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COMPARATIVE EFFECT OF HYDRO AND HALOPRIMING ON SEED GERMINATION AND SEEDLING GROWTH OF IPIL IPIL

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ABSTRACT

Ipil Ipil (*Leucaena leucocephala*) often called the lead tree, is a resilient and fast-growing plant native to Mexico and Central America. It's highly valued for its versatility, serving as animal fodder, a renewable source of timber, and for its ability to enhance soil fertility through nitrogen fixation. This species, with its distinctive foliage and flowers, plays a crucial role in sustainable agriculture an experiment was conducted during the year 2020 to examine the comparative effect of hydro and halopriming on seed germination and seedling growth of Ipil Ipil. The seeds of Ipil Ipil were primed in various solutions for 30 hours. The seeds were soaked in priming solutions viz. distilled water, lukewarm water and in NaCl solution @ 1% and 2%. The unprimed seeds were taken control. The results revealed that all the observed parameters studied in the present study were significantly (P<0.05) influenced by various seed priming treatments. The results of seed priming depict that seed germination (90.00%), germination index (0.43), germination time (5.22 days) and seedling vigor index (1498.0) were observed better in response to the lukewarm water. While the seeds primed with NaCl @ 1% had better results for fresh biomass of shoot (5.58 g), root (1.44 g), dry biomass of shoot (1.57 g) and root (0.29 g). Quality index of the seedlings and sturdiness quotient had better result from NaCl treatment. The quality of the seedlings was measured in terms of sturdiness quotient, quality index of the seedlings and leakage of the electrolytes. The sturdiness quotient (0.98), quality index of the seedling (24.85) was also better in response to the NaCl seed priming @ 1%. The only leakage of the electrolytes had no significant of seed priming. In conclusion, lukewarm water significantly impacted seed germination, while NaCl @ 1% mainly influenced seedling growth parameters. This study emphasizes the crucial role of seed priming in enhancing Ipil Ipil seedling quality.

Keywords*:* Ipil Ipil, seed priming, quality seedlings.

INTRODUCTION

Ipil Ipil (*Leucaena leucocephala*) is a small fast-growing perennial tree from dry lands and semi-drylands Regions of the world, belonging to the family Fabaceae. It has several known common names, such as White Popinac, White Lead tree, Wild Tamarind and Jumbay (Meena *et al*., 2013). *Leucaena leucocephala* mainly grows in tropical regions where there are consistently warm temperatures (25-30°C) for optimal growth. It also grows well in subtropical regions. (Hughes, 2002). It requires well drained fertile sandy loam soils. It can also survive well in saline soils. Unfavorable environmental conditions, such as those affecting seed germination and seedling emergence, are key factors leading to poor crop establishment and reduced agricultural yields in many agricultural areas (Ishiaku 2022). On the other hand, quickly sprouting seedlings have the potential to emerge and form deep roots prior to the upper soil layers being dry and crusted, which can lead to excellent crop establishment and increased crop output. Because they mechanically restrict embryo growth, thick seed coverings that envelop embryos have an impact on germination (Michael 2019). Despite great importance and wide assortment uses of Leucaena, its establishment is difficult. Its high degree of hard seed-caused by an impermeable waxy coat that needs to be broken to increase germination is one of the main obstacles to its successful establishment. Hard seeds that survive under adverse conditions are vital in the regeneration of many species, but too much hard seed at the start of plant sowing can significantly limit establishment. (Rusdy, 2016). Different priming methods are often used, such as nutripriming (submerging seeds in a nutrient solution), halo priming involves soaking seeds in saline solutions, osmopriming refers to immersing seeds in osmotic substances such as polyethylene glycol, and hydropriming is the process of submerging seeds in water. The main aim of priming is to shorten the duration between planting and germination Ahmed sher *et al*., 2019). Various pretreatments have been proposed to overcome hard seediness such as seed soaking in hot water, cold water, mechanical, chemical scarifications and seed priming. Seed priming is one strategy for solving the problem of slow or poor seed germination (Pandita and Nagarajan, 2007). Seed priming is considered a promising technology for improving the speed and uniformity of emergence, enhancing seed vigor, and ultimately boosting yields (Chiu *et al*., 2002). This helps safeguard seeds from both living organisms and environmental conditions during the crucial phase of seedling establishment. This strategy aims to synchronize emergence, resulting in a uniform stand and improved yield (Khan, 1992). Hydropriming, a specific priming method, involves soaking seeds in water before planting, with the option of subsequent air-drying (Pill and Necker, 2001). This approach is recognized as a simple, cost-effective, and safe means to enhance germination. A significant majority of farmers (over 80%) have reported benefiting from priming in terms of early germination, establishment, and yield. Moreover, an overwhelming majority (over 95%) expressed the intention to continue employing priming in the subsequent planting seasons (Singh, 2017). Hydro primed seeds are first conditioned with water to a predetermined moisture level, and then they can be dried completely or partially to reduce the moisture content. (lutts *et al*., 2016) The system's water supply might be either highly controlled or free. For some large-seeded species (such field beans or soybeans), where quick imbibitions may be harmful, the rate of water intake is crucial. (Rusdy, 2016). Halopriming is a technique wherein seeds are submerged in saline solutions to stimulate germination and foster uniform seedling emergence within unfavorable environmental conditions. (Nawaz *et al*., 2011).

The research study is based on two objectives: firstly, to evaluate the comparative effects of hydro and halopriming on seed germination and its related attributes; secondly, to explore the response of both primed and unprimed seeds towards better seedling growth of the Ipil Ipil.

MATERIALS AND METHODS

An experiment was carried out in 2020 at the horticulture nursery situated within Sindh Agriculture University Tandojam. to evaluate the effect of various seed priming strategies on the germination, growth, and qualitative features of Ipil Ipil. Ipil Ipil seeds were steeped in priming solutions such as distilled water, lukewarm water and NaCl solutions at 1% and 2%. The unprimed seeds were taken as control. The experiment followed a completely randomized design, with each seed priming treatment being replicated three times. The observations were recorded for seed germination, growth and quality related parameters of the seedlings.

Seed germination was consistently observed over the course of one month, and the germination percentage was computed using the formula provided by (Larsen and Andreasen, 2004). $GP = \sum n/N \times 100$ Where 'n' represents the count of germinated seeds at each assessment, while 'N' signifies the total number of seeds in each treatment.

The mean germination time was determined using the equation provided by Ellis and Roberts in 1981.

$$MGT = \frac{\sum Dn}{\sum n}$$

Where 'n' stands for the quantity of seeds that have sprouted on day 'D,' where 'D' denotes the number of days counted from the initial onset of growth.

The germination index was computed using the formula provided by the Association of Official Seed Analysts in 1983.

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \text{-------} + \text{-------} + \frac{\text{No. of germinated seeds}}{\text{Days of last count}}$$

The seedling vigor index was determined using the formula established by (Abdul-Baki and Anderson in 1970).

Seedling Vigor index (SVI) = [seedling length (cm) × germination percentage]

Fresh Biomass of shoot and root measured by taking fresh biomass of random samples by using an electronic weighing balance and the values were expressed in grams.

To determine the dry biomass of both the shoot and root, random samples from each set were subjected to a 24-hour drying process at 60°C in a hot air oven. Following the drying procedure, the weights were measured using an electronic balance, and the values were recorded in grams.

For quality of the seedlings, sturdiness quotient and quality index of the seedlings and leakage of the electrolytes were measured.

Sturdiness quotient reflects the stocky or spindly nature of seedlings (Thompson *et al*., 1985). It is calculated by dividing the seedling height (in centimeters) by the collar diameter (in millimeters), following the method described by Roller (1977) and Luna & Chamoli (2006).

$$SQ = \frac{\text{Seedling height (cm)}}{\text{Collar diameter (mm)}}$$

Quality index of the seedlings was measured by using the formula developed by Dickson *et al*., (1960).

$$DQI = \frac{TSDW}{\frac{H}{D} + \frac{SDW}{RDW}}$$

Electrolyte leakage was assessed by utilizing leaf discs measuring approximately 1 cm2. Leaf discs, weighing 0.5 g, were washed with distilled water, followed by incubation in 25 ml of deionized water at room temperature for three hours. Subsequently, the electrical conductivity of the so aking solution was assessed with a conductivity meter, recorded as value A. Subsequently, the leaf discs were boiled in the same solution at 100°C for 15 minutes to break down the cells. After cooling, the conductivity of the solution was measured again, noted as value B. The percentage of electrolyte leakage was calculated using the formula provided below.

$$\text{Leakage of electrolytes of leaf }\% = \frac{\text{Value A}}{\text{Value B}} \times 100$$

Statistical analysis of the data was performed using the Statistix 8.1 computer software (Statistix, 2006). Where required, the Least Significant Difference (LSD) test was employed to evaluate the superiority of treatments.

RESULTS:

Parameters related seed germination: The data in Table 1 showed that seed germination, seed germination time, germination index and seedling vigor index in Ipil ipil varied significantly due to the seed priming. The data indicates that the seed germination responds well to the seed priming. Priming seeds with lukewarm water showed the highest seed germination (90.00%) in response to seed priming. These results are significantly followed by the results (70.00%) obtained from the treatment where seeds were primed with NaCl @ 1%. The seeds also took lesser time to germination (5.22 days) in lukewarm water. Control seeds and seeds primed in 1% NaCl solution took more than 18 days to germinate. Seed priming with distilled water also had faster germination (9.00 days) than unprimed and NaCl treated seeds. The data in Table 1 also depicts that germination index ranged from 0.15 to 0.43 had maximum index in response to the lukewarm water treated seeds. The distilled water (0.34) and luke water (0.43) had similar results for germination index while the seeds treated with NaCl solution @ 1% (0.27) or 2% (0.26). For the seedling vigor index, each priming treatment exhibited similar response for the vigor index of the seedlings that ranges from 1450.6 to 1636.7. The unprimed seeds had minimum seedling vigor index 769.8 that is too much low than the primed treatments. The significant improvement in seed germination, particularly the highest germination rate of 90.00% observed with lukewarm water priming increased germination rates through seed priming, achieving notable advancements compared to control conditions (Jones & Smith, 2003). The results obtained with NaCl priming, particularly the 70.00% germination rate with NaCl @ 1%, increased germination percentages with sodium chloride priming in various plant species impact of moderate salinity on seed germination (Munir *et al*., 2010). The observed variation in germination index, ranging from 0.15 to 0.43, and seedling vigor index, ranging from 1450.6 to 1636.7, aligns with the concept emphasized by Bewley and Black (1985).

Table 1. Seed germination related parameters as affected by the hydro and halopriming.

Seed priming treatments	Seed germination	Germination time	Germination index	Seedling vigor index
Control	24.44 D	20.33 A	0.15 C	769.8 B
Distilled water	62.22 B	9.00 C	0.34 AB	1450.6 A
Lukewarm water	90.00 A	5.22 D	0.43 A	1498.0 A
NaCl 1%	70.00 B	18.78 B	0.27 BC	1636.7 A
NaCl 2%	46.67 C	18.56 A	0.26 BC	1492.9 A
Standard Error	4.4444	1.4572	0.0701	195.70
Probability	0.0000	0.0000	0.0078	0.0010
$LSD_{0.05}$	9.0768	2.9760	0.1432	399.68

Parameters related biomass of shoot and root: The fresh and dry biomass of shoot and root had significant effects in response to the seed priming. The seeds primed with NaCl @1% had better results for fresh biomass of shoot (5.58g), root (1.44g) dry biomass of shoot (1.57g) and root (0.29 g). This biomass decreased with the increase in NaCl concentrations from 1% and 2%. The distilled water and lukewarm water had similar results for fresh biomass of roots (1.11 and 1.13 g), dry biomass of shoot (1.24 and 1.13 g) and dry biomass of roots (0.19 and 0.24 g). The seedlings produced from unprimed seeds had minimum fresh biomass of shoot (0.76 g), fresh biomass of root (0.54 g), dry biomass of shoot (0.29 g) and dry biomass of root (0.11 g). The substantial impact of seed priming on fresh and dry biomass, particularly the superior results observed with NaCl @ 1% priming, seed priming, especially with saline solutions, can enhance plant biomass by improving nutrient uptake and stress tolerance showcasing the positive influence of NaCl priming on shoot and root biomass (Kaya *et al*., 2006). The minimal fresh and dry biomass observed in seedlings produced from unprimed seeds aligns with the general understanding of the positive effects of seed priming on plant growth. McDonald and Copeland (1997).

Table 2. Fresh and dry biomass of shoot and root in response to the hydro and halo seed priming.

Seed priming	Fresh Biomass of shoot	Fresh biomass of root	Dry biomass of shoot	Dry biomass of root
Control	0.76 D	0.54 C	0.29 C	0.11 C
Distilled water	3.68 B	1.11 B	1.24 B	0.19 BC
Lukewarm water	2.95 C	1.13 B	1.13 B	0.24 AB
NaCl 1%	5.58 A	1.44 A	1.57 A	0.29 A
NaCl 2%	3.43 BC	1.09 B	1.28 B	0.21 AB
Standard Error	0.2861	0.0650	0.1326	0.0431
Probability	0.0000	0.0000	0.0000	0.0025
$LSD_{0.05}$	0.5843	0.1327	0.2709	0.0881

Parameters related quality of the seedlings: Quality of the seedlings was measured in terms of sturdiness quotient and quality index of the seedlings and leakage of the electrolytes. The seeds of each priming treatment produced seedlings with minimum sturdiness quotient that ranges from 0.98 to 2.36. The seedlings produced from unprimed seeds had sturdiness quotient closer to (6.0) that is 5.62. These seedlings are also considered well as sturdiness quotient 6 or above 6 considered poor quality seedlings. The minimum sturdiness quotient (0.98) was observed from the seedling produced from seeds primed with NaCl @ 1% followed by lukewarm water (1.19). Quality index of the seedlings (24.85) was also better with the same NaCl @1% concentration. The rest of the priming treatments had similar quality index of the seedlings ranges from 15.39 to 17.19. The unprimed seeds produced seedlings with the lowest quality index (6.03). The variation in sturdiness quotient observed in seedlings from different priming treatments can significantly impact seedling quality and vigor particularly the minimum sturdiness quotient of 0.98 observed with NaCl @ 1% priming, are consistent with McDonald's findings, highlighting the positive influence of priming on seedling sturdiness (McDonald, 1999). The improvement in the quality index of seedlings, especially with NaCl @ 1% priming, resonates with the work of Jisha and Puthur (2016), demonstrated that seed priming positively influences the overall quality and performance of seedlings such as the quality index of 24.85 with NaCl @ 1%, align with Jisha and Puthur's findings, indicating the efficacy of priming in enhancing seedling quality (Jisha & Puthur, 2016).

The leakage of the electrolytes had no significant effects of seed priming. The seedlings produced from unprimed and primed seeds had similar leakage of the electrolytes ranges from 20.62 to 21.22. The electrolyte leakage serves as a physiological indicator of stress is linked to the disruption of cellular membranes, leading to the release of ions. The lack of significant variation in electrolyte leakage suggests that the priming treatments-maintained membrane integrity and mitigated stress-induced cellular damage (Mishra *et al*., 2006).

Table 3. Quality of the ipil ipil seedlings in response to the hydro and halo seed priming.

Seed priming treatments	Quality Index	Sturdiness Quotient	Electrolyte Leakage of Leaf
Control	6.03 C	5.62 A	21.22
Distilled water	15.39 B	1.77 C	21.27
Lukewarm water	17.19 B	1.19 D	22.92
NaCl 1%	24.85 A	0.98 D	22.62
NaCl 2%	16.06 B	2.36 B	24.05
Standard Error	1.2537	0.2099	3.1793
Probability	0.0000	0.0000	0.8066
$LSD_{0.05}$	2.5605	0.4288	6.4931

DISCUSSION

Several studies have demonstrated that halopriming can considerably improve seed germination, seedling emergence, establishment, and overall crop yield in soils affected by salinity. By priming with NaCl and KCl, the detrimental effects of salts were effectively eliminated (Iqbal *et al*., 2006). Since there is not much evidence about seed priming of Ipil Ipil, this research was aimed to examine the comparative effect of hydro and halopriming on seed germination and seedling growth of the seedlings.

Ipil Ipil *(Leucaena leucocephala)* holds significance as one of the most crucial trees due to its ability to thrive in diverse environments. Recognized for its rapid growth, this tree species boasts high proficiency in nitrogen fixation, transferring atmospheric nitrogen to the soil through its roots (Singh, 2012). However, the germination of its seeds is notably hindered by the presence of a tough seed coat. It requires pre-sowing seed treatments before sowing of the seeds (Silvertown, 1999; Tadros *et al*., 2011). Many studies have been conducted on the pre-sowing seed treatments of ipil ipil includes scarification, soaking of the seeds in water and chemical treatment. Rare studies have been observed on the seed priming. (Thomas Okoh 2019). In the present study, hydro and halo priming techniques were used. In hydro, priming was done with water and in halo priming with salt solutions. All germination related parameters were observed better in hydro priming while seedling growth includes plant biomass such fresh biomass of shoot and root, dry biomass of shoot and root, quality index of the seedlings and sturdiness quotient were observed better in NaCl solutions. The germination percentage, germination time, germination index, seedling vigor index were observed better in response to the priming of the Ipil Ipil seeds in lukewarm water as compared to normal water. Time period of seed priming also affected all the related parameters of seed germination. Seed germination responded better more than 90% in lukewarm water treatment. Hamad & Anwar (2021) observed better seed germination (50%) in response to the hot water treatment of Ipil Ipil seeds for 12 hours as compared to scarification and control. These differences may be due to hot and lukewarm water and different time period. Koobnye *et al*. (2018) observed seed germination of 84.3% in response to the seed soaking in hot water at 800C for five minutes. Valente *et al*. (2017) The application of hot water at a temperature of 100°C for a duration of ten minutes resulted in the highest observed germination speed. Hot water treatment has been investigated for its ability to promote seed germination by influencing several factors such as increasing seed coat permeability to facilitate water absorption, facilitating gaseous exchange (Longer and Degago, 1996), and releasing inhibitors (Mohamed-Yaseen *et al*., 1994). The current study discovered that soaking seeds in tepid water for 30 hours increased germination and associated characteristics. Teles *et al*. (2000) reported that heating water to 80°C for 5 minutes disturbed seed dormancy in leucaena seeds while having no effect on germination or vigor. Similarly, Paulino *et al*. (2004) found that combining hot water at 60°C or 80°C with mechanical scarification had no effect on leucaena seed germination. Although immersing the seeds in water for 24 hours is considered a straightforward method, its effectiveness is contingent on rapid water penetration into the seed coat. The hot water method is used to break seed dormancy, although the outcomes vary depending on the legume, as discovered by Nascimento and Oliveira (1999).

Valente *et al*. (2017) observed the lowest seed germination index when they used acetone for ten minutes, ethyl alcohol for ten minutes and intact seeds (control). Rusdy (2016) observed maximum seed germination in sulfuric acid treatment regardless of soaking time. He also observed the lowest seed germination in cold water.

The seedling vigor index was observed better in lukewarm water and NaCl @ 1% treatment. However, plant biomass such as fresh shoot and root biomass, dry shoot and root biomass were observed maximum in response to the priming where seeds were primed in NaCl solution @ 1%. These results are in contrast with the results reported by Hamad & Anwar (2021). They observed better fresh plant biomass 0.88 g and 0.26 dry plant biomass in response to the hot water treatment of Ipil Ipil seeds for 12 hours as compared to scarification and control. This may be due to the varied priming technique and time period. The application of halo priming has been noted to enhance seed germination, promote the growth of robust seedlings, and result in a higher quantity of plants per unit area. Nonetheless, with the increasing salinity of NaCl, there was an observed decline in germination percentage and seedling emergence, both in treated and untreated seeds. Notably, the decrease was less pronounced in the seeds subjected to priming. The priming process involving NaCl and KCl was found to be beneficial in mitigating the harmful effects of salts, as documented in the study by Iqbal *et al*. (2006).

CONCLUSION

According to the findings of this investigation, all seed germination related parameters are greatly affected by the lukewarm water treatment. However, parameters related with the seedling growth are affected by the NaCl treatment @ 1%. It is suggested from the present study, to consider future based studies on the various lowest and highest concentrations of NaCl for better seed germination, quality and seedling growth of Ipil Ipil. However, parameters related to seedling growth are affected by the NaCl treatment at a concentration of 1%. Salt (NaCl) at moderate concentrations has been reported to have a positive impact on plant growth by enhancing nutrient absorption and osmotic regulation (Munns and Tester, 2008). The 1% NaCl concentration in this study appears to promote seedling growth in Ipil Ipil (*Leucaena leucocephala*).

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