INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 2, p. 454-463, 2020

RESEARCH PAPER OPEN ACCESS

A response of tropical tree *Terminalia arjuna* (arjun) [Combretaceae-Myrtales] seeds and seedlings towards the pre-sowing seed treatment in forest nursery of Sargodha, Pakistan

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Key words: *Terminalia arjuna*, Combretaceae, Myrtales, Tropical tree spp, pre-sowing seed treatment, nursery raising, chemical treatment, Pakistan.

http://dx.doi.org/10.12692/ijb/16.2.454-463 Article published on February 24, 2020

Abstract

A tropical evergreen woody tree *Terminalia arjuna*Roxb. (*T. arjuna*), native to India and commonly found along the river banks and canals, has the high medicinal value especially for heart related diseases. A present study was conducted over the period of 4 months to check the pre-sowing seed treatment effects on *T. arjuna* seeds in nursery and to identify which treatment was better to use. Seeds were collected from three different sites (S1, S2, S3). Best results using analysis of variance (ANOVA) were expressed by the seeds treated with $conc.\ H_2\ SO_4$ for 10 minutes and Gibberellic Acid (G.A) 75mg/l for 24hours. Maximum mean seed germination was (7.66 ± 0.45) at T5, maximum mean seedling survival was (6.00 ± 0.32) at T5, maximum mean seed germination % was (76.66 ± 5.29) at T5, maximum mean seedling survival % was (63.33 ± 4.25) at T7 and maximum mean germination energy % was (79.64 ± 7.78) at T5. Seeds collected from S2 site performed significantly better than S1 and S2. Use of $conc.\ H_2\ SO_4$ and Gibberellic Acid (G.A) effected the growth of *T. arjuna* significantly.

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Introduction

Terminaliaarjuna Roxb. (T. arjuna) is an evergreen forest woody tree, belonging to the family Combretaceae. It produces large orthodox seeds as propagules (Chandrashekar *et al.*, 2013),commonly found in tropical and sub-tropical parts of India (Kumar and Prabhakar, 1987) and also plays an important role in sericulture industry (Orwa, 2009). AntheraeamylittaTasar silkworm really like T. arjuna leaves as food (Dutta, 1995). In Indian mythology Arjun tree has great importance(Agarwal, 1981).

Literature history described that around last three centuries T. arjuna stem bark has been using for preparing ayurvedic medicines(Kumar and Prabhakar, 1987), primarily as a cardiac tonic and also as a potent antioxidant for ischemic heart diseases (Sultana *et al.*, 2007).From last few decades many clinical researches have been reported the significant efficiency for the patients with ischemic heart disease, hypertension, and heart failure, beside it also has potential for ethnomedicinal importance (Bone, 1996; Kapoor, 1990; Maulik and Talwar, 2012). Under natural conditions in India Terminalia arjuna grows along streams and rivers, from sea-level up to 1200 m altitude. It grows well on fertile, neutral (pH 6.5–7) soils, especially loose, moist, alluvial loam with good water supply and drainage (Atal and Kapur, 1982; Handa and Kaul, 1996; Nadkarni and Nadkarni, 1976).

Terminalia arjuna can be propagated by seed, and also by root-suckers, stumps and air-layering (Kumari, 1998.). But using the traditional method, propagation of T. arjuna is very difficult because of its hard seed coat and viability. So that cutting and air-layering methods were used for this plant (Pandey *et al.*, 2006). This is one of the main reasons that why most of the peoples don't want to raise the T. arjuna nurseries because of its low and very slow germination rate under normal conditions but if seeds are treated with chemicals like sulfuric acid (H2SO4) and growth hormones like gibberellic acids (C19H22O6) before sowing then it can be very helpful to increase the germination rate (Naik *et al.*, 2010).

Moisture level of the soil is one of the key factor which directly influencing germination and speed of germination at the seed stage (Foster, 1986; Khurana and Singh, 2004), amount of water uptake by a seed depend upon its seed size (Benleyand Black, 1978; Harper *et al.*, 1970) and area of seed contacting with soil. Interior of the seed contain food and nutrients for the survival and also to energize the seed for germination and survival for future growth.

Seed with bigger size and weight contain more reserve food than smaller one (Athaya, 1985.). So bigger the seed size and weight, more chances of seed survival will be possible. Under laboratory conditions, higher germination rate has been reported for the large seeds of T. arjuna compared with medium and small seeds (Negi and Todaria, 1997).

One of the main purposes to select this topic for research was that no research evidence founded on internet relate to *Terminalia arjuna* to check out the effect of pre sowing seed treatment on seeds regarding to Pakistan. So, under the present circumstances the research work has been designed to explore the seed germination period, Survival seedling percentage, germination percentage, germination energy percentage and initial growth performance of *T. arjuna* under a number of easily applicable low-cost pre-sowing treatments.

The main objectives were to check out the seed germination and seedling growth attribute of *Terminalia arjuna* after pre-sowing seed treatments and to identify the best possible treatments which can be used to establish *Terminalia arjuna* nursery specially in Pakistan. And also, to check the effect of seed collection sites on the seed germination and seedling growth.

Materials and methods

Materials used during experiment

270 viable seeds, 5litre water, 400ml Gibberellic acid (G.A), 60ml Sulphuric acid ($conc.\ H_2\ SO_4$), Burner, Fertile soil, Farm yard manure (FYM), p-bags, 10 500ml Beakers, 10 1000ml Beakers.

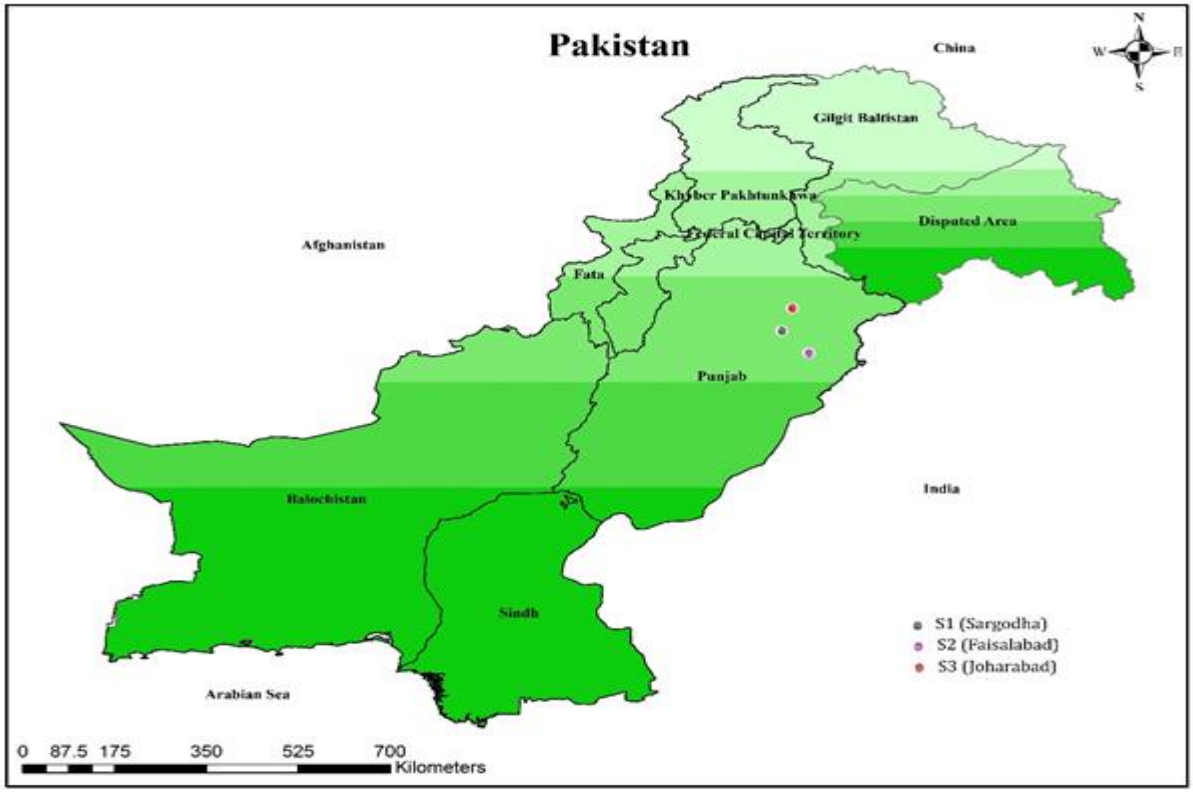

Fig. 1. Seed collection sites marked with three different colors, grey representing S1 (Sargodha), pink representing S2 (Faisalabad) and red representing S3 (Joharabad). All sites are present in Punjab, one of the provinces of Pakistan.

Experimental site

Whole experiment was designed in forest nursery of University College of Agriculture, University of Sargodha (UCA, UOS), Punjab, Pakistan during year 2018 from April to September.

Preparation of growing media

The soil for the experiment was collected from the canal situated near to the forest nursery. The soil was sandy loam (Rana, 2012). The soil was cleaned thoroughly by removing unwanted material like plant parts, stones and weeds. To increase the fertility of the soil, soil media for the experiment was prepared by using, sand, vermicompost (VC) and well-decomposed farmyard manure (FYM). All these ingredients were mixed in proper proportions. The composition for the growing media comprised Soil: Sand: VC: FYM (1: 1: 1: 1).

Seed collection

Seeds were collected from three different localities described in (table-1). Total 500 seeds were collected from all those three sites. Collected seeds were subjected to grading. Physically healthy and fresh seeds with average weight ranging (2.3g – 4.6g) (Negi and Todaria, 1997) were selected to carry out experiment. After grading only 270 seeds were selected which further used in experiment.

Selected seeds were placed in open space for 1 month before applying any pre-sowing treatment for drying.

Pre-sowing seed treatments

Following were the treatment methods used for pre-sowing seed treatment as shown in (Table-2).

Sowing of seeds

The seeds were sown in polythene bags 8 x 4 inches (containing media in same proportion) in June 2018. Light irrigation was provided in the polythene bags after sowing and timely weeding and hoeing was done until the harvesting of seedlings. Watering of polythene bags was done as per requirement. Sowing of the seeds were done in such a way that their

contact area with soil had maximum (Harper *et al.*, 1970).

Collection of data

Temperature, Humidity and Rainfall data were counted (Figure-2) every day before sowing to the end of the experiment (May- September, 2018).

After 2nd week of sowing, seed germination was observed and counted until the germination process stopped. Seed survival data was counted in (September, 2018) after 14-weeks of sowing.

Following formulas were also used during data analysis:

Seed germination % (SG%) equation

Germination percentage was calculated by dividing the total number of seeds germinated by total number of seeds sown and multiplied by 100.

$$Seed\ Germination\ \% = \frac{Total\ no.\ of\ seed\ germinated}{Total\ no.\ of\ seeds\ sown} \times 100$$

Survival seedling % (SS%) equation

The survival seedling on each treatment was calculated at 100 days after seed sowing. The survival percentage was calculated by using formula as given below.

$$Survival\ Seedling\ \% = \frac{No.\ of\ survived\ seedlings}{Total\ no.\ of\ seedlings} \times 100$$

Germination energy % (GE%) equation

Germinating energy (GE) was calculated on the basis of the percentage of the total number of seeds that had germinated when the germination reached its peak.

$$Germination\ Energy\ \% = \frac{No.\ of\ seeds\ germinated\ upto\ the\ time\ of\ peak\ germination}{Total\ no.\ of\ seeds\ sown} \times 100$$

Randomized Complete Block ANOVA with factors "pre-sowing treatments and seed collection site". Regression analysis was used to check the interrelationship between (MSG% and MGE%) and (MSS% and MGE%). 2-tail t-test was also used to checked the significance level of different variables. All data were analyzed using *Statistix 8.1* and demographical work was done using *Origin soft. 2016* and *Microsoft excel 2016*.

Results and discussion

The experimental results on the effect of pre-sowing seed treatments on different factors are presented in Table-3. Pre-sowing seed treatment was performed and in response significant results produced.

Table 1. Chart of seed collection sites.

Title	Site Name
S1	Sargodha
S2	Faisalabad
S3	Joharabad

There was significant different in mean seed germination (F=5.62, df=8, *P*=0.001). Seed treated with T5 performed best (7.66 ± 0.45)and seeds without treatment To performed poorly (3.00 ± 0.95) while seeds treated with T7 (6.66 ± 0.66) and T4 (6.00 ± 0.11) also performed well.Mean seed germination % differentiate significantly (F=5.96, df=8, *P*=0.001), with the highest percentage on T5 (76.66 ± 5.29 %) and lowest percentage on To (30.00 ± 6.98 %) and seeds also performed well treated with T7 (66.66 ± 6.58) and T4 (60.00 ± 7.54).

Table 2. Chart of pre-sowing treatments.

Title	Name of Treatment	Duration	No of Seeds
To	Control		10+10+10=30
T1	Cold Water (10°)	36 hours	10+10+10=30
T2	Hot water (100°)	8 minutes	10+10+10=30
T3	Hot water (100°)	10 minutes	10+10+10=30
T4	Sulphuric Acid / 99.9% $conc.H_2SO_4$	8 minutes	10+10+10=30
T5	Sulphuric Acid / 99.9% $conc.H_2SO_4$	10 minutes	10+10+10=30
T6	Gibberellic Acid (G.A) 50mg.l^{-1}	18 hours	10+10+10=30
T7	Gibberellic Acid (G.A) 75mg.l^{-1}	24 hours	10+10+10=30
T8	Cow dung (1day old)	32 hours	10+10+10=30

For experimental results seedling survival was the 2nd very important factor after seed germination. There was significant difference in mean seedling survival (F=7.92, df=8, *P*=0.000), which exhibited the great difference within the seedling survival on different treatments. Peak performance was showed off by the seeds treated with T5 (6.00 ±0.32), lowest by the seeds without treatment To (1.33 ± 0.92) and seeds treated with T7 (5.33 ± 0.54) and T4 (4.33 ± 0.53) also performed well. And the results for mean seedling survival % was also significant (F=5.24, df=8, *P*=0.002), with the highest percentage at T7 (79.64 ± 7.7 8%), T5 (76.85 ± 6.32 %), lowest at T1 (34.44 ± 6.64 %) and midrange at T4 (72.22 ± 7.28 %).

Table 3. Response of Seeds and Seedlings growth towards the Pre-sowing seed treatment, Mean ± SE (*P*≤0.05).

Treatments	MSG ± SE	MSS ± SE	MSG% ± SE	MSS% ± SE	MGE% ± SE
To	3.00 ± 0.95^{C}	1.33 ± 0.92^{C}	30.00 ± 6.98^{C}	44.44 ± 7.55^{C}	26.66 ± 6.85^{C}
T1	3.33 ± 0.12^{C}	1.66 ± 0.81^{C}	33.33 ± 5.55^{C}	34.44 ± 6.64^{C}	30.00 ± 5.29^{C}
T2	3.66 ± 0.35^{B}	1.66 ± 0.66^{C}	36.66 ± 6.59^{C}	42.22 ± 7.90^{B}	30.00 ± 6.34^{C}
T3	4.00 ± 0.56^{B}	1.66 ± 0.62^{C}	40.00 ± 5.52^{B}	41.11 ± 5.34^{B}	36.66 ± 5.65^{C}
T4	6.00 ± 0.11^{B}	4.33 ± 0.53^{A}	60.00 ± 7.54^{A}	72.22 ± 7.28^{A}	36.66 ± 6.95^{C}
T5	7.66 ± 0.45^{A}	6.00 ± 0.32^{A}	76.66 ± 5.29^{A}	76.85 ± 6.32^{A}	63.33 ± 4.25^{A}
T6	5.00 ± 0.85^{B}	3.33 ± 0.67^{B}	50.00 ± 7.58^{B}	65.55 ± 8.75^{A}	40.00 ± 6.57^{B}
T7	6.66 ± 0.66^{A}	5.33 ± 0.54^{A}	66.66 ± 6.58^{A}	79.64 ± 7.78^{A}	50.00 ± 4.35^{A}
T8	4.33 ± 0.65^{B}	2.33 ± 0.72^{B}	43.33 ± 6.55^{B}	50.00 ± 8.27^{B}	40.00 ± 5.25^{B}
Grand Mean	4.85	3.07	48.51	56.27	39.25

*MSG=Mean Seed Germination. *MSS=Mean Seedling Survival. *MSG%=Mean Seed Germination%. *MSS%=Mean Seedling Survival%. *MGE%=Mean Germination Energy%. *A*B*C=Different level of significance at (*P*≤0.05).

Germination energy % was also an important factor to determine the growth percentage with the significant difference (F=4.70, df=8, *P*=0.004). Maximum percentage range was performed by the seeds treated with T5 (63.66 ± 4.25 %), minimum was performed by the seeds without treatment To (26.66 ± 6.85 %) and midrange was performed by the seeds treated with T7 (50.00 ± 4.35 %).

For getting better understanding about the relationship and interaction between mean germination energy % vsmean seed germination % and mean germination energy % vs mean seedling survival %, a scatterplot technique was used (Figure-3).

The relationship between MGE% and MSG% was significantly positive (y=0.68x + 6.03, R^2 = 0.76, *P*≤0.05), as linear line indicating upward positive trend soMSG% strongly affected by the MGE%. The 5th week after sowing was time of peak germination at that time maximum seed germination was recorded with highest value for the seed's treatment with T5 and lowest at To. The percentage growth rate of SG% (M=48.51, df=19.35, n=27) was hypothesized to be greatlyaffected by GE%(M=39.25, df=15.17, n=27). This hypothesis was significant, t(49)=1.95, *P*=0.05, (*P*≤0.05).

The conditions for the MSS% in relation with MGE% was also same and significant (y = 0.55x + 7.96, R^2 = 0.55, *P*≤0.05), as linear line indicating upward positive trend. Those seeds which were germinated faster within 1-5th week after germination expressed the best results for the MSS%. Maximum value was recorded at T5 and lowest at T1. The percentage growth rate of SS% (M=56.27, df=20.25, n=27) was hypothesized to be greatly affected by GE % (M = 39.25, df = 15.17, n = 27). This hypothesis was significant, t (48) = 3.49, *P* = 0.001, (*P*≤0.05).

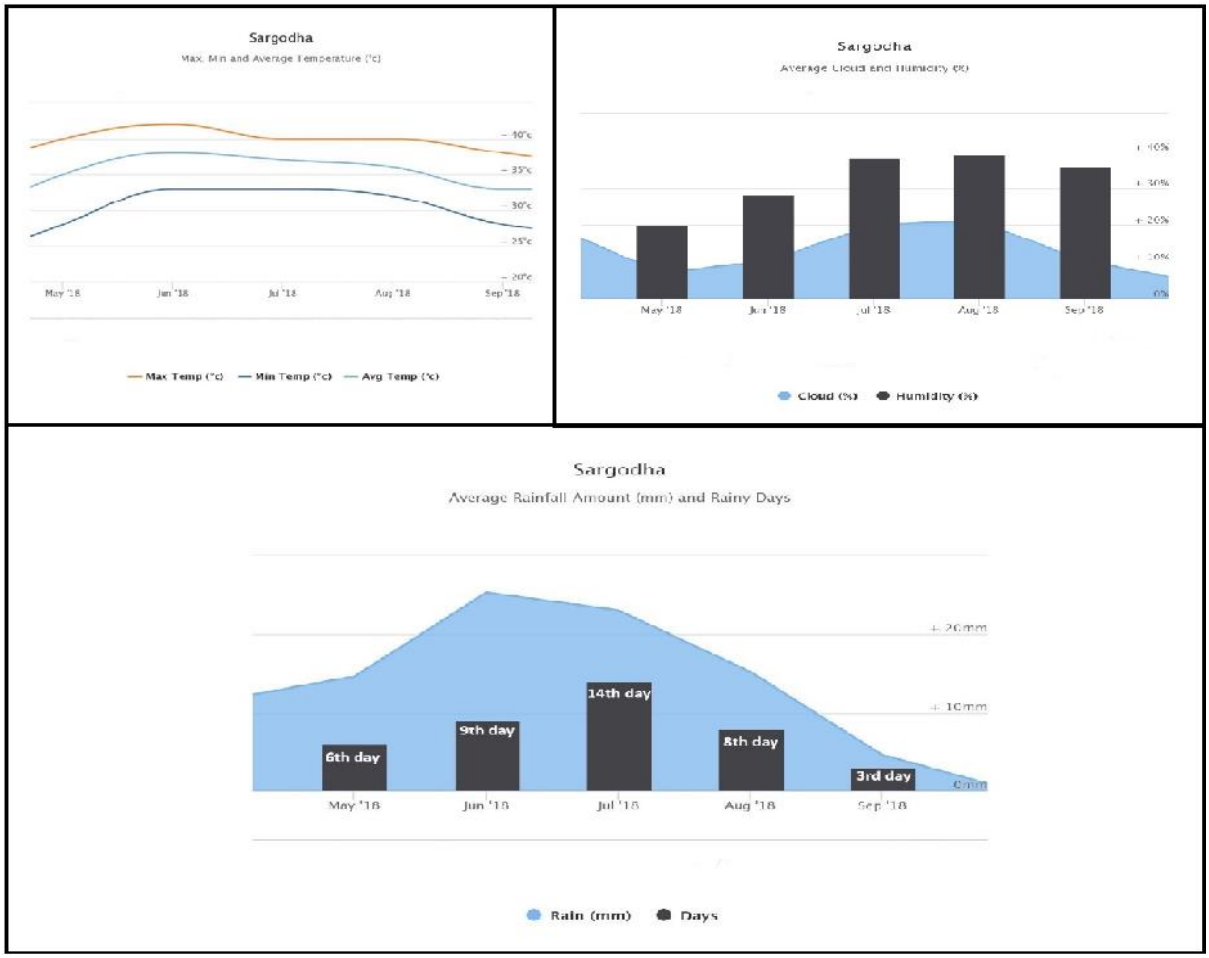

Fig. 2. Data from May to September, 2018 of Sargodha describing; average temperature with minimum and maximum range, average humidity and cloud formation % and average rainfall and rain days of each month.

Seed collection site also had a significant role in the whole experiment. As seeds were collected from three different sites as describe before having almost same climatic conditions. Effect of seed collection sites on seed germination % and seedling survival % were interesting and unique (Figure-4). In both figures A & B of figure-3, left side three colors are indicating sites (S1-S2-S3) and on right side of both figures different colors indicating seed germination % and seedling survival % under pre-sowing treatment. Results for the S1 were neither convincing nor significant ($F = 4.47$, df = 1, $P = 0.06$). The results for seed germination % for S1 were as following; maximum (70 ± 5.63 %) at T7, minimum (20 ± 5.63 %) at T0 and T1 and mid-range (60 ± 5.63 %) at T5. And results for seedling survival % for S1 were as; maximum (75 ± 6.9 %) & (71.42 ± 6.9 %) at T4& T7, minimum (10 ± 6.9 %) at T1 and mid-range (66.67 ± 6.9 %) at T5. In case of S2 the results were significantly different ($F = 5.05$, df = 1, $P = 0.05$) from rest of other two sites. The results for seed germination % for S2 were as; maximum (90 ± 8.01 %),(80 ± 8.01 %) & (80 ± 8.01 %) at T5, T4 & T7, minimum (30 ± 8.01 %) at T0, T2 & T3 and mid-range (60 ± 8.01 %) at T6 & T8. And the results of seedling survival % for S2 were as following; maximum (88.89 ± 7.56 %) & (87.5 ± 7.56 %) at T5 & T7, minimum (33.33 ± 7.56 %) at T0, T2 & T3 and mid-range (75 ± 7.56 %) & (66.67 ± 7.56 %) at T4, T6 & T8. And the results for S3 were not-significant ($F = 3.33$, df = 1, $P = 0.11$). The seed germination % for S3 was as; maximum (80 ± 5.15 %) at T5, minimum (30 ± 5.15 %) at T1 and mid-range (60 ± 5.15 %) & (50 ± 5.15 %) at T2 & T3, T4, T7. And seedling survival % for S3 was as; maximum (80 ± 6.37 %) & (75 ± 6.37 %) at T6, T7 & T5, minimum (33.33 ± 6.37 %) at T1 and mid-range (66.67 ± 6.37 %) & (60 ± 6.37 %) at T4 & T2 respectively.

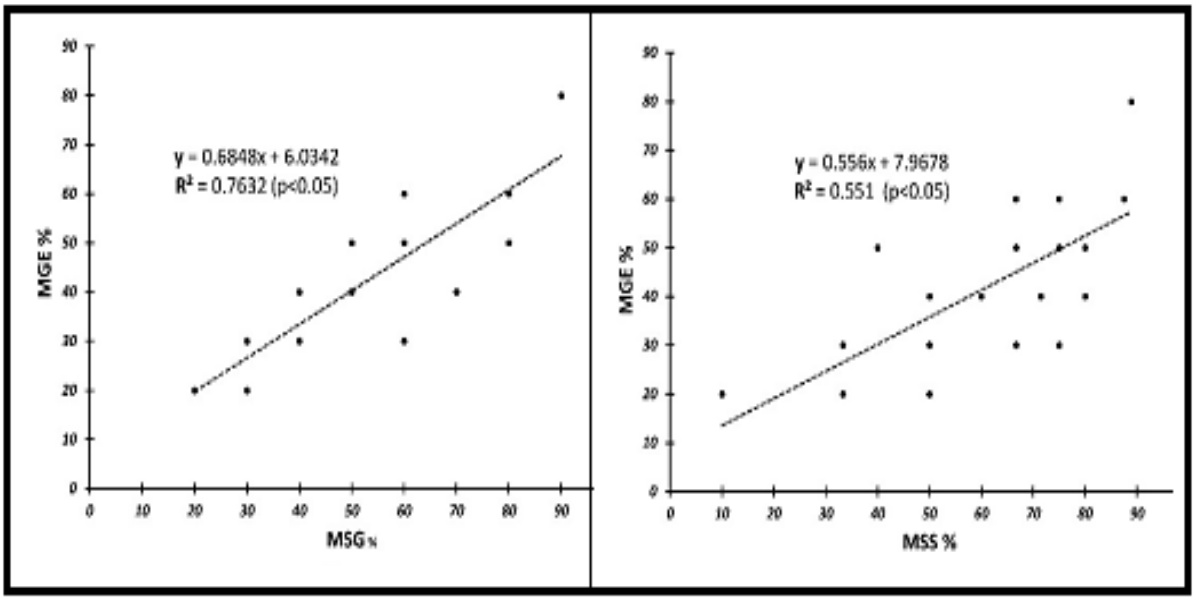

Fig. 3. Scatterplot in relation with mean germination energy % (MGE%) vs mean seed germination % (MSG%) and mean germination energy % (MGE%) vs mean survival seedling % (MSS%) at level of significance (P≤0.05).

The experimental results described clearly about the significant effects of pre-sowing seed treatment and seed collection site at on the seed germination rate and seedling survival rate of *T. arjuna*.

As the pre-sowing treatment strongly effected the growth rate of *T. arjuna* seeds in nursery and also helped to enhance the seed germination rate and seedling survival rate which has proved the findings of (Das, 2015).

All those three factors mean seed germination %, mean seedling survival % and mean germination energy % were interlinked with each other although mean germination energy % was the factor, stronger than both other factors and had strongest influence on both of them. Germination energy % also strongly affected by the pre-sowing seed treatment and produced best results under T5 and T7 treatments.

The results are in harmony with those reported for *Ribesorientale*(Sharma, 2005), *Acacia auriculiformis* (Marunda, 1990), *Indigoferapseudotinctoria* (Jinnui, 2008), *Grewiaoptiva* (Singh, 1997) and *Terminalia bellerica*(Chakraborty, 1992).

As germination energy % influenced strongly on the seed germination % and seedling survival %, so we concluded that greater the germination energy % more positively seed germination % and seedling survival % effected. All three sites selected for the seed collection were not very different from each other neither ecologically nor climatically.

Comparing the results of all three sites (S1, S2, S3), seeds collected from the S2 were performed better than the seeds collected from S1 and S3.

Although the weight and size of the seed also matter for the seed germination and for seedling survival that's why only healthy and well weighted seeds were selected for the experiment, so that best result for the experiment would be obtained.

Which later on proved by the statistical results that we got non-significant result (F = 0.85, df = 8, P = 0.57) in relation between seed weight and other growth variables like seed germination and seedling survival because the average mean seed weight was (3.65 ± 0.06) almost equals to the ideal seed weight, which clearly indicate that the seed weight selected for the experiment was perfect and we can conclude here that size and weight really matter for the seed and seedling growth (Negi and Todaria, 1997) and also that seed weight was recorded almost equivalent for each site and treatment.

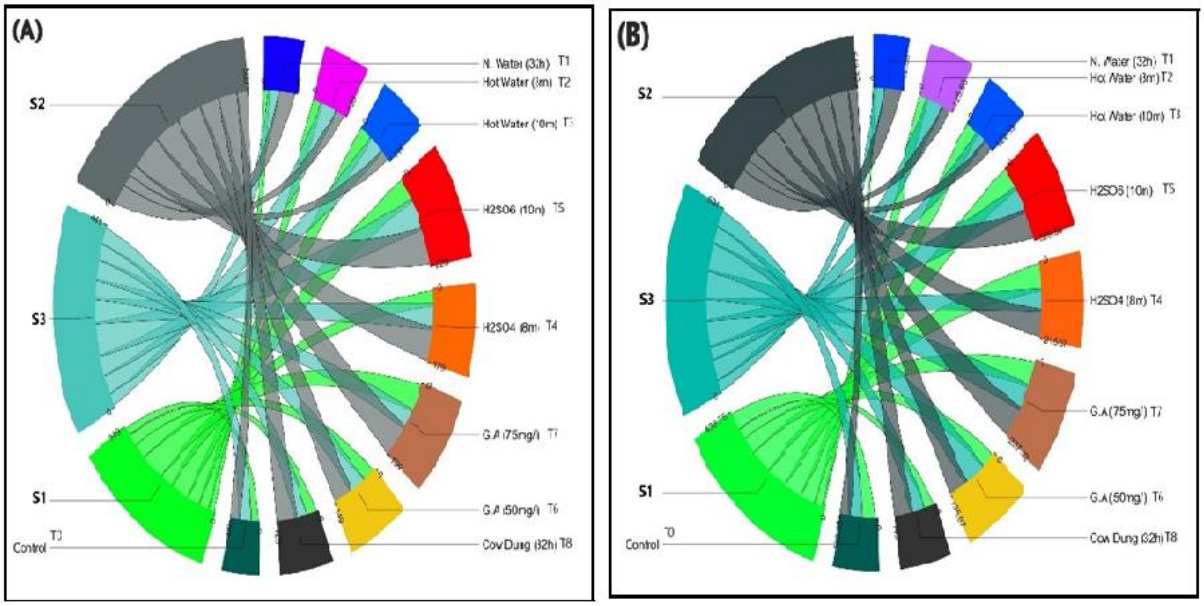

Fig. 4. Effect of seed collection sites on the (A) seed germination % and (B) seedling survival % at significance level (P≤0.05).

Similarly seed collection site also effected the growth rate, although results were not as stronger as like pre-sowing treatment but significantly positive.Overall experiment results have explained and proved that the mean seed germination, mean seedling survival strongly affected by the use of treatment T5, T7 (Agbogidi, 2007; Gupta, 2002; Naik *et al*., 2010) and selection of seed collection sites.

T. arjuna has hard seed coating which made seeds difficult to germination so use of chemicals and growth hormones help seeds to germination quite easily. One of the main reasons that why seed germination and seedling survival rate were high for T4 to T8 pre sowing seed treatments than for T0 to T3 pre sowing seed treatments, as Sulphuric acid has ability to burn any substance so it helped to burn the outer coat of the seed so the germination rate of seeds increased, GA is growth regulator which ultimately helps seed to germination easily cause it soften the outer coat of the seed and primary root and shoot to grow faster, therefore ultimately enhanced seed germination rate and as we all know cow dung has maximum amount of moisture with high temperature and high rate of bacterial actives inside, so putting seeds into the cow dung for 32h really helped to soften the seed coat and in result seed germination rate enhanced.

Experimental results also explained that T1 (normal water for 32h) was not a very useful treatment in case of *T. arjuna* seeds however it produced great results in case of *Acacia senegal* seeds (Marimuthu *et al*., 2001) this difference could be possible because of difference in seeds shape, size and hardness of seed coat.

The new and great finding from this experiment was the T8 (cow dung for 32h), seeds treated with T8 produced satisfactory results although in comparison with T5 and T7 treatments results were not very much impressive but it showed a new direction which is very easy and cheap to adopt and has protentional to produce the results like T5 and T7 treatments. Only 2-3 more experimental trials are required to verify the significant results of T8 treatment with the shifting of duration from 32h to 42h or 48h.

Conclusion

Use of chemicals and growth hormones were significantly useful to enhance the germination rate of seeds and seedlings. Pre-sowing seed treatments were also influenced positively on growth germination %, seedling growth % and especially for the germination energy %, which imposed the direct effect on the growth germination % and seedling growth %. Another important factor was seed collection site, all

three sites performed in a different and unique way produced some interesting and knowledgeable results. Although overall results for the seeds treated with the T8 (cow dung 32h) was not very significant but seeds collected from S2 performed very well and gave out some good results. So, increasing the duration of the treatment from 32h to 42h may can produce much better and significant results for *T. arjuna*seeds.

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2)

Acknowledgement

We are very thankful to the whole forestry department of UCA, UOS for helping us to carried out this experiment and special thanks to the Dr. M. Sajjad Haider (Chairman of forestry department) and Mr. Khursheed Allam (Lecturer of forestry department) for their supervision andhelpful efforts beyond the limits. We are also very thankful to the dean and principal for providing some extra hands for preparation and maintaining of forest nursery. And also, thankful to the all labor staff of UCA, UOS for working day and night with us. All the expenses of whole experiment were arranged and managed by ourselves.

Conflicts of interest

The authors declare no conflict of interest.

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