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ENHANCING SEED GERMINATION AND SEEDLING GROWTH OF CHEBULIC MYROBALAN (*TERMINALIA CHEBULA* RETZ.) BY APPROPRIATE SOAKING AND SOWING TIME AFTER COLLECTION OF FRUITS

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Abstract

The experiment was designed to enhance seed germination and seedlings' growth of chebulic myrobalan *(Terminalia chebula* Retz.) by proper seed soaking and sowing time after collection of fruits during March to July 2020 at BAU-GPC of Bangladesh Agricultural University, Mymensingh. In total 900 seeds were subjected to five pre-sowing treatments e.g. control or without soaking of seeds (C_1), soaking in water for two days (C_2), four days (C_3), six days (C_4), and soaking cow dung slurry for six days (C_5) and four sowing times: on collection date and after 10, 20 and 30 days of collection respectively. The field experiment was conducted following RCBD with three replications. Seeds soaked in cow dung slurry (C_5) showed the highest germination percentage (85.25%), germination energy (43.41%) and survival percentage (85.65%). Seeds sowing after 10 days of collection (P_2) recorded as the highest germination percentage (81.37%) and germination energy (39.60%) whereas the highest survival percentage was observed after 30 days of collection (P_4) (76.71%). After 30 days of collection, seeds soaked in cow dung slurry for six days (C_5P_4) showed highest germination (93%), survival percentage (92.86%), leaf number (60) and shoot length (32cm). The lowest germination percentage (60%) and leaf number (32.50) was observed in the treatment C_1P_4 and the lowest survival percentage (40%) and shoot height (12 cm) in C_1P_1.

Keywords: Cow dung slurry, Sowing time, Soaking, *Terminalia chebula* Retz.

Introduction

Chebulic myrobalan in Bengali horitoki (*Terminalia chebula* Retz.) belongs to the family 'Combretaceae' is an important medicinal tree species used for several purposes in the Indian sub-continent. It is a medium to large-sized tree distributed throughout the tropical and sub-tropical Asia including China and Tibet (Kannan *et al*., 2009). In Bangladesh, it is found in the hill forest of Chottogram, Gazipur, Tangail and in the dry areas of Sal forest in Mymensingh and Dinajpur (Das and Alam, 2001). This species is widely used in combination with *Terminalia belerica* and *Emblica officinalis* in

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Triphala which is believed to remove toxins and other undesirable accumulations from the body, improve digestion, assimilation and acts as an antioxidant. It is routinely used as traditional medicine by tribes of Tamil Nadu to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection, and wound infections (Dash, 1991). Due to tremendous population pressure, poverty, absence of appropriate government policy and unsustainable utilization of forest resources, the population of this valuable tree species is declining rapidly. The success of plantation programs largely depends on the germination of seeds and the growth of seedlings in the nursery. The nursery technique should be efficient enough to produce an adequate number of quality seedlings within a reasonable time (Hossain *et. al.*, 2013).

Poor natural regeneration due to a lower rate of seed germination has led to the scarcity of this species in their natural habitat (Richa and Sharma, 1994). People don't get interested in raising the seedlings of this species in nursery due to poor germination percentage. Low germination percentage, as well as long time, requirement is believed due to the hard seed coat and thick fleshy pulp of fruits (Luna, 1996). Germination of seeds with hard seed coats could enhance by pre-sowing treatments (Palani *et al.,* 1996). Delayed and irregular germination of seeds in the nursery is a serious constraint of efficient nursery management and plantation establishment. However, literatures which have examined the effect of seed treatments of this medicinal plant are very scarce. So, under the present circumstances, the research work has been designed to explore the seed germination percentage and growth performance of *Terminalia chebula* Retz. Seedlings following different seed soaking and sowing time treatments. The purpose of this study is to enhance seed germination and grow vigorous seedlings of *Terminalia chebula* Retz.

Materials and Methods

The study was carried out at BAU-GPC, Bangladesh Agricultural University, Mymensingh, Bangladesh situated at the intersection of the 23°29´27.3768´´N latitude and 89°25´16.0860´´E longitude during the period from March to July 2020. Optimum matured, 900 uniform and disinfected fruits of Terminalia chebula Retz were collected from BAU campus and fruits were depulped at two ends with a sharp knife in such a way that the embryo was not damaged before soaking or sowing uniform and disinfected fruits of Terminalia chebula Retz were collected from BAU campus and fruits were depulped at two ends with a sharp knife in such a way that the embryo was not damaged before soaking or sowing. The pre-sowing treatments used in the experiment were: C_1 = without soaking seeds; C_2 = Seeds soaking in normal water for two (02) days, C_3 = for four (04) days; C_4 = for six (06) days; C_5 = soaking in normal cow dung slurry for six (06) days. Four sowing times, viz on collection date (P_1), after 10 days of collection (P_2); after 20 days of collection (P_3) and after 30 days of collection (P_4). A Randomized Complete Block Design (RCBD) was adopted for the study with three replications.

Growing media, seed sowing, intercultural operations and protection measures

The sandy loamy soils were collected, sieved (≤3 mm) and mixed with decomposed cow dung at a ratio of 3:1, after that filled in polybags (12.5 cm × 15.25 cm). To facilitate

aeration and proper drainage, several perforations were made in the polybags before filling them. One seed was sown in each polybags by the dibbling method in the germination media with a depth of 0.5 cm. Seeds and seedlings were protected from hot sun, heavy rain, birds, rodent pest, ants, termites and fungal infection. Watering, weeding and loosening of soil were done regularly to obtain maximum germination and growth of seedlings.

Data collection

Germination percentage

Seed germination percentage was calculated using the following formula:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of Seeds sown}} \times 100$$

Germinating energy

Germinating energy (GE) was calculated based on the percentage of the total number of seeds that had germinated when the germination reached its peak (Zazai *et al.*, 2018).

$$\text{Germinating energy (GE)} = \frac{\text{Number of seeds germinated up to time of peak germination}}{\text{Total number of seeds sown}} \times 100$$

Survival percentage

The survival percentage of each treatment was recorded at 120 days after seed sowing. The survival percentage was calculated by using formula as given (Zazai *et al.*, 2018) below:

$$\text{Survival percentage of seedlings} = \frac{\text{Number of survived seedlings}}{\text{Total number of seedlings}} \times 100$$

Shoot height, leaf number and biomass

At the end of the experiment, all seedlings were measured for height and a total number of leaves in each seedling was counted. Ten seedlings from each replication were randomly selected and uprooted very carefully to estimate the seedling biomass. The uprooted seedlings were then separated into leaves, shoot and root components and were dried in an electric oven at 70°C until the constant weight was obtained for studying biomass productions in different pre-sowing treatments.

Vigor index

Seedling vigor index (VI) was calculated according to Abdul Baki and Anderson (1973) as germination percentage × (shoot length + root length).

Statistical analysis

Data were statistically analyzed by using computer software Microsoft Excel and Statistix.10 to explore possible treatment variations. The Least Significant Difference values were used for the analysis.

Results and Discussion

Germination percentage

Significant variation was observed on the germination percentage of *Terminalia chebula.* The highest germination percentage (85.25%) was ascertained from seeds soaked in cow dung slurry for six days and the lowest (63.00%) was recorded from control (Table 1). Hossain *et. al*. (2005a) noticed that seeds soaking in water for various periods significantly enhanced seed germination and seedling growth of *Terminalia chebula* and the result support the preset findings. Vigorous and higher survival percentage was observed in seeds treated by cow dung. The highest germination percentage (81.37%) was observed when seeds were sown after 10 days of collection and the lowest (70.20%) in seeds sown in 30 days after collection (Table 1). For combined effect of several pre-sowing treatments and seeds sowing time, the highest germination percentage (93.00%) was noticed in sown seed after 30 days of collection with six days soaking in cow dung slurry and the lowest germination percentage was recorded (60) from no treatment and sown seeds after 30 days of collection (Fig. 1). Our results are in conformity with similar study in other tree species like *Zizyphus mauritiana* (Singh *et al.*, 2004). Usually the seeds of legumes with hard seed coats show enhanced germination when various pre-sowing treatments are used (Ajiboye *et al.*, 2009).

Germination energy

Germination energy varied from 28.33% to 43.41% among the treatments. The highest germination energy was found in soaking in normal cow dung slurry for six days (43.41%) and the lowest 28.33% was in Control (Table 1). In case of sowing time after seeds collection, the highest germination energy (39.60%) was in seeds which were sown after 10 days of collection and lowest 34.46% was in 20 days of collection (Table 1). For combined effect of several pre-sowing treatments and seeds sowing time, the highest germination energy (53.67%) was observed in C_5P_4 followed by C_5P_2, C_3P_4 and C_4P_2 which was significantly higher than control. The lowest germination energy was recorded (20.00%) from C_1P_4 (Fig. 1). The findings of the present study showed the similarity of Jackson (1994) that germination speed, germination percentage, and seedling growth of *T. chebula* Retz. Significantly increase when seeds are soaked in water for 48h compared to without soaking. The results of the present study are consistent to Rashid *et al*., 1990.

Survival percentage of seedlings

The highest survival percentage of seedlings was found in soaking seeds in normal cow dung slurry for six (06) days (85.65%) and lowest (47.50%) was in Control (Table 1). Sowing times according to days after collection of seeds, the highest survival percentage (76.71%) was noted in seeds sown after 30 days of collection and the lowest (65.83%) was in seeds sown on collection date (Table 1). For combined effect of several pre-sowing treatments and seeds sowing time, the highest survival percentage (92.86%) was observed in C_5P_4 and the lowest was (40.00%) recorded from C_1P_1 (Fig. 1). Vijayalakshmi and Renganayaki (2017) conducted study about survival percentage of

Pterocarpus santalinus L. that the week acids, digestion of thin and strong veins by the microbes present in cow dung, both together might have resulted in the opening of pores; entry of growth stimulants of cow dung and adequate water through the opened pores might have resulted in positive performance.

Table 1. Effect of seed soaking and collection time on germination, survival percentage, leaf number and shoot length of *Terminalia chebula* Retz

Treatment	Germination percentage	Germination energy (%)	Survival percentage	Number of Leaf	Shoot height (cm)
C_1	63.00	28.33	47.50	36.00	15.04
C_2	67.75	37.71	59.13	42.60	20.06
C_3	74.24	39.99	82.13	41.11	24.00
C_4	81.91	32.83	84.19	43.65	22.55
C_5	85.25	43.41	85.65	51.38	24.75
$LSD_{0.01}$	1.64	1.08	1.45	1.45	1.43
Level of significance	**	**	**	**	**
P_1	71.80	35.70	65.83	38.70	18.60
P_2	81.37	39.60	69.82	47.51	23.00
P_3	73.40	34.46	74.19	42.45	20.18
P_4	70.20	36.66	76.71	43.90	25.40
$LSD_{0.01}$	1.35	0.97	1.30	1.38	1.28
Level of significance	**	**	**	**	**

** = Significant at 1% level of probability

C_1 = without soaking seeds; C_2 = seeds soaking in normal water for two (02) days, C_3 = for four (04) days; C_4 = for six (06) days; C_5 = soaking in normal cow dung slurry for six (06) days.

P_1 = Seeds sowing on collection date; P_2 = sowing after 10 days of collection; P_3 = sowing after 20 days of collection; P_4 = sowing after 30 days of collection

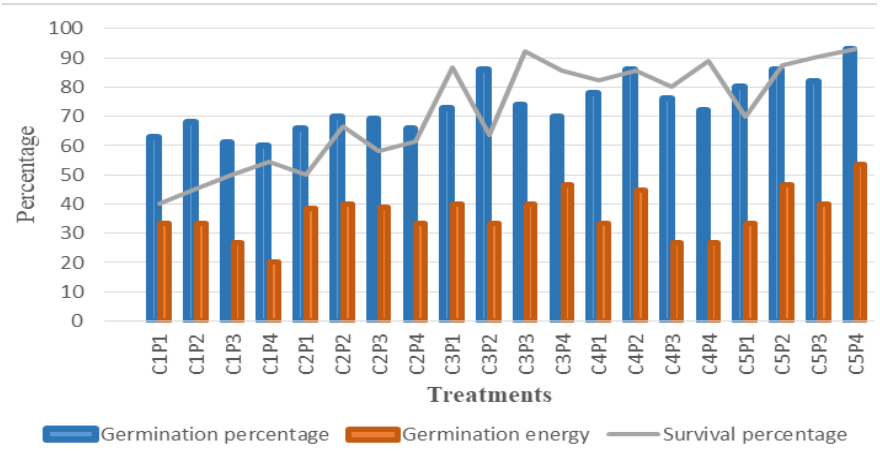

Fig. 1. Germination percentage, germination energy and survial percentage under several pre-sowing treatments and sowing time of *Terminalia chebula*

Number of leaves

The highest number of leaves (51.38) of the seedlings was found in soaking seeds in normal cow dung slurry for six (06) days and the lowest (36.00) in control (Table 1). For several seed sowing time, the highest leaf number 43.90 was in seeds were sown after 10 days of collection and the lowest 38.70 in seeds sown in collection date (Table 1). In case of several pre-sowing treatments and seeds sowing time, the highest leaf number was in C_5P_4 (60) and the lowest 32.50 was recorded in C_1P_4 (Fig. 3). Hossain *et al.,* (2005b) reported that average number of leaf per seedling of *Terminalia bellirica* varied significantly when the seeds were treated. Hossain (2006) mentioned that the number of leaves was positive response by seed treatment of *Gliricidu sepium* (Jacq.).

Shoot height

Shoot height of the seedlings developed under treatments was the highest 24.75 cm in soaking seeds in normal cow dung slurry for six (06) days and the lowest (15.04 cm) in control (Table 1 and Plate 1). Hossain *et al.,* (2013) observed the maximum total height of the seedlings of *Terminalia chebula* Retz. in depulped seeds soaked in cold water for 72 hrs. For several seed sowing times, the highest shoot height 25.40 cm was in P_4 and the lowest 18.60 cm was in seeds sown in collection date (Table 1). For combined effect of several pre-sowing treatments and seeds sowing time, the highest shoot height (32.00 cm) was observed in C_5P_4 followed by C_5P_2, C_3P_2 and C_3P_4 (Fig. 2). The lowest shoot height of seedlings was recorded (12.00 cm) from C_1P_1. Nadukeri *et al.,* (2018) investigated that seeds of *Annona reticulata* L soaked in water for 96 hours can be used to induce better germination and seedling growth. The rapid growth seedlings in cow dung slurry treatment might be due to the presence of growth promoting substances (auxin) and nutrition (Shinde and Malshe 2015).

Root length

Root length of the seedlings was the highest (28.00 cm) when seeds were soaked in normal cow dung slurry for six (06) days and the lowest in control (22.05 cm). For seed sowing times, the highest root length (26.05cm) of seedlings were observed when seeds were sown after 20 days of collection and the lowest 22.65 cm was in collection date (Table 2). For combined effect of several pre-sowing treatments and seeds sowing time, the highest root length (36.50 cm) was observed in C_5P_4 and lowest root length was recorded (18.00 cm) from C_1P_1 (Fig. 2).

Vigor index

The highest vigor index of the seedlings under treatments was the highest in soaking seeds in normal cow dung slurry for six (06) days (4496.94) and the lowest was in control (2336.67). For several seed sowing times, the highest vigor index (3845.10) was in sown seeds after 30 days of collection and the lowest was found in collection date (2961.75). For combined effect of several pre-sowing treatments and seeds sowing time, the highest vigor index (6217.00) was observed in C_5P_4 and the lowest was recorded

(1890.00) from C_1P_1. Pampanna and Sulkeri (2001) mentioned that the use of bio-regulators by cow dung enhance seed germination and seedling vigor of sapota.

Table 2. Effect of seed soaking and collection time on vegetative growth of *Terminalia chebula* Retz

Treatment	Root length (cm)	Vigor Index	LDW (g)	SDW (g)	RDW (g)
C_1	22.05	2336.67	1.41	2.12	0.84
C_2	23.84	2974.23	1.97	2.96	1.02
C_3	26.00	3712.00	1.81	2.86	0.95
C_4	25.00	3894.70	1.94	3.05	1.12
C_5	28.00	4496.94	2.04	3.37	1.17
$LSD_{0.01}$	1.67*	179.75	0.09	0.09	0.04
Level of significance	*	**	**	**	**
P_1	22.65	2961.75	1.51	2.48	0.94
P_2	24.25	3845.10	2.04	3.22	1.12
P_3	26.05	3393.28	1.74	2.65	1.02
P_4	25.50	3573.18	1.96	3.01	1.07
$LSD_{0.01}$	1.49	160.77	0.08	0.08	0.04
Level of significance	*	**	**	**	**

** = Significant at 1% level of probability

C_1 = without soaking seeds; C_2 = Seeds soaking in normal water for two (02) days, C_3 = for four (04) days; C_4 = for six (06) days; C_5 = soaking in normal cow dung slurry for six (06) days.

P_1 = Seeds sowing on collection date; P_2 = after 10 days of collection, P_3 = after 20 days of collection and P_4 = after 30 days of collection

LDW= leaf dry weight, SDW= shoot dry weight, RDW= root dry weight

Fig. 1. Growth performances of *Terminalia chebula* Retz. seedlings germinated under several sowing time

Biomass production

Statistically significant difference was found on dry weight of leaves, shoot and root of *Terminalia chebula Retz.* seedlings (Fig. 3). The highest leaf dry weight (2.04 g), shoot dry weight (3.37 g) and root dry weight (1.13 g) were in the treatment of cow dung slurry for six (06) days and the lowest in control (1.41 g, 2.12 g, 0.84 g respectively). For several seed sowing times, dry weight of leaves, shoot and root of *Terminalia chebula Retz.* seedlings were found the highest 2.04, 3.45, 1.42 g, respectively in the treatment sown seeds after 10 days of collection and the lowest recorded in seeds sown in collection date (1.51, 2.48 and 0.94 g respectively). The results of some study regarding the seedling growth and biomass production of *Terminalia chebula Retz.* seedlings mentioned by Hossain *et al.*, 2005a that the leaf dry weight, and shoot dry weight of *Terminalia chebula Retz.* seedlings were significantly enhanced by soaking of seeds in water.

The highest dry weight of leaves, shoot and root of *Terminalia chebula* Retz. seedlings under combined effect of soaking duration and seeds sowing times were found 2.74 g, 4.36 g and 1.34 g respectively in the treatment C_5P_4 and the lowest in C_1P_1 (Fig. 3). Kumar (2016) reported that the highest dry weight of root and shoot of *Terminalia bellirica* were recorded when seeds were soaked in cow dung slurry for 72 hrs. Hossain *et al.*, (2013) observed the average biomass of *Terminalia chebula* Retz. seedlings in depulped seeds soaked in water for 48 hours.

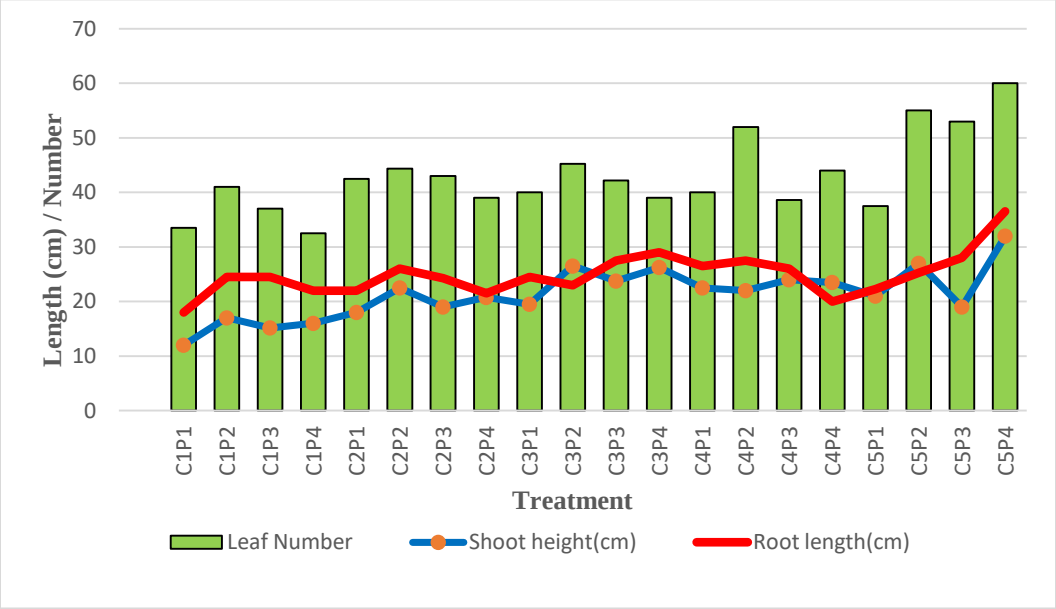

Fig. 2. Leaf number, shoot length and root length under several treatments of *Terminalia chebula Retz.*

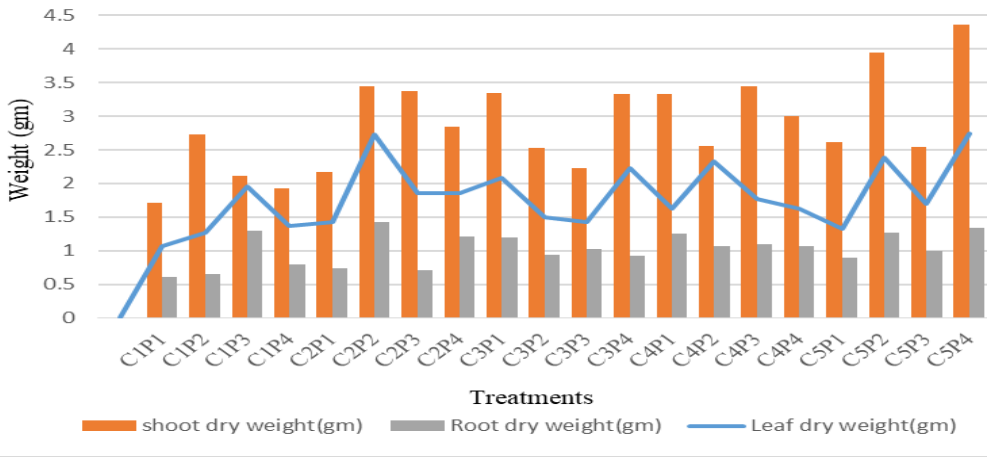

Fig. 3. Shoot dry weight, root dry weight (gm) and leaf dry weight of *Terminalia chebula*

Conclusion

From the study, it can be concluded that after 30 days of fruit collection, seeds soaked in cow dung slurry gave better germination rate (93%) and vigorous seedling growth of *Terminalia chebula* Retz. Considering the initial seedling growth performance, vigor index and dry matter of the seedlings, seeds soaked in cow dung slurry for six (06) days may be recommended for this species for maximum seed germination and seedling growth performance in the nursery. In summary, the results have suggested that seeds soaking in cow dung slurry for six days after 30 days of fruit collection can be used for propagation of *Terminalia chebula* Retz.

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