Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP3: 165-169

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National conference on "Conservation, Cultivation and Utilization of medicinal and Aromatic plants"
(College of Horticulture, Mudigere Karnataka, 2018)

Enhancement of germination and seedling growth attributes of a medicinal tree species *Pterocarpusmarsupium* Roxb. through pre sowing seed treatments

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Abstract
Pterocarpus marsupium Roxb. is an important tree species belongs to family Fabaceae and it is distributed in deciduous forests throughout India which is a good source of dye, timber and also used as medicine for treating leprosy, leukoderma, inflammation, excessive urination etc. It is mainly propagated through seeds but the problem in seed germination is due to hard seed coat which limits seed germination. So the present study was carried out in College of Horticulture, Mudigere with an aim to find out the best treatment for enhancement of germination in *P. marsupium.* Seeds were treated with different treatment includes growth regulators (GA_3, NAA, Cytokinine), acid scarification (KNO_3, HCl, H_2SO_4) and organics (hot water, cow urine, cow dung slurry), among all the treatments seeds treated with H_2SO_4 (2%) recorded the higher germination percentage (65.83%) and seedling attributes compared to untreated seeds in control. It helps in the multiplication of a large number of plants meanwhile increase the availability and conservation.

Keywords: medicinal tree species, pre sowing seed treatments

Introduction
Pterocarpus marsupium Roxb. is commonly called as Indian Kino tree and in Kannada it is called as hone, Bijasara, Asana (Devgun *et al.*, 2009) [6] is distributed in deciduous and evergreen forests of central, western and southern regions of India. It can grow up to 30 m tall, bark 10-15 mm, the surface of bark is grey or greyish-black, rough, deeply vertically cracked, Oleo-resin obtained from tree trunk is called kino-gum, which is fragrant, brittle, almost black in colour. Leaves are compound, imparipinnate and have 5-7 leaflets which are oblong, obtuse with emarginated apex. Flowers are yellow in terminal panicles. Fruits are roundish, 4 to 5 mm in diameter which is flat and winged pods. Seeds are 1 to 2 cm and hard (Khan, 2015) [9]. The heartwood is strong, tough, very hard, golden brown or reddish brown on exposure with darker streaks. Occurs throughout India in deciduous and evergreen forests, particularly in Southern India, Madhya Pradesh, Orissa and Bihar.
The heartwood, leaves, flowers and gum are used in medicine. The heartwood is used in the treatments of kapha and pitta, inflammation, excessive urination, intestinal worms, haemorrhages, fractures, bruises, leucoderma, leprosy, skin diseases, elephantiasis, erysipelas, pain in the rectum, diarrhoea, dysentery, rheumatoid arthritis, cough, asthma, diabetes, bronchitis and greyness of hair (Maneesha *et al.*, 2015) [10]. The leaves are used to treat boils, sores and many skin diseases. The flowers are used in the treatment of fever, loss of appetite and vitiated conditions of pitta. The gum obtained from the barkis useful in pitta, diarrhoea, psoriasis, wounds, ulcers, diseases of the liver, boils, gleet, intermittent fevers and inflammations. It is a very good liver tonic. All active principles of *P. marsupium* are thermostable. The plant contains pterostilbene (45%), alkaloids (0.4%) and tannins (5%) (Maneesha *et al.*, 2015) [10].
Pterocarpus marsupium Roxb. Is important medicinal and multipurpose tree. Due to the tremendous population pressure and absence of appropriate government policies,

unsustainable utilization of valuable tree species leads to the natural stands of this tree is disappearing. The natural regeneration through seeds is difficult due to the physical limitation to moisture diffusion caused by hard and thick seed coat which are responsible for seed dormancy (Das and Chatarjee, 1993) [5], the seeds of fabaceae family have impermiable seed coat (Al-Menaie *et al.,* 2010) [3]. Presence of palisade layer in the seed coat of certain plants belong to the family fabaceae is assumed to be casually connected with their high degree of impermeability to water diffusion (Rolston, 1978) [14]. The dormant seed creates a serious problem in germination. Therefore, the present investigation was undertaken with an objective of enhancement of seed germination and seedling attributes through different pre-sowing treatments.

Materials and methods

The experiment was carried out for seed dormancy breaking studies in *P. Marsupium* during 2017-18 at College of Horticulture, Mudigere, Chikkamagaluru, Karnataka. Seeds of *P. Marsupium* were collected during August 2017 from well established plantation in farmer field from Shivamogga

Before sowing, polybags having size of 8 X 6 inches were filled with mixture of sand, soil and well rotten FYM in the ratio of 1:2:1 respectively. To facilitate aeration and proper drainage, six perforations were made to the polybags before filling them with prepared media mixture. After seed treatment, one seed per polybag was dibbled and then covered with a thin layer of soil.

Treatment details

T_1 - GA_3 (100 ppm)
T_2 - GA_3 (200 ppm)
T_3 - NAA (100 ppm)
T_4 - NAA (200ppm)
T_5 - Cytokinin (20 ppm)
T_6 - Cytokinin (30 ppm)
T_7 - KNO_3 (1 %)
T_8 - KNO_3 (1.5 %)
T_9 - HCL (1 %)
T_{10} - HCL (2 %)
T_{11} - H_2SO_4 (1%)
T_{12} - H_2SO_4 (2%)
T_{13} - Hot water (50 ℃ for 20 min)
T_{14} - Hot water (80 ℃ for 20 minutes)
T_{15} - Cow urine (1:1)
T_{16} - Cow urine (1:2)
T_{17} - Cow dung slurry (1:1)
T_{18} - Water soaking for 3 hours (control)

There were 18 treatments; in each treatment 60 seeds were sown. The seeds were treated with growth regulators (GA_3, NAA, Cytokinin), acids (KNO_3, HCL, H_2SO_4) and organics (cow urine, cow dung slurry) with duration of 6 hours, 20 minutes and 48 hours respectively.

Record keeping

Observation for germination parameters were recorded each day up to three months from the day of sowing and for seedling attributes, three seedling from each treatment and from two replications were recorded at monthly intervals.

Days to initiation of germination: The polybags were observed daily, for seedling emergence. The days on which the first seedling emerged was expressed as days to initial germination (Sada*et al*., 2014) [15].

Days to 50 percent germination

A number of days taken for 50 percent of the seeds to germinate in entire lot was considered as 50 percent germination.

Days to final germination

The number of days taken for the last seedling emergence was recorded and expressed as days to final germination (Mauromicale and Cavallaro, 1995) [11].

Germination percent

The number of normal seedlings produced in each treatment was counted and average was expressed in percent (ISTA, 2003) [8].

Germination percentage = number of normal seedlings X 100
Total number of seeds sown

Assessment of seedling growth

Morphological data such as seedling height, fresh weight and dry weight of the areal part and roots were recorded up to 90 days after sowing.

Vigour Index

Vigour index (VI) was computed using the following formula and expressed as whole number (Vijayalakshimi and Renganayaki, 2017) [17].

VI = Germination percentage X dry weight (g/seedling).

Statistical analysis of data

The experiment was carried out in randomized complete block design. Data were analyzed by analysis of variance (ANOVA) to detect significant differences between mean. Significantly differing mean were tested based on F test value at 0.05 probability level. Variance in data has been expressed as mean ± standard error.

Results and discussion

Seed germination

Germination period

Seed germination was started on the 10th day after sowing which is the earliest germination and it is continued up to 69 days. The fastest initiation of germination (10th day), days taken for 50 percent germination (24 days) and minimum period for germination (39.5 days) in case of seeds treated with H_2SO_4 at 2% concentration and delayed germination (26th day) maximum days taken for 50 percent germination (46.5 days) and total germination period (69.5 days) was maximum in untreated seeds (control) which is shown intable 1.The similar results were reported by Khan (2015)[9] in *P. marsupium* and Afshar, *et al*., (2014)[1] in dormant seeds of *Canna indica*. This might be due to the H_2SO_4 made the hard seed coat permeability to water. Apart from H_2SO_4, the seeds treated with cow dung slurry and concentrated HCl also shown better results and these results are in confirmation with the results obtained in case of *Melia azedarach* (Sujatha and Manjappa, 2015) [16].

Germination percentage

The treatment with H_2SO_4 at 2% concentration was effective in inducing highest germination percentage (65.83) followed by 1:1 of cowdung slurry (60.83%) and the lowest germination percentage was observed in case of seeds treated with hot water at 80 °C for 20 minutes showed lowest germination percentage of 9.17 percent followed by control 19.17 percent. The acid scarification with H_2SO_4 enhanced

the seed germination was also supported by the reports of Sadat *et al.* (2014) in *Cassia fistula* [15], Mensah and Agbagwa (2004) in seeds of *Gmelina arborea* [12] and khan (2015) in *P. marsupium* [9]. The treatment with hot water at 80 °C for 20 minutes was not effective to induce germination however longer duration at temperature may cause the damage to the embryo to germinate which is also reported by Ahire *et al.* (2009) in case of *Uraria picta* [2]. Usually the seeds of fabaceae with hard seed coat show enhanced germination when various pre-sowing treatments are used, it was also reported by Palani*et al.* (1996) in *Albizia lebbeck* [13], Anand *et al*. (2012) in *Melia dubia* [4].

Plate 1: Developmental stages of seed germination in *P. marsupium*

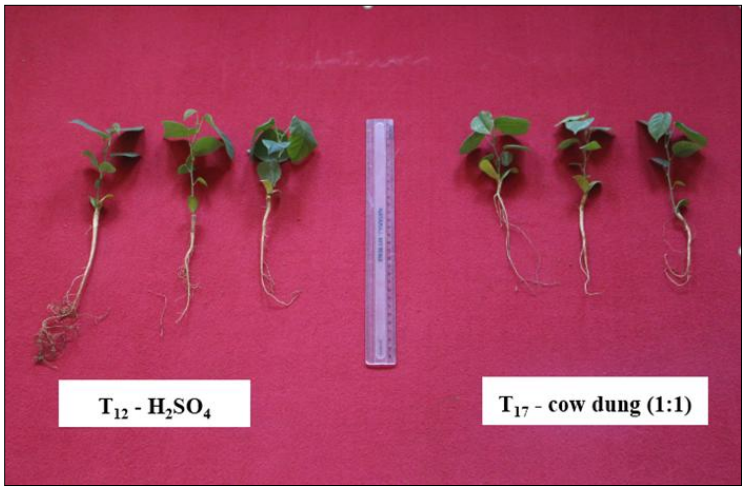

Plate 2: Pre sown seed treated Seedling at 90 days after sowing

Table 1: Effect of pre-sowing seed treatments on germination characteristics.

Treatments	Days to initiation of germination	Days to 50 % germination	Days to final germination	Germination %
T_1 - GA_3 (100 ppm)	19.00	35.00	56.50	37.50
T_2 - GA_3 (200 ppm)	15.50	32.00	51.50	47.50
T_3 - NAA (100 ppm)	19.00	39.50	63.00	32.50
T_4 - NAA (200ppm)	20.00	35.50	58.00	39.17
T_5 - Cytokinin (20 ppm)	21.50	41.50	67.00	26.67
T_6 - Cytokinin (30 ppm)	24.00	39.50	62.50	31.67
T_7 - KNO_3 (1 %)	19.50	40.00	61.50	40.00
T_8 - KNO_3 (1.5 %)	16.50	37.00	52.00	54.17
T_9 - HCL (1 %)	14.50	34.50	56.50	46.67
T_{10} - HCL (2 %)	12.00	30.50	54.00	58.33
T_{11} - H_2SO_4 (1%)	15.00	30.50	51.00	53.33
T_{12} - H_2SO_4 (2%)	10.00	24.50	39.50	65.83
T_{13} - Hot water (50℃)	19.00	36.00	60.00	40.00
T_{14} - Hot water (80℃)	22.50	32.50	65.50	9.17
T_{15} - Cow urine (1:1)	16.50	36.00	55.50	44.17
T_{16} - Cow urine (1:2)	19.00	31.00	60.50	43.33
T_{17} - Cow dung slurry (1:1)	11.50	31.50	47.00	60.83
T_{18} - (control)	26.50	46.50	69.50	19.17
S.EM	1.15	1.47	2.03	2.27
CD @ 5%	3.44	4.39	6.07	6.77

Seedling growth attributes

The seedling growth characters such as length of shoot, root, the fresh and dry weight of shoot root and seedling was recorded at 90 days after sowing. For all the growth parameters, T_{12} - H_2SO_4 at 2% showed highest length of seedling (37.77 cm), fresh weight of seedling (5.16 g), dry weight of seedling (1.69g) and seedling vigour index (118.39 g/seedling) which is followed by T_{17} - Cow dung slurry (1:1) compare to all other treatments and the lowest was recorded in control (Table 2). This may be due to germination characters were directly correlated with the seedling growth and vigour. Increased seedling biomass through seed treatment of cow dung slurry was also reported by Hossain *et al.*, (2013) in *Terminalia chebula* [7]. The findings of the present study show that germination percentage and seedling growth of *P. marsupium* significantly increased when seeds were treated with H_2SO_4 (2%) followed by Cow dung slurry (1:1).

Table 2: Effect of pre-sowing seed treatments on seedling characters.

Treatments	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Vigour index (g/seedling)
T_1 - GA_3 (100 ppm)	31.02	4.12	1.48	55.56
T_2 - GA_3 (200 ppm)	32.20	4.45	1.56	74.18
T_3 - NAA (100 ppm)	28.98	3.92	1.50	48.59
+T_4 - NAA (200ppm)	29.75	4.08	1.53	59.79
T_5 - Cytokinin (20 ppm)	30.79	3.67	1.57	41.96
T_6 - Cytokinin (30 ppm)	28.29	3.78	1.54	48.82
T_7 - KNO_3 (1 %)	31.40	4.24	1.58	63.13
T_8 - KNO_3 (1.5 %)	37.88	4.69	1.59	86.03
T_9 - HCL (1 %)	36.79	5.14	1.59	73.97
T_{10} - HCL (2 %)	41.49	5.16	1.69	98.78
T_{11} - H_2SO_4 (1%)	37.77	4.87	1.61	85.87
T_{12} - H_2SO_4 (2%)	45.66	6.40	1.80	118.39
T_{13} - Hot water (50℃)	31.26	4.74	1.54	61.73
T_{14} - Hot water (80℃)	28.61	4.48	1.35	12.41
T_{15} - Cow urine (1:1)	35.30	4.70	1.54	68.09
T_{16} - Cow urine (1:2)	31.38	4.46	1.57	68.15
T_{17} - Cow dung slurry (1:1)	40.57	5.64	1.66	100.98
T_{18} - (control)	24.57	3.76	1.34	25.72
S.EM	1.29	0.20	0.08	4.21
CD @ 5%	3.86	0.59	0.23	12.56

Conclusion

The results of the present study demonstrated that the beneficial effects on improving the germination in *P. marsupium*. Among all the pre-sowing treatments with H_2SO_4 (2%) and cow dung slurry (1:1) showed significantly improved germination and seedling vigour than all other treatments. Since these treatments are cheap it can be utilized for large scale propagation of *P. marsupium* an endangered medicinally important tree.

References

1. Afshar FI, Ali SS, Mozhgan S. Effect of H_2SO_4 on seed germination and viability of *Canna indica* L. ornamental plant. Int J Adv Bio Biomed Res. 2014; 2(1):223-229.
2. Ahire ML, Goga GS, Dayaram NT. Seed viability and influence of pre-sowing treatments on germination and seedling development of *Uria picta* (Jacq.) Seed Sci Biotech. 2009; 3(2):48-53.
3. Al-Menaie HS, Al-Ragam O, Al-Shatti A, Mathew M, Suresh N. The effects of different treatments on seed germination of the *Cassia fistula* L. and *Cassia nodosa* Buch. -Ham. Ex Roxb. In Kuwait. Afr J Agric Res. 2010; 5(3):230-235.
4. Anand B, Devagiiri GM, Gurav M, vasudev HS, Khaple AK. Effects of pre-sowing seed treatments on germination and seedling growth performance of *Melia dubia* CAV: An important multipurpose tree. Int J Life Sci. 2012; 1(3):59-63.
5. Das T, Chatarjee. *In vitro* studies of *Pterocarpus marsupium*- An endangered tree. Indian J plant physiol. 1993; 36:269-272.
6. Devgun M, Nanda A, Ansari SH. *Pterocarpus marsupium* Roxb. - A comprehensive review. *Phacog Rev.*, 2009; 3(6):359.
7. Hossain MA, Uddin MS, Rahman MM, Shukor NA. Enhancing seed germination and seedling growth attributes of a medicinal tree species *Terminalia chebula* through depulping of fruits and soaking the seeds in water. J Food Agric Environ. 2013; 11(3-4):2573-8.
8. International Seed Testing Association (ISTA). ISTA Working Sheets on Tetrazolium Testing Agricultural, Vegetable and Horticultural Species. Edited by Leis, N; Kramer, S; Jonitz, A. Basserdorf, CH-Switzerland. 2003; 1:200.
9. Khan MR. The Effect of Different Treatments on the Seed Germination of *Pterocarpus marsupium* Roxb. Weekly Sci Res J. 2015; 2(28):22.
10. Maneesha T, Manik S, Khare HN. Chemical constituents and medicinal uses of *Pterocarpus marsupium* Roxb. Flora and Fauna. 2015; 21(1):55-59.
11. Mauromicale G, Cavallaro V. Effects of seed osmo priming on germination of tomato at different water potential. Seed Sci Technol. 1995; 23:393-403.
12. Mensah SI, Agbagwa IO. Breaking dormancy in *Gmelina arborea* Roxb. Through treatment of seeds with chemical substances and alternating temperature. J Bio Res Biotech. 2004; 2(1). http://dx.doi.org/10.4314/br.v2i1.28542
13. Palani M, Dasthagir MG, Kumaran K, Jerlin R. Effect of presowing treatments on growth attributes of *Albizia lebbeck* L Benth. An For. 1996; 4(1):85-88.
14. Rolston MP. Water impermeable seed dormancy. Bot Rev. 1978; 44:365-398.
15. Sadat E, Babalola O, Shonubi O, Olubukanala TO. Seed dormancy in *Cassia fistula* Linn. Population from Nigeria. J Am Sci. 2014; 10:85-93.
16. Sujatha VN, Manjappa K. Effect of pre-sowing treatment

on seed germination of *Melia azedarach* L. Karanatka J Agric Sic. 2015; 28(2):269-270.
17. Vijayalakshimi KP, Renganayaki PR. Effect of pre-sowing treatment on germination of red sanders. Int J Microbiol App Sci. 2017; 6(4):168-173.