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# Enhancement of seed germination in Chironji (*Buchanania lanzan* Spreng) through physical and chemical treatments

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**Abstract**
Germination is one of the main problems in plant propagation propagated by seed. A clear understanding of seed germination and dormancy helps in plant propagation and conservation of germplasm. Due to the very hard seed coat on chironji seed makes it an impermeable to imbibition and gases exchange resulting in low germination and vigour. In present investigation, chironji seeds were treated with different physical and chemical treatments. The fastest and highest germination percentage was recorded in the treatment of alternate wetting (24 hrs.) and drying (12 hrs.) of seed (86.7%) followed by 3 days dipping in water (81%). Based on the present study, it can be suggested that increased rate of imbibition and inducing cracking on hard seed coat would be the most effective way to enhance the seed germination in chironji; alternate wetting and drying of seed and seed dipping in water were the easiest, economic and most reliable treatments for enhancement of chironji seed germination.

**Keywords:** Chironji, germination, $GA_3$, $H_2SO_4$, HCl, $KNO_3$, scarification

## Introduction
Chironji (*Buchanania lanzan* Spreng) belongs to family Anacardiaceae and commonly known as charoli in Gujarati. The plant was first described by Francis Hamilton in the year 1798. Being a dryland agricultural crop, it is mostly grown in the dry deciduous forest area of Chhattisgarh, Jharkhand, Rajasthan, Gujarat, Uttar Pradesh and Madhya Pradesh states of India [1]. This fruit is under cultivation/underutilized but it has lots of culinary and medicinal properties. Kumar *et al*., 2012 reported that chironji is a good source of oil (52%) [2]. The seeds consists of moisture (3.0%), fat (59.0%), protein (19.0-21.6%), carbohydrate (12.1%), fibre (3.8%), calcium (279.0 mg), phosphorus (528.0 mg), iron (8.5 mg), thiamine (0.69 mg), ascorbic acid (5.0 mg), riboflavin (0.53 mg), niacin (1.50 mg) and also contain 34-47% fatty oil and 650 kcal/100g of kernal as caloric value [3].

Chironji plants flowers during the month of January to March based on the different agro climatic zone of the country and fruits ripen in the month of April-June; seed collection is mostly done during the fruit ripening time. Early harvesting has negative influence on their seed quality and germination. Most of the farmers follow the harvesting before fully ripening of fruits. As a result, there is an over-exploitation and unorganized methods of harvesting adopted by fruit collectors resulting in the chironji population decline [4].

Chironji is propagated through seed as well as vegetative method (soft wood grafting, chip budding, root cutting). Germination is one of the main constraints in the propagation and cultivation of chironji because of hard seed coat present on the kernels. Seeds of Anacardiaceae species usually possess physical dormancy type, which is promoted by an impermeable endocarp mentioned by Li *et al*., 1999 [5]. Seed germination is broadly control by two factors that is external factors (factors outside the embryo) and internal factors (embryo associated) [6]. Scarification on stony endocarp is very much necessary before sowing for good germination [7]; sowing of scarified chironji seeds during the month of June can achieved the 83.0% germination within 18 days revealed by Shukla and Solanki in 2000 [8]; scarification followed by conc. $H_2SO_4$ (5%) treatment also resulted in increased germination percentage (61.5%) within 25 days [9].

Popularization of this crop particularly in the rainfed regions and supplying of ample quality planting materials of chironji seedling is very much needed. In this context, the present investigation was carried out to enhance the seed germination through different physical and chemicals treatments.

**Material and Methods:** The experiment was conducted during the *kharif* 2019 at Medicinal and Aromatics Plants Research Station, Anand Agricultural University, Anand, Gujarat under the net house condition. Matured seed were randomly selected from the collected seed lot and selected seed were undergoes the surface sterilized with 1% aqueous sodium hypochlorite (NaOCl) solution for 3 minutes followed by rinsing the treated seed with thrice in distilled water. The seeds of each treatment were selected randomly and treated with as per specific treatment designed. The treated seeds were sown in the germinating plastic tube filled with thoroughly mixed of soil and cocopeat (50:50) during the 1st week of July 2019. The climatic conditions of the Research Station, Anand, Gujarat from the last week of June (26th Metrological Standard Week) to July (30th Metrological Standard Week) of year 2019 are presented in table 1.

**Table 1:** Meteorological data of research station

| Metrological Standard Week (MSW) | Rainfall (mm) | Mean temp. (°C) | Mean RH (%) |
|---|---|---|---|
| 25 (18th to 24 June) | 10.0 | 31.1 | 69.2 |
| 26 (25th June to 1st July) | 94.2 | 29.8 | 81.0 |
| 27 (2nd to 8th July) | 62.8 | 29.1 | 83.1 |
| 28 (9th to 15th July) | 0.0 | 30.7 | 70.5 |
| 29 (16th to 22nd July) | 12.4 | 31.4 | 69.8 |
| 30 (23th to 29th July) | 43.2 | 30.4 | 80.3 |

The texture of the soil used in the experiment has loamy sand type with very deep and fairly moisture holding capacity. The experiment was laid out in Completely Randomized Design (CRD) with two replications under the net house condition. The treatments comprised of various physical and chemicals treatments are presented in table 2. The scarification treatment was done by using sand paper having the 50 grade in a uniform way.

**Table 2:** Physical and chemical treatments on chironji seeds

| Sr. No. | Physical/Chemical Treatment |
|---|---|
| $T_1$ | Dipping in tap water for 1 day |
| $T_2$ | Dipping in tap water for 3 days |
| $T_3$ | Dipping in tap water for 5 days |
| $T_4$ | Dipping in hot water (100 ±2 °C) for 10 sec. |
| $T_5$ | Dipping in hot water (100 ±2 °C) for 30 sec. |
| $T_6$ | Dipping in hot water (100 ±2 °C) for 50 sec. |
| $T_7$ | Alternate wetting (24 hrs.) and drying (12 hrs.) |
| $T_8$ | Alternate wetting (12 hrs.) and drying (24 hrs.) |
| $T_9$ | Alternate wetting (24 hrs.) and drying (24 hrs.) |
| $T_{10}$ | Dipping in $H_2SO_4$ (5%) for 1 min. |
| $T_{11}$ | Dipping in $H_2SO_4$ (5%) for 3 min. |
| $T_{12}$ | Dipping in $H_2SO_4$ (5%) for 5 min. |
| $T_{13}$ | Dipping in $KNO_3$ @ 0.5% for 24 hrs. |
| $T_{14}$ | Dipping in $KNO_3$ @ 1.5% for 24 hrs. |
| $T_{15}$ | Dipping in $KNO_3$ @ 2.5% for 24 hrs. |
| $T_{16}$ | Dipping in $GA_3$ @ 300 mg/l for 24 hrs. |
| $T_{17}$ | Dipping in $GA_3$ @ 600 mg/l for 24 hrs. |
| $T_{18}$ | Dipping in $GA_3$ @ 900 mg/l for 24 hrs. |
| $T_{19}$ | Dipping in HCl (35.4%) for 1 min. |
| $T_{20}$ | Dipping in HCl (35.4%) for 3 min. |
| $T_{21}$ | Dipping in HCl (35.4%) for 5 min. |
| $T_{22}$ | Scarification with sandpaper |
| $T_{23}$ | Control |

The observation recorded were germination percentage (%), speed of germination, shoot and root length (cm), shoot and root dry weight (mg), vigour index-I & II, mean germination time, mean daily germination, peak value and germination value. Seed with at least 2 mm long radical emergence from soil surface was considered as germination and recorded daily.

**1. Germination percentage** (%): It is calculated based on the following formula [10]

$$\text{Germination \%} = \frac{\text{Number of germinated seeds x 100}}{\text{Total number of seeds sown}}$$

**2. Speed of Germination**: It is calculated by using formula given by Czabator, 1962 [11].
Speed of germination = n1/d1 + n2/d2 + n3/d3 +…..+ n/d

Where, 'n' are the number of seeds germinated on days 'd'

**3. Shoot and root length (cm)**: Length of five normal seedlings was measured in 'cm' on the final count.

**4. Shoot and root dry weight (mg)**: The weight of the five seedlings excluding the cotyledons was taken on final count day after oven drying at 80°C for 24 hrs.

**5. Vigour index-I & II**: This was calculated by determining the germination percentage, length and weight of seedling[12].
Vigour index-I = Germination Percentage x Seedling length
Vigour index-II = Germination Percentage x Seedling dry weight

**6. Mean germination time**: Mean germination time was calculated by using the formula given by Edward, 1932 [13].

$$\text{Mean Germination Time (MGT)} = \frac{N_1T_1 + N_2T_2 + M_3T_3 + \ldots + N_nT_n}{N_1 + N_2 + N_3 + N_4 + \ldots + N_n}$$

Where, N= No. of new seeds germinated
T= Time from the beginning of the experiment

**7. Mean Daily Germination**: It is an average number of seed germination per day during the test period and calculated with the help of formula given by Gordon, 1973 [14].

$$\text{Mean Daily Germination (MDG)} = \frac{\text{Final germination (\%)}}{\text{Total number of days of test}}$$

**8. Peak value (PV)**: It was calculated by the formula given by Czabator, 1962 [11].

$$\text{Peak value (PV)} = \frac{\text{Peak germination (\%)}}{\text{Days on which maximum germination occurred}}$$

**9. Germination value (GV):** It was calculated by the formula given by Crabator, 1962 [11].

Germination value (GV) = PV x MDG

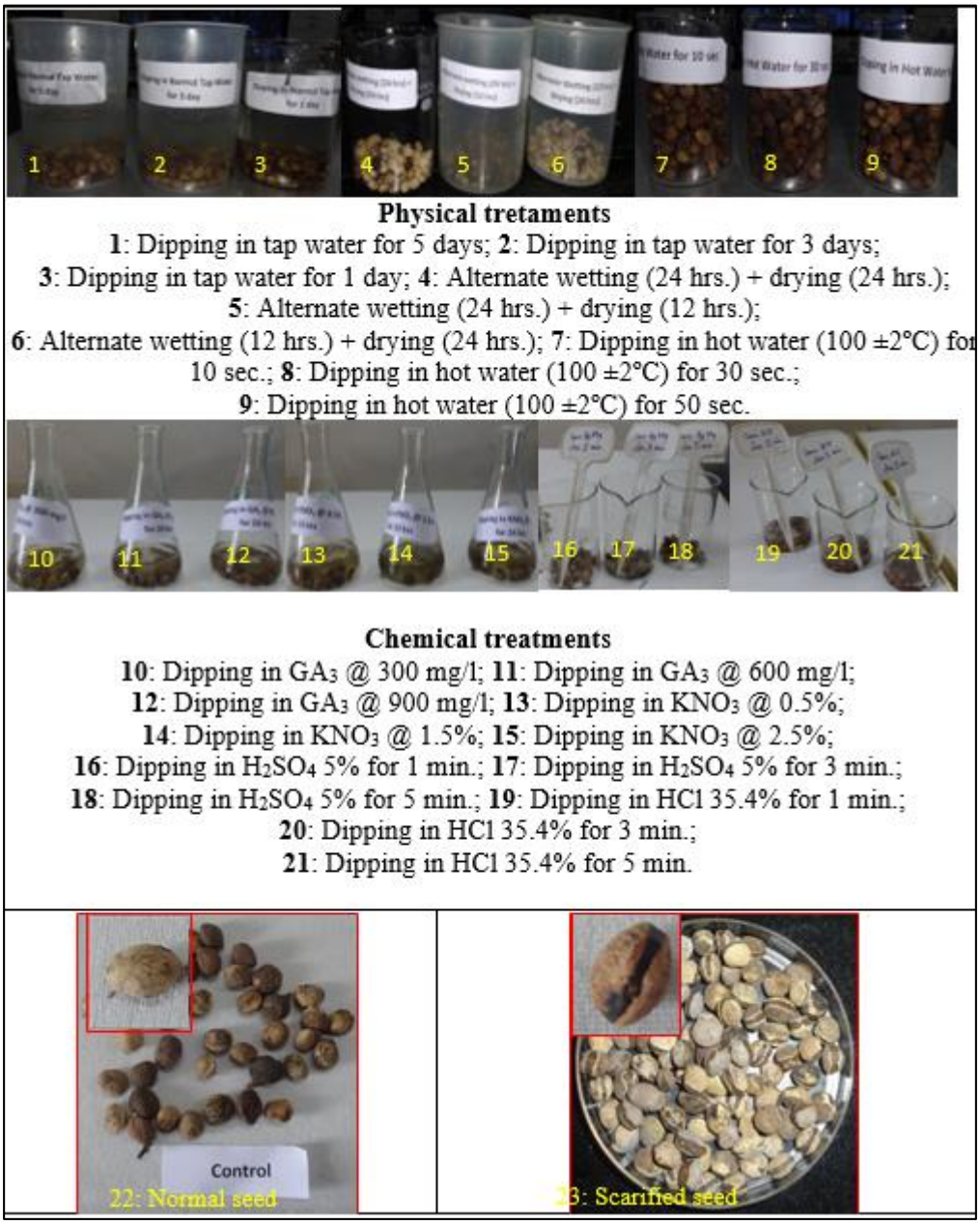


**Fig 1:** Treatments set-up

**Results:** In present investigation, germination percentage and other germination related parameters were observed significantly differences in chironji seed due to the different physical and chemical treatments. The data pertaining to various treatments after statistical analysis is presented in table 3**.** The significantly higher germination percentage (86.7%) was recorded by the treatment $T_9$, alternate wetting (24 hrs.) and drying (24 hrs.) followed by $T_2$, dipping in tap water for 3 days (81%) and $T_{12}$, dipping in $H_2SO_4$ 5% for 5 min. (50%). The lowest seed germination percentage was recorded by $T_1$, dipping in tap water for 1 day (10.2%) and $T_5$, dipping in hot water (100 ±2°C) for 30 sec. & $T_6$, dipping in hot water (100 ±2°C) for 50 sec. (12%). The treatment $T_9$, alternate wetting (24 hrs.) and drying (24 hrs.) exhibited significantly higher speed of germination (1.07), root length (9.7 cm), vigour index I (1664.33), vigour index II (6485.30), mean daily germination (2.89), peak value (0.69) and germination value (2.00) as compared to others treatments and control. In case of shoot length, significantly higher value was observed in the treatment $T_{18}$, dipping in $GA_3$ @900 mg/l for 24 hrs. *i.e.* 10.9 cm which was close to the treatment $T_9$ (9.5 cm), $T_{12}$ (9.3 cm) $T_{17}$ (9.1 cm) and $T_{16}$ (9.0 cm). The treatment $T_{16}$, dipping in $GA_3$ @300 mg/l for 24 hrs. was registered the same root length (9.7 cm) with the treatment $T_9$. Shoot and root dry weight was found great variations among the treatments. Significantly maximum shoot dry weight (72.45 mg) was occurred in $T_{16}$, dipping in $GA_3$ @300 mg/l for 24 hrs. which was as par with the treatment $T_{15}$ (72.32 mg); and significantly higher root dry weight (15.27 mg) was obtained in $T_{12}$, dipping in $H_2SO_4$ 5% for 5 min. Differences in mean germination (MGT) time in different treatments was found significant and maximum MGT (13.06) was observed in treatment $T_2$, dipping in tap water for 3 days which was followed by $T_9$ (12.55), $T_{10}$ & $T_{16}$ (10.50), $T_{15}$ (10.00).

Based on the analysis of variance of data the different physical and chemical treatments including scarification with sandpaper on chironji seed showed the significant differences at 5% in all the parameters of germination.

**Table 3:** Effect of different physical and chemical treatments on seed germination of chironji (*Buchanania lanzan*)

| Treatments | GP % | SG | SL (cm) | RL (cm) | SDW (mg) | RDW (mg) | VI-I | VI-II | MGT | MDG | PV | GV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | 10.2 | 0.18 | 6.7 | 6.0 | 43.95 | 6.64 | 128.00 | 516.38 | 8.25 | 0.34 | 0.13 | 0.04 |
| $T_2$ | 81.0 | 1.00 | 7.5 | 7.7 | 68.70 | 7.49 | 1229.38 | 6164.31 | 13.06 | 2.70 | 0.34 | 0.91 |
| $T_3$ | 36.2 | 0.66 | 7.6 | 7.6 | 65.67 | 7.19 | 548.75 | 2635.57 | 8.45 | 1.21 | 0.27 | 0.32 |
| $T_4$ | 15.7 | 0.40 | 6.6 | 6.3 | 48.55 | 6.61 | 201.23 | 864.12 | 8.79 | 0.52 | 0.22 | 0.12 |
| $T_5$ | 12.0 | 0.17 | 6.8 | 6.4 | 46.71 | 8.51 | 158.00 | 661.89 | 8.50 | 0.40 | 0.17 | 0.07 |
| $T_6$ | 12.0 | 0.32 | 5.8 | 6.0 | 45.65 | 6.33 | 184.60 | 815.25 | 9.33 | 0.52 | 0.16 | 0.09 |
| $T_7$ | 19.5 | 0.32 | 7.1 | 7.5 | 55.75 | 7.56 | 283.60 | 1234.15 | 9.25 | 0.65 | 0.17 | 0.11 |
| $T_8$ | 20.0 | 0.39 | 7.8 | 7.3 | 56.65 | 10.12 | 302.00 | 1335.40 | 7.83 | 0.67 | 0.27 | 0.18 |
| $T_9$ | 86.7 | 1.07 | 9.5 | 9.7 | 60.47 | 13.82 | 1664.33 | 6485.30 | 12.55 | 2.89 | 0.69 | 2.00 |
| $T_{10}$ | 20.0 | 0.29 | 7.5 | 8.9 | 53.90 | 10.88 | 327.00 | 1295.63 | 10.50 | 0.67 | 0.19 | 0.13 |
| $T_{11}$ | 16.7 | 0.27 | 7.5 | 7.9 | 58.40 | 8.08 | 256.70 | 1107.00 | 9.17 | 0.56 | 0.22 | 0.12 |
| $T_{12}$ | 50.0 | 0.88 | 9.3 | 9.5 | 64.56 | 15.27 | 936.98 | 3923.28 | 8.78 | 1.67 | 0.38 | 0.63 |
| $T_{13}$ | 16.7 | 0.34 | 7.7 | 7.2 | 58.05 | 11.94 | 247.45 | 1165.79 | 9.00 | 0.56 | 0.13 | 0.07 |
| $T_{14}$ | 23.3 | 0.43 | 7.7 | 8.6 | 61.39 | 13.93 | 380.30 | 1788.12 | 9.38 | 0.78 | 0.21 | 0.16 |
| $T_{15}$ | 23.3 | 0.35 | 7.8 | 9.6 | 72.32 | 11.10 | 403.83 | 1952.98 | 10.00 | 0.78 | 0.17 | 0.13 |
| $T_{16}$ | 20.0 | 0.29 | 9.0 | 9.7 | 72.45 | 11.01 | 373.00 | 1663.20 | 10.50 | 0.67 | 0.19 | 0.13 |
| $T_{17}$ | 18.5 | 0.33 | 9.1 | 8.1 | 52.00 | 10.10 | 318.35 | 1143.85 | 6.50 | 0.62 | 0.15 | 0.10 |
| $T_{18}$ | 20.0 | 0.40 | 10.9 | 8.3 | 66.68 | 12.36 | 383.00 | 1490.60 | 6.85 | 0.67 | 0.21 | 0.14 |
| $T_{19}$ | 13.3 | 0.26 | 6.3 | 5.8 | 56.50 | 7.50 | 160.00 | 853.27 | 5.75 | 0.44 | 0.13 | 0.06 |
| $T_{20}$ | 13.3 | 0.20 | 6.6 | 6.4 | 52.50 | 6.75 | 172.00 | 789.93 | 5.49 | 0.44 | 0.10 | 0.04 |
| $T_{21}$ | 13.3 | 0.24 | 6.1 | 5.8 | 55.45 | 6.70 | 158.00 | 828.67 | 5.43 | 0.44 | 0.13 | 0.06 |
| $T_{22}$ | 36.7 | 0.71 | 8.4 | 8.6 | 64.57 | 9.17 | 621.45 | 2704.03 | 8.49 | 1.22 | 0.25 | 0.31 |
| $T_{23}$ | 13.3 | 0.20 | 6.2 | 7.0 | 49.60 | 6.83 | 176.00 | 752.33 | 5.91 | 0.44 | 0.10 | 0.04 |
| S.Em± | 1.05 | 0.02 | 0.29 | 0.30 | 2.05 | 0.38 | 18.90 | 69.36 | 0.42 | 0.03 | 0.01 | 0.02 |
| C.D. at 5% | 3.08 | 0.05 | 0.86 | 0.88 | 5.99 | 1.12 | 55.28 | 202.18 | 1.22 | 0.10 | 0.03 | 0.05 |
| C.V. % | 5.79 | 5.60 | 5.44 | 5.61 | 5.00 | 5.76 | 6.39 | 5.35 | 6.83 | 5.61 | 7.62 | 8.98 |

GP: Germination Percentage; SG: Speed of Germination; SL: Shoot Length; RL: Root Length; SDW: Shoot Dry Weight; RDW: Root Dry Weight; VI-I&II: Vigour Index-I & II; MGT: Mean Germination Time; MDG: Mean Daily Germination; PV: Peak Value; GV: Germination Value.

**Discussion:** The chironji seed are enclosed by a very hard seed coat [15]; embryo extrusion is one of the main barriers in seed germination particularly in those seeds having such hard seed coat. Due to the hard seed coat it makes an impermeable to water and oxygen as results it ceases/slow down the metabolic processes [16, 17]; makes very difficult to break the seed coat. The first very much necessary step in the seed germination process is the water imbibition[18]. Cellular constituents of seed are hydrated resulting in swelling of the seed due to the development of huge turgor pressure inside the seed. Following an alternate wetting and drying condition might be design to help in cracking the seed coat; more penetration of water and exchange of gases which ultimately enhance the seed germination process. Dipping the seed in normal water which might be also enhanced the imbibition process. Chironji seed were started germination as early as 7$^{th}$ days after sowing was observed under this experiment. The seedlings were developed more vigorously in terms of length and dry matter accumulation. Concentrated sulphuric acid ($H_2SO_4$) has been used as chemical scarification in various seed for overcoming seed dormancy of *Buchanania lanzan* [19], *Schinus molle*[20], *Canna indica* [21], *Tetrapleura tetraptera* [22], *Vigna* species [23], *Cercis siliquastrum* [24], Carex seeds[25] *etc*. Mesocarp structure has been changes after acid scarification digesting parenchyma cells and removes unwanted chemicals compounds from both parenchyma cells and the secretary cavities; this improved the water absorption during imbibition [20]. In the present investigation, $H_2SO_4$ (5%) for 5 minutes ($T_{12}$) treatment induces higher seed germination percentage (50%) and other germination related parameters as compared to controls; significantly higher root dry weight was also observed under this treatment (13.93 mg).

In the present investigation, $GA_3$ doesn't influence much on seed germination but it increases length of shoot and root compared to other treatments and control. In year 1930s, it was confirmed that the gibberellin (GA) promoting on stem growth by studies of the Bakanae (foolish seedling) disease in rice. And, $GA_3$ also stimulates seed germination by helping in *de-novo* synthesizing of the α-amylase enzymes which helps in conversion of insoluble starch into soluble carbohydrates [26]. However in chironji seed the treatments of $GA_3$ were found not much affected on seed germination but increases the shoot as well as root elongation. There, it is evident that the promotion of stem elongation by gibberellins is almost entirely due to increase in cell elongation. But it is the total effect of the increased in cell division in apical and subapical meristematic regions, greater production of auxin in the said regions [27] and increasing osmotic uptake of nutrients [28]. Resulting in increasing stem elongation and stem diameter. The production of more number of leaves might be due to higher growth of seedlings and also due to activity of $GA_3$ at the apical meristem resulting in more synthesis of nucleoprotein responsible for increasing leaf initiation [29]. As a result, it produces more dry matter weight in stem portion. Significantly higher shoot dry weight was also occurred in the treatment $T_{16}$ (Dipping in $GA_3$ @ 300 mg/l for 24 hrs.) *i.e.* 72.45 mg which was at par with the treatment $T_{15}$ (72.32 mg), $T_2$ (68.70 mg) and $T_{18}$ (66.68 mg).Mechanical scarification using sandpaper was found quite effective in chironji seed germination (36.7%). This is might be due to the damaging of lignified palisade cell layer which enables more penetration of water. Similarly it was reported in *Medicago scutellata* and *Medicago polymorpha* [30], *Prosopis koelziana* and *Prosopis juliflora* [31] and *Capparis ovata*[32] that scarification with sandpaper was effective seed dormancy breaking method.

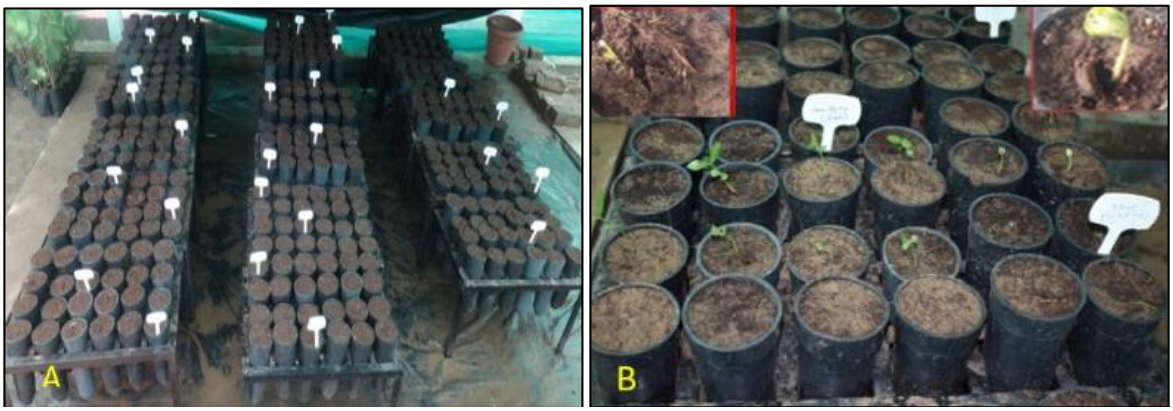


**Fig 2:** A. Experimetntal view B. Initiation of chironji seed germination


Alternate Wetting (24 hrs.) + Drying (24 hrs.) Dipping in $H_2SO_4$ (5%) for 5 minutes


Dipping in tap water for 3 days Scarification with sandpaper

**Fig 3:** Germinated seedlings at final count stage

**Conclusion:** Being an economically important species, it is very much necessary to multiply the species. However, germination is the one of the main constraint in propagation and large scale cultivation. Chironji seed has very hard seed coat making impermeable to water imbibition and exchange of gases. A very high percentage of chironji seed germination was recorded in the treatment of alternate wetting (24 hrs.) and drying (24 hrs.) followed by 3 days dipping in tap water, scarification with $H_2SO_4$ 5% for 5 minutes and with sandpaper. Therefore, the present investigation shows quite economic treatment for breaking of chironji seed dormancy and it will help in species conservation and cultivation.

**Conflict of Interest:** The study did not examine any specific types of conflict of interest separately.

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