed seeds. Bekele-Tesemma (1993) also reported that cracking the seed stones and subsequently soaking them in cold water for at least six hours is suggested to improve the germination of *Z. mucronata*. In other study by Hassen *et al*. (2005), hot water treatment was also reported to reduce the seed germination of *Z. mucronata*, but not apparently observed in the current study. In this study, compared to *Z. spina-christi*, the seeds of *Z. mucronata* were generally observed to be less sensitive to water stress without exhibiting noticeable marks of soaking impairment even after an extensive soaking period. This is possibly an adaptation strategy for species adapted to arid and semi-arid ecosystems helping the seed dispersal along drainage lines after flash floods (Zietsman & Botha, 1987).

Conclusions

This study identified the best possible seed dormancy-breaking techniques for *Ziziphus* species (*Z. spina-christi* and *Z. mucronata*). The coupling of mechanical and chemical treatments (e.g., cracking seed stone + soaking with water) generally enhance the germination of *Z. spina-christi* as well as *Z. mucronata*. It is, therefore, essential to first crack so as to open the seed stones, or totally extract the seed germplasms, and subsequent scarification (e.g. abrasion with sandpaper) and soaking with biochemical reagents mainly water for 24 to 48 hours and/or using concentrated Sulfuric acid for 10 to 20 minutes. The study has also proven that *Z. spina-christi* can be easily propagated and planted, compared to *Z. mucronata*. The findings of this study have greater implications for wider large-scale propagation and production of the seedlings of *Ziziphus* in forestry development works including restoration, rehabilitations as well as domestication and conservation projects in Ethiopia. Overall, the findings of this study will contribute to the conservation and sustainable utilization of the species in the agricultural and pastoral communities of Ethiopia. Furthermore, for large-scale propagation, the application of recommendable pretreatment outputs from the current and other studies could also be employed for cultivation and domestication as well as commercialization of the species to meet many social and economic objectives.

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