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Effect of Seed size and pre-sowing treatment on seed germination of *Terminalia bellirica*

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ABSTRACT

Terminalia bellirica Roxb is a large tree up to 40 meters high belongs to family Combretaceae. Due to over-exploitation (because of its multipurpose uses), tremendous population pressure, rural poverty, absence of appropriate government policy, accelerated growth of synthetic drugs and inappropriate utilization of forest products, these vital plat resources are being declined besides these, natural regeneration through seeds is very poor, uncertain and required long time to germinate, thus the State Forest Departments avoid the species in the afforestation and/or reforestation programs. Seeds from different locations in Rajasthan were analyzed for seed quality as well as germination potentials with different treatments. GA_3 was found to increase seed germination in comparison to others. A detailed work with review work on its reproductive biology is presented in the present study.

Keywords: Medicinal value; Seed germination; Substrate; Nursery techniques; Containerized seedlings; Propagation challenges; Forest conservation.

1. Introduction

Terminalia bellirica Roxb is a large tree upto 40 meter high belongs to family Combretaceae. Its leaves are petiolate, broadly elliptic, clustered towards the end of branches. It flowers greenish yellow, solitary, simple axillary spikes. It is found in deciduous forests throughout the greater part of India except in arid zone. It is found in upper Gangetic plains, Chota Nagpur, Bihar, Orissa, West Bengal, Konkan region and South India (Sharma *et. al.*, 2005, Deb A *et. al*., 2016). The word Bellirica is taken from the scientific name to tree and distinguish this myrobalan from the ulha one (*Chebulic myrobalan*) (1999). It is the secondary host of tasar silkworm (Anonymous, 1976). *Terminalia bellirica* is known as Vibhitaka and vibhitaki in Sanskrit, Tanni and Tanrikkkai in Tamil, Bahera, Birha in Punjabi and Bellirica myrobalan in English. It contains different chemical constituents in different parts viz. stem, bark, fruit, etc. Its flowering starts in March to May and fruit ripens in December to February.

It occurs throughout the sub-Himalayan tract upto 1200-1300 mela and is usually common in sal (*Shorea robusta* Gareth.) and miscellaneous forest of the sub-Tropical zone (Osmaston, 1978).

Importance: *Terminalia bellirica* is important tree from commercial as well as traditional indigenous medicinal value. Its seeds are edible and are important components of "**Triphla churn**" used in bronchitis eouyl pebble in dyspepsia, cortication, impurity of bloods and stomach problem, etc. (CSIR 1985).

It leaves are highly valued as fodder for milk cattle and are fed to tsar silkworm (Bhatia *et al.*, 1977) and they are lopped repeatedly for this purpose (Luna 2005). It poles are used for making the beams of houses, handloom of agricultural implements and furniture to Species has been over-exploited for to multiple uses. Its seed germination is poor and in seeds. However, seeds are viable for one year (Luna 2005). Its germination is poor due to its hard seed coat. Owing to high demand for its seedlings in agroforestry plantations & its medicinal uses it can be used for economic upliftment of the local in inhabitants.

However, due to over-exploitation (because of its multipurpose uses), tremendous population pressure, rural poverty, absence of appropriate government policy, accelerated growth of synthetic drugs and inappropriate

utilization of forest products, these vital plat resources are being declined besides these, natural regeneration through seeds is very poor, uncertain (Luna, 2005) and required longtime to germinate, thus the State Forest Departments avoid the species in the afforestation and/or reforestation programs. This delay and irregularity in germination of seeds is believed to be due to the hard seed coat and thick fleshy pericarp of the fruits of the species (Shivanna *et al.*, 2007). The pericarp of the fruit contains very high level of phenolic compound (Bajpai *et al.*, 2005). Seed germination may be reduced by the presence of impermeable seed coats that restricts the availability of water, which can penetrate into seeds at their optimum concentration (Kumar *et al.*, 2015a,b). Fruit length was significantly and positively correlated with seed length. Seed diameter had significantly positive correlation with fruit weight and seed weight. Although commercial exploitation of *T. bellerica* is hampered by the shortage of superior plants, hard seed coat and heavy insect infestation of seedlings and primarily due to the difficulties experienced in propagating this species using the traditional method of grafting and the poor rooting ability of shoot cuttings. There are some studies which examined that positive correlation between *T. chebula* and *T. bellirica* seed germination and de-pulping of dry fruits by knife and soaking the seeds in cold water for various time periods (Hossain *et al.*, 2005a,b). De-pulping of the *T. bellirica* fruits by rotting fleshy pulp in water would be one of the important options beside de-pulping the dry fruits with knife or other mechanical scarifications but the technique and its subsequent effect on seed germination have not been sufficiently investigated.

2. Review of literature

Substrate plays a significant role in seedling emergence, because seeds have characteristic requirements for moisture and oxygen for germination. A substrate needs to be non-toxic, free of moulds with adequate aeration and moisture for germinating seeds (Justice 1972). Farmyard manure gave highest germination percent while minimum days to complete germination were recorded in the case of goat manure. Higher germination in FYM may be due to the fact that compost is non-toxic, rich in NPK and has adequate aeration and moisture for germination of seeds (Justice 1972). In a similar investigation, germination of *Albizia lebbek* Benth was found best in FYM followed by sand, sand + soil and sand + soil + FYM (Thakur *et al.*, 2000). FYM was the richest in NPK.

In order to maximize survival and growth, it is essential to raise seedlings in nurseries with proper care. Watering, weeding around the out planted seedling and the protection against big herbivores enhance seedling growth (Bognounou *et al.*, 2010). In the nursery, raised beds are used in areas with high water tables, sunken ones are used in semi-arid and arid areas while flat beds are used in intermediate areas (Anonymous 1996). Nature of the bed affects the conditions for germination; sunken beds in our study yielded the highest germination percent. The large seeds (as in *Terminalia bellirica*) need higher moisture and deeper soil for proper germination than small seeds. The texture or particle size distribution of nursery soils and that of potting medium for containerized planting stock is an important soil physical property influencing root and shoot growth (Dickson *et al.*, 1960).

ISTA (1993) has suggested sand as a suitable medium for seed germination in *Acacia spp*. In *Acacia nilotica* (L.) Wild. ex Del. the maximum germination (97 %) was observed in the sand medium as compared to other soil media (Venkatesh *et al*., 2000). (Maithani *et al.*, 1988) recorded significant differences in seed germination in *Dalbergia sissoo* Roxb under 1:2:1 (sand:soil:FYM) and 1:1:1 (sand:soil:FYM). In *Picea smithiana* (Wall.) Boiss maximum seed germination was recorded in humus medium (Lavania *et al.*, 2007). Sand as a germination substratum is

preferred for tree species having large seeds (Magini 1962) because aeration in sand medium is best. However, *T. bellirica* did not behave similarly. It may be due to hard seed coat, which may need constant moisture conditions to soften it, which is not possible in sand.

Seedlings grown in containers have many advantages such as better survival rate, easier to plant, immediate growth response benefits, cheaper to produce and plant than bare-root seedlings (*Landis et al.*, 1990). However, we found no significant effect of container volume on germination percent of *T. bellirica*. Derby & Hinesley (2005) reported that germination percent of *Chamaecyparis thyoides* (L.) (atlantic white cedar) was affected by container volume with the maximum germination percent in Hiko Trays (V - 530 model, 15 cells/tray, volume = 530 cm^3).

Ginwal *et al.*, (2002) found that sand and compost combination (20 % sand + 80 % compost) gave best result in *Dalbergia sissoo* Roxb. We did not find significant effect of manure type on seedling growth. On the other hand, Thakur *et al.*, (2000) found sand + soil + FYM as the best potting medium for development of healthy seedlings with nodulated roots and better growth in *Albizia lebbeck* Benth. Nandeshwar & Patra (2004) also suggested that soil, sand and compost in the ratio of 1:1:2 is the best for growth and survival of *Acacia catechu* Willd seedlings.

Studies carried out by Annapurna *et al.*, (2004) to determine the effects of container type and size on the growth and quality of seedlings of Indian sandalwood (*Santalum album* L.) revealed that survival and overall growth of 6 month old sandalwood seedlings, in terms of height, collar diameter, seedling biomass and root-shoot ratio, were best in root trainers (600 ml) and next best in plastic containers (1500 ml).

In *Dendrocalamus strictus* Nees. and *Bambusa bambos* (L.) Voss. raised in root trainers and polythene bags of different sizes, significant variations were observed in almost all seedling morphological parameters (Gera *et al.*, 2007). Large container seedlings tend to maintain their size advantage over time as compared to smaller container seedlings (Kope *et al.*, 1996; Simpson 1994; Sutherland & Day 1988) while the absolute growth of large stock may be one year ahead of smaller stock (Simpson 1994).

Based on our findings the following combinations are proposed for optimum germination and growth in nursery for *T. bellirica* silt, loam, soil + FYM in sunken beds or 4000 ml-plastic pots. Further research to develop suitable techniques for breaking the seed coat dormancy easily is recommended.

2.1. Seed collection and processing

Fresh and mature fruits of *T. bellirica* were collected in January 2004 from Dugadda (Tehri Garhwal) at 29º 48’ N latitude, 78º 36’ E longitude and 670 m asl, a natural sub-tropical habitat for the species in Garhwal Himalaya. Seeds were extracted from fruits by removing the pulp manually. Thereafter, seed were sun dried for period of one week and packed in jute bags for experimentation. Before sowing, seed viability was checked by floating method. In this method, three replicates of 100 seeds each were soaked in a bucket containing tap water. Non-viable and dead seeds are generally light in weight, therefore, they floated on water and were removed. Other seeds, which settled at the bottom of the bucket, were considered viable. Seed viability varied between 85 and 92 %. Seeds were given a pretreatment to overcome the problem of hard seed-coat. They were soaked in boiled water for a period of 24 h (water was boiled, seeds were dipped into it and then the vessel was removed from the hot plate and kept at room temperature for 24 h) (Todaria & Negi 1992).

2.2. Effect of manure on germination and seedling growth

Four types of manure viz. bio-organic soil enricher (CelrichTM), farm yard manure (FYM), poultry manure (chicken manure) and goat manure were used as medium for germination and growth. CelrichTM is a bio-organic soil enricher made from biodegradable organic substances, mainly of plant origin. It is a rich source of plant nutrients. It is a dark brownish powder having earthy smell. It has a pH of 7.5, and contains 1.8 % organic carbon, 1.75 % nitrogen, 1.25 % phosphorus and 1.20 % potash (Khadder *et al.*, 2009). FYM is prepared using cow dung, cow urine and crop waste and other dairy wastes and is a rich source of nutrients; it contains 13 % nitrogen, 11 % phosphorus and 11 % potash (Kipkosgei *et al.*, 2003). Poultry manure contains 10 % nitrogen, 8 % phosphorus and 5 % potash, and the goat manure contains 1.44 % nitrogen, 1 % phosphorus and 0.22 % potash (Mariakulandai & Minickam 1975). Pretreated seeds were sown in polythene bags containing a mixture of sand, garden soil and the concerned manure in 1:2:1 ratio. A single seed was sown in each polythene bag and for each type of manure 50 polybags were used. The group of 50 polythene bags for each type of manure was divided into 5 rows, each row containing 10 polythene bags, each row was considered as a replicate. Manual irrigation was applied daily.

2.3. Effect of soil texture on germination and seedling growth

Seeds were sown in sandy, sandy loam and silt loam soils. Soil was collected from nearby sites. Soil was placed in polythene bags (soil: FYM, 2:1); 50 pretreated seeds were used for each soil texture treatment with a single seed in each polybag. Thus each texture treatment contained 50 polythene bags. Ten polybags were arranged in each row to make five replicates as described earlier. Manual irrigation was applied daily.

2.4. Effect of type of nursery beds on germination and seedling growth

Three types of nursery bed viz. flat, raised and sunken, each 1 x 1 m in size, were used for this study. Each type of nursery bed was replicated thrice. In each bed, pretreated seeds were sown in three replications with 50 seeds each. FYM was used as germinating/planting material as a mixture of sand: garden soil: FYM in the ratio of 1:2:1. Generally, flat beds were laid out at the ground level, they are easily irrigated and drained out. Raised beds were laid out 10 - 15 cm above the ground level, they are easy to use for seedlings transplanting from the nursery. Sunken beds were laid out 30 - 45 cm deep from the ground level, they help in collecting run-off water from adjoining areas and reduce evaporation loss from the sides (Luna 2006). Manual irrigation was applied daily until the completion of germination and thereafter, weekly till the commencement of the rainy season. Weeding and hoeing were done manually.

2.5. Effect of container volume on germination and seedling growth

Three types of container (different capacity), viz. polybags (1600 ml), plastic pot (4000 ml) and root trainer (350 ml) were used. Seeds were sown in containers having 1:2:1 ratio of sand, garden soil and FYM. Single pretreated seed was sown in each container, 50 replicates were used for each container type. Other details are same as given in the manure treatment. Seed germination under different treatments as described above was recorded up to 90 days from the date of sowing and plumule emergence was taken as the criterion for successful germination. Survival percentage under different treatments was calculated at the time of recording of seedling height and collar diameter

at an interval of 3 months, the data collection continued for one year. Temperature varied from 18.8 ºC to 35.2 ºC and relative humidity from 50 to 90 % during the study period.

2.6. Survival and growth performance of nursery seedlings under field condition

Nursery growth of seedlings can be taken as an indicator of survival and potential growth when planted in field. One year old seedlings raised under different treatments viz. four types of manure, three types of bed, three types of soil, and three types of container were transplanted in an experimental garden in randomized block design in July 2005. Ten healthy seedlings, with three replicates from each treatment (total 30 seedlings) were used for out-planting in the field. Data on growth performance (height and collar diameter) and survival percent of transplanted seedlings were recorded under field condition up to one year at six-month interval.

2.7. Effect of growing media containing ions on seed germination

In an experiment used 4 different manures mixed with soil viz. colic (Bio-organic soil enriched) farmyard manure (FYM) goat and poultry manure There types of nursery beds viz sunken, flat & raised, there types of containers heavy different volumes & there of soil viz silt brown sandy brown and sandy seedlings were raised and studied in nursery for 12 months & planted in field.

Results indication maximum seed germination in FYM & minimum in poultry manure treatment similar maximum germination peasant age was recorded in sunken beds as compared to flat & raised nursery beds. Among different types of contends 4000 ml plastic pots showed maximum germination where as minimum germination percent was in 350 ml root trainers and 1600 ml polybags servile percent was higher for seedlings raised in FYM, sinker beds. Silt brown soil and 4000 ml plastic pods compact to other treatment. The best combination for optimum germination and growth in nursery for *T. bellirica* was silt brown soil FYM sunken beds or 4000 ml plastic pots. Result show that other year of growth height was great in seedlings.

Raised in soil mixed with FYM Sunken beds, silt brown soil & 4000 ml plastic pots as compared to other treatments. However coller diameter was highest in seedling's raised in goat manure, sunken bed silt brown and root trance. Survival percent under field conditions was higher in seedlings raised in FYM & celrich (bio-organic soil enricher) compared to other treatments viz goat & poultry manure. Maximum height & collar diameter were refolded in seedling raised in plastic pots.

3. Material and Methods

Seeds of *Terminalia bellirica* were collected and after proper cleaning and drying they were stored for further analysis. Seed length, width and thickness were recorded for 100 seeds of each seed lot. The seed germination tests were performed in seed germination Laboratory of Silviculture and Forest Management, ICFRE- Arid Forest Research Institute, Jodhpur. With the help of seed counter machine seeds per kilogram were calculated. Laboratory test on the germination response of seeds to pre-germination treatments of Hot water, GA_3 (500 and 1000 ppm) and IBA GA_3 (500 and 1000 ppm) compared to untreated seeds **(**control**)**.

Soaking Hundred seeds in hot water for 15 min. Twenty seeds were also soaked in GA_3 (500 and 1000 ppm) and IBA GA_3 (500 and 1000 ppm) for 6 hours. All the pre-treated and untreated seeds were rinsed thoroughly in

distilled water and were placed in germination tray. The experiment was carried out at room temperature in the laboratory. Seeds were considered germinated upon plumule emergence. The number of seeds that germinated was recorded while the percentage seed germination was calculated. The following procedure was made for different parameters determinations:

3.1. Formulas for various calculations

(a) GP (Germination percentage) = (Total number of seeds germinated/total number of seeds tested) × 100

Final Germination Percentage (FGP %) = Final no. of seeds germinated in a seed lot × 100

The higher the FGP value, the greater the germination of a seed population (Scott *et al.*, 1984).

(b) MGT (Mean germination time) = total (daily germination) × 1 day / total seed sowing

Mean Germination Time (MGT day) =Σf ·x/Σf

f=Seeds germinated on day x

The lower the MGT, the faster a population of seeds has germinated (Orchard, 1977).

- First Day of Germination FDG day = Day on which the first germination event occurred.

Lower FDG values indicate a faster initiation of germination (Kader, 1998).

- Last Day of Germination LDG day = Day on which the last germination event occurred Lower LDG values indicate a faster ending of germination (Kader, 1998).

- Germination Rate Index GRI (%/day) =G1/1 + G2/2 +· · ·+ Gx/x

G1=Germination percentage × 100 at the first day after sowing, G2=Germination percentage × 100 at the second day after sowing.

(c) AVG MGT (Average Mean germination time) = Total MGT/Total number of days.

(d) GV (Germination Value) = (Total MGT/total germination) × (GP%/10).

4. Seeds Germination in Rajasthan seedlots

Seeds of *Terminalia bellirica* were collected from 4 locations in Rajasthan during 2021 to 2023. After proper cleaning seeds were stored for further testing seed size viz. length, width was recorded. Seed analysis shows mean length in the range of 10.91 in Barawada, Pratapgarh and Karamdiya forest nursery, Pratapgarh while highest 16.27 mm in Shahbad, Baran seed lot. Mean seed length in Van Naka, Dhariyawad, Pratapgarh was 11.3 mm. Mean seed width was in the range of 6.8 mm in Barawada. Pratapgarh to 12.23 mm in Shahbad nursery area, Baran. In vennaka, Dhariyawad, Pratapgarh mean seed width was 7.55 mm while in Karamdiya forest nursery, Pratapgarh it was 7.37 mm.

Seeds were treated with Hot water, GA_3 500 ppm and GA_3 1000 ppm with control. Under control condition germination was 30 to 73.3 percent. It was 30 percent in Shahbad, Baran, 40 percent in Karmadiya, forest nursery, Pratapgarh, 60 percent in van naka, Dhariyawad, Pratapgarh and 73.3 percent in Barawada, Pratapgarh. With hot

water treatment germination percentage was 10 percent in Shahbad, Baran 60 percent in karamdiya, Pratapgarh and van naka, dhariyavad, Pratapgarh and 73.3 percent in Barawada, Pratapgarh seed lot.

Using GA_3 500 ppm germination was found 10 percent in Shahbad, Baran, 80 percent in van naka, Dhariyawad, Pratapgarh and karamdiya forest nursery, Pratapgarh. It was 46.7 percent in Barawada, Pratapgarh seed lot. With GA_3 1000 ppm germination was found 40 percent in Shahbad, Baran 60 percent in Karamdiya forest nursery, Pratapgarh and barawada, Pratapgarh. It was highest 80 percent in van naka, Dhariyawad, Pratapgarh. Under lab condition germination percentage was in the range of 10 to 80 percent in different seedlots. Total MGT was in the range of 8.29 to 180 while total GV was in the range of 93.1 to 5400. Average MGT was in the range of 0.38 to 8.18 while average GV was in the range of 4.23 to 305.45.

Under laboratory condition and germination percentage gas found in the range of 10-80 percent in different treatment. Total MGT was highest as 180 in GA_3 500 ppm treated seedlot of Karamdiya Forest nursery, Pratapgarh while it was lowest as 8.29 in GA_3 1000 ppm seed lot of Shahbad nursery, Baran. Total GV was minimum in GA_3 500 ppm seed lot of Shahbad nursery, area as 93.1 while it was highest in GA_3 500 ppm treated seed lot of Karamdiya forest nursery, Pratapgarh seed lot with value 5400.

Average MGT was 0.6 in GA_3 500 ppm treated of seed lot of Shahbad nursery area while it was 8.18 in GA_3 500 ppm treated seed lot of Karamdiya forest nursery, Pratapgarh seed lot.

Average GV value was lowest as 4.23 in GA_3 500 ppm treated seed lot of Shahbad nursery area, Baran while it was highest as 305.45 in GA_3 1000 ppm treated seed lot of Karamdiya forest nursery, Pratapgarh.

5. Conclusion

The present study revealed that there is wide variation in seed size and germination percentage with reference to different treatments. However, GA_3 is found to substantially increase germination percentage. Various parameters viz Average MGT value, Average GV value of different seedlots was calculated. The variation may be due to different edaphic, environmental factors as well as due to genetic make up of seed lots. A detailed study with reference to its reproductive biology need to be studied to better understand the mechanism.

6. Future Recommendations

A detailed study with reference to its reproductive biology needs to be studied to better understand the mechanism. To produce quality seedlings various parameters should be studied thoroughly. Similarly to increase germination and field performance various treatments and edaphic factors, microclimate also need attention.

Declarations

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Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis, and manuscript writing equally.

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Table 1. Effect of Seed Size on Germination in *Terminalia bellirica*

S.No.	Location	GPS	Date of Collection	Seed Analysis		Germination Percentage			
				Mean Length (mm)	Mean Width (mm)	Control	Hot Water	GA_3 500 PPM	GA_3 1000 PPM
1.	**Barawada, Devgarh, Pratapgarh**	N24°10’645” E74°43’312”	24-03-2022	10.91	6.8	**73.3**	**73.3**	46.7	60
2.	**Vaan Naka, Shiyakheda, Dhariyawad, Pratapgarh**	N24°16’635” E74°43’818”	24-03-2022	11.3	7.55	60	60	**80**	**80**

3.	**Karmdiya Forest Nursery, Paratpgarh**	N 24°00.420' E 74°77.123'	22-03-2023	10.9	7.37	40	60	**80**	60
4.	**Shahabad Nursery Area**	N:25.264555 E: 77.203191	03-02-2021	16.27	12.23	30	10	10	**40**
	Mean ± S.D.			12.34 ± 2.62	8.48 ± 2.51	50.82 ± 19.49	50.82 ± 27.92	54.17 ± 33.37	60 ± 16.32

Table 2. *Terminalia bellirica* with Germination Value and Mean Germination Time

S.No.	**Location**	**GPS**	**Date of Collection**	**Germination in Tray**					
				Treatments	**P%**	**Total MGT**	**Total GV**	**Average MGT**	**Average GV**
1.	**Barawada, Devgarh, Pratapgarh**	N24°10'645"	24-03-2022	**Control**	**73.3**	**45.7**	**238.75**	**2.18**	**11.37**
		E74°43'312"		**Hot Water**	**73.3**	**137**	**2021.03**	**6.52**	**96.24**
				GA_3 500 PPM	46.7	78.5	964.49	3.74	45.93
				GA_3 1000 PPM	60	105.87	2597.3	5.04	123.68
2.	**Van Naka, Shiyakheda, Dhariyawad, Pratapgarh**	N24°16'635"	24-03-2022	Control	60	59.20	1480	3.95	98.67
		E74°43'818"		Hot Water	60	59.20	1480	3.95	98.67
				GA_3 500 PPM	**80**	**76**	**2264.67**	**5.07**	**150.98**
				GA_3 1000 PPM	**80**	**74**	**2232**	**4.96**	**148.80**
3.	**Karmadiya Forest Nursery, Pratapgarh**	N 24°00.420'	22-03-2023	Control	40	88.4	2828.8	4.02	128.58
		E 74°77.123'		Hot Water	60	116.4	3957.6	5.29	179.89
				GA_3 500 PPM	**80**	**180**	**5400**	**8.18**	**245.45**
				GA_3 1000 PPM	60	130.2	6720	5.92	305.45
4.	**Shahabad Nursery Area**	N:25.264555	03-02-2021	Control	30	62.3	1027.95	2.83	46.73
		E: 77.203191		Hot Water	10	21.7	303.8	0.99	13.81
				$GA_3$500PPM	10	13.3	93.1	0.6	4.23
				GA_3 1000 PPM	**40**	**8.29**	**229.35**	**0.38**	**10.43**
	Mean ± S.D.				53.95 ± 22.87	78.50 ± 47.04	2114.92 ± 1896.23	3.97 ± 2.17	106.80 ± 87.03

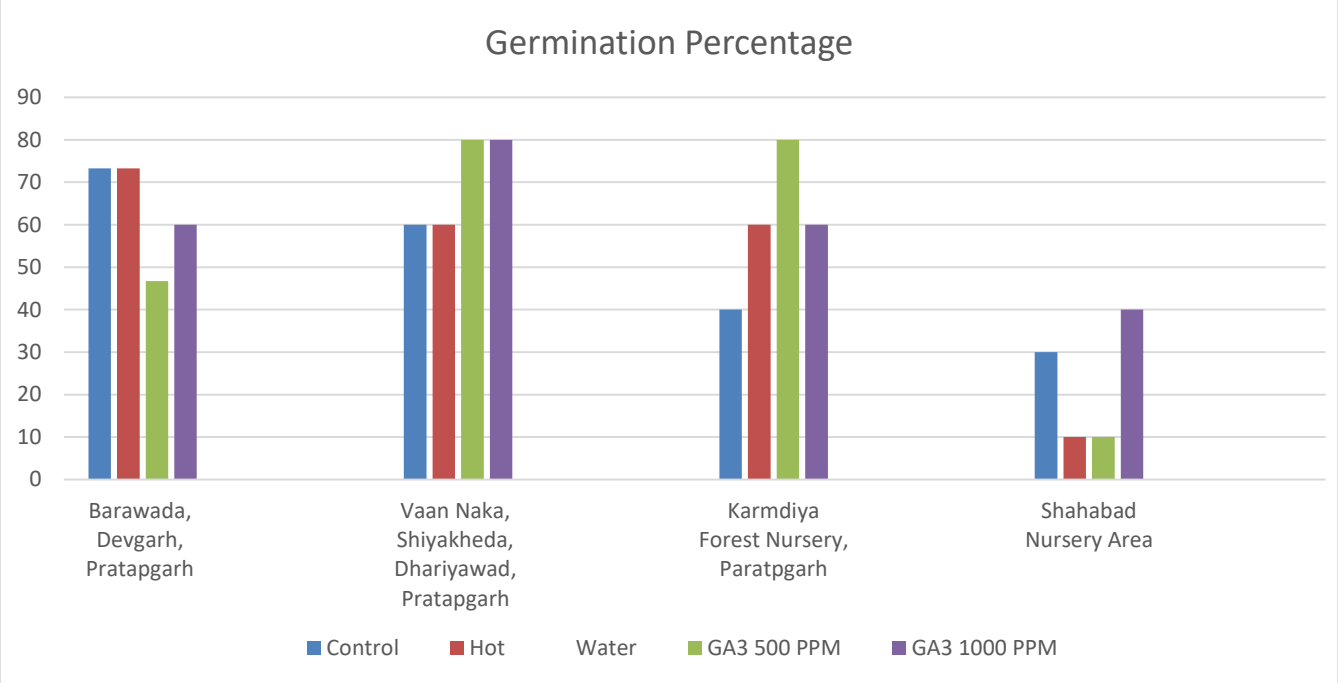

Figure 1. Seed collection of *Terminalia bellirica*

Figure 2. Germination of *Termination bellirica*