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EVALUATION OF INDIGENOUS METHODS OF NURSERY TECHNIQUES FOR MEDICINAL PLANTS

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Project Proposal

Title of the Project	Evaluation of indigenous methods of nursery techniques for medicinal plants
Principal Investigator	AV Raghu
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Funding Agency	KFRI Plan Grants
Objective	To test, standardize and compare traditional and conventional methods of seed handling & nursery techniques & to suggest a better & cost effective method for raising plantable propagules.

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Evaluation of indigenous methods of nursery techniques for medicinal plants

Abstract

Effect of indigenous methods on seed germination of selected medicinal plants such as *Embelia ribes, Streospermum colais, Celastrus paniculatus and Oroxylum indicum* was studied at Kerala Forest Research Institute, Peechi during 2013-15. Seeds were collected, processed and pre-treated with indigenous organic formulations like panjagavyam, cow dung slurry, cow's urine, cow's milk, and rice water. All the experiments were followed as per ISTA rules. The treated seeds were subjected to germination trials. The results were compared with control where no pre-treatment was given. Among the experiments, cow dung slurry showed a good response in E. *ribes* and C. *paniculatus*. In the other species like O. *indicum* and S. *colais*, water soaking and panjagavyam were given remarkable results. Indigenous treatments lead good results in the seed germination and ensured uniform seedling emergence. Thus it can be concluded that the traditional Indian practices have relevance and applicability in seed propagation methods.

Keywords: Pre-sowing treatments, Panjagavyam, Indigenous see treatment

INTRODUCTION

Medicinal plants play a crucial role in the health care of people around the the plants are world especially in developing countries (Rao et. al., 2004). Medicinal plants in the Kerala part of Western Ghats are mostly in the form of herbs and shrubs, both annual and perennial. A large proportion obtained from wild sources (Gupta, 1986). Majority of the people in developing countries for medicinal plants have been met their demand by indiscriminate harvesting of spontaneous flora including those in forests. Overexploitation of the wild sources had led to many species being extinct, threatened or endangered (Omobuwajo et. al., 2008). Indiscriminate exploitation has been the trend for decades in developing countries and therefore generates conservation issues for important plants. As markets increase for herbal products, wild population are been depleted. Medicinal plants are playing enormous roles in treating various diseases throughout the world since time immemorial. Plant-based drugs are now being increasingly used in traditional medicines because of their efficacy, cheap and lower side effects. World health organization (WHO) has estimated that 80 per cent of the total populations in developing countries rely on traditional medicines and mostly plants are derived for their primary health care. Rests of the people are also dependent substantially on plant-based medicines or on chemicals derived from plants (Sharmaand Govind, 2009).

Seed germination and seedling survival are critical stages in the life cycle of plant populations (Kitajimaand Fenner, 2000). Seed viability may affect the germination period and percentage. Pre-sowing treatments may influence seed germination rate and germination process. Effect of pre-sowing treatments on seed germination of some tropical forest species has been reported in earlier studies (Ali et. al., 1997; Altamira and Hossai., 2005; Azad et. al., 2006). Indigenous pre-sowing treatments were used to enhance and improve seed germination in many medicinal plants. The present work deals with the experience of efforts of promoting conservation and cultivation of some selected plant species. On the basis of our knowledge and available literature, the present study was conducted to see the effect of pre-treatments with various organic inputs on seed germination of selected medicinal plants such as *Embelia ribes* Burmf., *Streospermum colais* (B Ham.ex Dille.)Mabb., *Celastrus paniculatus* Willd. and *Oroxylum indicum* Benth.ex Kurz. Considering the facts the study was

undertake investigate the effects of pre-sowing treatment on seed germination seedling growth under laboratory and nursery condition. Keeping a view of their therapeutic potential, economic value and need for cultivation their seed quality parameters, four experimental procedures were chosen for the present study.

OBJECTIVES OF THE STUDY

To test, standardize and compare traditional and conventional methods of seed handling & nursery techniques & to suggest a better & cost effective method for raising plantable propagules.

The studies were done in the following four selected medicinal plant S

Species selected for the study

Sl. No.	Species	Local name	Family
1	Celastrus *paniculatus*	Jyothishmathi	Celastraceae
2	Embelia *ribes*	Vizhal	Myrcinaceae
3	Oroxylum *indicum*	Palakappayyani	Bignoniaceae
4	Sterospermum *colais*	Pathiri	Bignoniaceae

MATERIALS AND METHODS

Seed collection

The seeds of selected species used in the experiment were obtained from mature fruits from different locations. Seeds were extracted from fruits and dried in shade and stored in airtight containers. The moisture content of seeds was determined at beginning of storage and at every 30 days, using four subsamples of 25 seeds each, for each treatment.

Thousand seed weight, length, width, thickness, contents were determined (ISTA 1985). The moisture content (MC) is seed and moisture determined using a hot air oven (ISTA, 1999).

Moisture Content (MC) of the whole seed sample was determined on fresh weight basis followed by placing the seeds in hot air oven at 103°C for 17 hours and is calculated using the formula (ISTA, 2003): Percentage of moisture content = (Fresh weight- Dry weight)/Fresh weight x 100.

Storage treatments

The seed lots were sprinkled with water at room temperature until all the seeds were wet and dried for 2-5 days in the shade until completely dry. Seeds are mixed with Bavistin before storing. Seeds were mixed thoroughly, packed in sealed polyethylene bags, in large plastic bins with airtight lids and stored in cold rooms maintained at a temperature of 4 and 16 degree Celsius and 45% humidity. Another set of seeds were stored in ambient conditions at the KFSC seed storage rooms until the tests were carried out. Seeds in cold rooms were brought to ambient conditions prior to all tests.

Total number of seeds used in the study (Each Species)	
Initial germination	400
Initial MC% determination	100
MC% determination at 3 months	300
Germination trials at 3 months	1080
Total	**1880**

In the case of *E. ribes*, the seeds were collected from natural populations of Ponmudi (08˚.45.324'N, 077˚06.444, 691msl) Thiruvananthapuram district of Kerala during the month of June. Random sampling was followed during the collection. Seed quality was assessed visually whereby damaged; infested or deformed seeds were discarded. The weight of 100 seeds in triplicate was taken by electronic balance and size, comprising length and breadth (10 replicates) were determined by vernier caliper. Uniform seeds were used for the treatments to reduce non treatments varies since the germination percentage and seedling vigor was found positively correlated with seed size (Jackson, 1994).

Seeds were stored at five different storage conditions like ambient temperature (25±2°C), 4°C and 16°C temperature, Cotton bag with sawdust, Earthen pot with sawdust in Forest Seed Centre situated at KFRI, Peechi. In both the rooms (4°C, 16°C) the relative humidity maintained was 45 %.

Initial moisture content and germination percentage were tested as per the ISTA rules (ISTA, 1999). Germination study was conducted in the nursery on vermiculite medium. Four replicates of 100 seeds each per treatment were maintained in germination trials. The seeds were lined sown in medium trays. Watering was done daily to moist the substratum. The whole experiment was conducted in lab conditions under a shade house. At the 3- or 4-leaf stage the seedlings were pricked out and bagged using polybags of size 14 X 7 cm. The potting mixture, consisting of soil, sand, and manure in the ratio 2:1:1, was used in the polybags.

Germination was observed daily. All the germinated seeds were counted, at the end of the tests; the cumulative germination percentage was calculated for each treatment. Germination test was carried out in each month from the date of storage. Mean Emergence Time (MET) was calculated by the following formula (Butola and Bodola, 2004) MET $=\Sigma(fx)/\Sigma x$, *where x is number of newly emerged seedlings on each day, f the number of days after the seedlings were set to germinate and Ex the total number of seedlings emerged at the end of the experiment)*

Statistical Analysis

All the experiments were carried out in a randomized block design with four replications of 100 seeds each. In this study, it is to be tested if there is any statistically significant difference in MC, G%, and MET among different conditions - Ambient, 4°C, 16°C, Cotton bag within sawdust, earthen pot in sawdust. One-way multiple analysis of variance (MANOVA) is used to test the null hypothesis that there is no statistically significant difference in MC, G%, and MET among the conditions - Ambient, 4°C, 16°C, Cotton bag with sawdust, earthen pot in sawdust. The error bars are also drawn below. In all the analysis significance level is taken to be 0.05 (i.e., if the p-value is less than 0.05, reject the null hypothesis, or it can be concluded that the null hypothesis is statistically significant). Statistical Analysis was carried out using the statistical package, SPSS (version 22.0.0.0).

Seed Germination tests

All germination tests were conducted in KFSC seed laboratory. An initial germination test is carried out in four replications with each replication containing 100 seeds (ISTA. 1996). Germination tests were carried out with the seeds stored in each condition at an interval of two months.

The seedling emergence test was carried out under lab conditions with four replications of 30 seed each, for each treatment, which were evenly distributed in plastic trays, containing, vermiculite which were then daily watered for substrate moisture maintenance and were kept under the test conditions of 25° ± 1° and 95° ± 3% percent relative humidity maintained in a germination room illuminated with fluorescent light. A seed was considered to have germinated when a normal seedling had developed a seedling was classified as normal when it had a well-developed primary root and intact hypocotyl and cotyledons (ISTA, 2007). After the test period, normal seedlings were counted and the mean values expressed as a percentage (ISTA, 1999) to the total number of seeds placed for germination.

Mean Emergence Time (MET=Σ(fx)/Σx, where x is number of newly emerged seedlings on each day. f the number of days after the seedlings were set to germinate and Σx the total number of seedlings emerged at the end of the experiment) (Butola, J. S. and Badola, H. K., 2004). The Germination Index which expressed as the speed of germination was also calculated (AOSA, 1983).

GI = No. of germinated seed + ... + No. of germinated seed

Days of the first count Days of Final count

The counts were daily performed from the eighth to the 25th day after seeding, by computing the only seedling that had emitted the epicotyl; and results were expressed in percentage. After the germination period is over (when there is no further germination observed in the test trays) the trays removed from the germination room. The details of the germination test are recorded in germination sheet. Seedling at 3-4 leaf stages is planted into polythene bags of size 20 x 8cm filled with a soil based potting mixture. Regeneration from seeds remains the most common method of propagation in these species. Delayed and irregular germination is a serious constraint in the large scale propagation,

Vegetative propagation

Vegetative propagation of selected medicinal plants using traditional and conventional method for raising the seedlings.

The study was conducted in the Kerala Forest Research Institute, Thrissur District, Kerala (10° 31' 47" N; - 76° 22' 7.5"E) during 2012-15. Fresh mature seeds of E.*ribes* were collected from Ponmudi, Thiruvananthapuram district, during July 2013. The fruits were depulped by rubbing the fruits on a rough surface after keeping the seeds in water for two days. Decorating is done by grinding the depulped seeds in motor and pistil to remove the pulp. C. *paniculatus* fruits were collected from Bangalore during November 2013 and the seeds were processed after assessing the quality. The well mature pods of O. *indicum* were collected from Cherpulassery, Palakkad District during December and the seeds were extracted. The seeds of S. *colais* used in the experiment were obtained from mature fruits from Pallanad, Idukki District in early December 2012. All the seed lots were brought to the laboratory where it was subjected to basic seed technology tests such as fruit weight, seed weight, moisture content etc. The fruits were sun-dried and preserved in air tight container till the treatments were applied. The processed seeds were tested for moisture content on a fresh weight basis by oven-dry method (ISTA, 1999). The quality assessment of seeds through rapid viability test (Cutting test & Tetrazolium test).

The seeds were subjected to the following treatments:

1) Control- TO
2) Soaking seeds in water for 1 day- T1
3) Soaking seeds in rice water for 1 day- T2
4) Soaking seeds in rice wash water for 1 day-T3
5) Soaking seeds in Cows' milk for 1 hour - T4
6) Soaking seeds in Cows' milk for 1 day - T5
7) Soaking seeds in Cows' urine for 1 hour - T6
8) Soaking seeds in Cow dung slurry for 1 hour - T7
9) Soaking seeds in Cow dung slurry for 1 day - T8
10) Soaking seeds in Panchagavyam 3% for 1 hour-T9
11) Soaking seeds in Panchagavyam 3% for 1 day-T10

The treated seeds were washed with distilled water and then sown for germination in vermiculate medium (Chacko and Pillai, 1997). Seeds (n = 100 seeds in 4 replicates) were sown in trays in a Randomized Block Design and kept in germination chamber (30°C; 95 % RH) for germination.

RESULTS & DISCUSSION

In the case of E. *ribes*, the moisture content of fresh seeds was 39.43% with 55% germination. A linear reduction of seed moisture content was observed after the different storage conditions. The seeds stored in 4°C shows good results in terms of moisture content and germination percentage. The seeds stored in ambient condition and 16°C and shows promising results in mean emergence time. The descriptive statistics of the study variables in MC, G%, and MET among different conditions like ambient, 4°C, 16°C, cotton bag in sawdust, earthen pot in sawdust were calculated in terms of Mean, Standard deviation and number of cases (Table 1). From Table 1. It can be observed that the mean value of MC is highest (34.3543%) for 4°C storage and lowest (22.2971%) when stored cotton bag within saw dust. G% shows highest at 4°C (34.91%) and lowest (19.3186 %) in the cotton bag. MET is highest (115.9457) in ambient and lowest (77.8871) in the earthen pot within saw dust. From statistical studies significant difference in mean MC ($F(4, 30) = 144.838$, p-value = 0.019) was observed among the different germination conditions. But there is no. statistically significant difference in mean G% and MET. As there is a significant difference in mean MC, Turkey's posthoc test was conducted to test which pair of a group has a significant difference (Table 3). From this, significant difference in mean MC between the storage conditions of 4°C and cotton bag (MD= 12.0571, p-value = 0.012) has confirmed. And there was no significant difference observed for mean G% and MET. Error bars are also drawn to compare the mean study variables for each group visually. The error bar represents the variability of the data. In the graphs, the data point rounded is the mean of the study variables of each group, and the length of the bar represents the confidence interval (95%). From these graphs we can compare two values visually; if they are significantly different they will not overlap. (Fig. 1 to 3)

The loss of viability of recalcitrant seeds could be either due to the moisture content falling below a certain value or simply a general physiological deterioration with time (Chin et al., 1984), Recalcitrant seed loss viability when their moisture content falls below 20-30% since they are sensitive to desiccation, Even under ambient temperature and low relative humidity, their post-harvest

life is very short, either days or a month, depending on the species. Also, they cannot be stored in subzero temperatures due to ice formation (Robert, 1973; Farrant et al., 1988). Mechanical damage by drying recalcitrant seeds is associated with volume reduction and the collapse of vacuoles which act as an influential factor in causing damage to recalcitrant seeds (Berjak and Pammenter, 2013). The membrane-related physiological damages or an accumulation of by-products of biochemical enzymatic breakdown may be the basic cause for seed death (King and Roberts, 1980). According to a study on the effect of temperature on seed storability of E. *ribes*, seeds stored at 0 ± 2°C, failed to germinate even after one week of storage, which might be due to freezing damage (Sivalingam et al., 2011). In this study seeds stored at 10°C retained viability for 3 weeks with 12% germination. The results envisage that lower temperature is comparatively superior in maintaining the seed viability of recalcitrant seeds of E. *ribes*. Agreement with our observations earlier studies has been reported that germination percentage of recalcitrant seeds declines rapidly as seed moisture content decrease (King and Roberts, 1982; Pritchard et al., 1995; Tompsett and. Pritchard, 1998; Xia et al., 1992).

In tree species, seed germination is difficult due to hard seed coats and dormant seed embryos (Jaiswal, Chaudhary 2005) and they often fail to germinate even under favourable moisture, oxygen and soil conditions (Urgenc, Cepel, 2001). The moisture content of seeds is one of the factors influencing germination, as it is a test in retention of viability of seeds (McDonald, Copeland 1999), Storage of seed is an important factor on which the seed quality greatly depends. Without proper storage, seed quality is degraded. Many factors determine the longevity of seeds during storage. These include seed moisture content, temperature, relative humidity, initial viability, stage of maturity at harvest, storage gas and initial moisture content of seed entering into the storage. (Harrington, 1972).Seed production is reduced as a result of destructive harvesting practices, which

also caused gradual erosion in the natural population. The species is mainly propagated through seeds and collecting them becomes a laborious process` s as their pericarps are winged. Another difficulty it faces is poor germination rate and thus propagation through seeds in the wild is limited (Baul 2006). Hence, steps have to be taken to conserve this tree of great economic value by finding suitable methods for its large scale propagation. Knowledge of the behavior of forest seeds under different storage conditions is extremely important for the rational management of the species, and it also allows increased availability period for seeds with high germination and vigor so that new seedlings are continuously produced with satisfactory quality.The temperature affects the germination and the state of dormancy of the seeds and the seasonal changes of the dormancy state of the seeds of some species is directly related to the seasonal temperature changes (Pons, 1933).

Table.1. Descriptive Statistics of *E. ribes* seeds on MC, G%, and MET

Germination	Storage condition	Mean	Standard deviation	N
MC	Ambient	25.8229	6.23775	7
	4 °C	34.3543	5.54929	7
	16°C	25.8600	5.77744	7
	Cotton Bag	22.2971	5.39516	7
	Earthen Pot	24.8743	8.72402	7
	Total	25.8229	7.33474	35
G%	Ambient	20.7471	20.7471	7
	4 °C	34.9100	34.9100	7
	16°C	24.1614	24.1614	7
	Cotton Bag	19.3186	19.3186	7
	Earthen Pot	22.3600	22.3600	7
	Total	24.2994	24.2994	35
MET	Ambient	115.9457	23.91461	7
	4 °C	96.5114	22.49308	7

	16°C	100.0029	23.39921	7
	Cotton Bag	93.1314	27.46252	7
	Earthen Pot	77.8871	15.87331	7
	Total	96.6957	24.86737	35

Table 2. Test between-subject effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	MC	579.351a	4	144.838	3.477	.019
	G%	1076.546b	4	269.137	1.207	.328
	MET	5236.001c	4	1309.000	2.487	.065
Intercept	MC	24842.333	1	24842.333	596.313	.000
	G%	20666.178	1	20666.178	92.708	.000
	MET	327252.141	1	327252.141	621.792	.000
Condition	MC	579.351	4	144.838	3.477	.019
	G%	1076.546	4	269.137	1.207	.328
	MET	5236.001	4	1309.000	2.487	.065
Error	MC	1249.796	30	41.660		
	G%	6687.511	30	222.917		
	MET	15789.134	30	526.304		
Total	MC	26671.480	35	-		
	G%	28430.235	35	-		
	MET	348277.276	35	-		
Corrected Total	MC	1829.147	34	-		
	G%	7764.057	34	-		
	MET	21025.135	34	-		

Table 3. Tukey's post hoc test

Group (I)	Group (J)		Mean Difference (I-J)	Std. Error	Sig.
MC	Ambient	4 degree	-8.5314	3.45005	.124
		16 degree	-.0371	3.45005	1.000
		Cotton bag	3.5257	3.45005	.843
		Earthen Pot	.9486	3.45005	.999
	4°C	4 degree	8.5314	3.45005	.124
		16 degree	8.4943	3.45005	.127
		Cotton bag	12.0571*	3.45005	.012
		Earthen Pot	9.4800	3.45005	.070
	6°C	4 degree	.0371	3.45005	1.000
		16 degree	-8.4943	3.45005	.127
		Cotton bag	3.5629	3.45005	.838
		Earthen Pot	.9857	3.45005	.998
	Cotton bag	4 degree	-3.5257	3.45005	.843
		16 degree	-12.0571*	3.45005	.012
		Cotton bag	-3.5629	3.45005	.838
		Earthen Pot	-2.5771	3.45005	.943
	Earthen Pot	4 degree	-.9486	3.45005	.999
		16 degree	-9.4800	3.45005	.070
		Cotton bag	-.9857	3.45005	.998
		Earthen Pot	2.5771	3.45005	.943

Fig. 1. Error Bars of MC

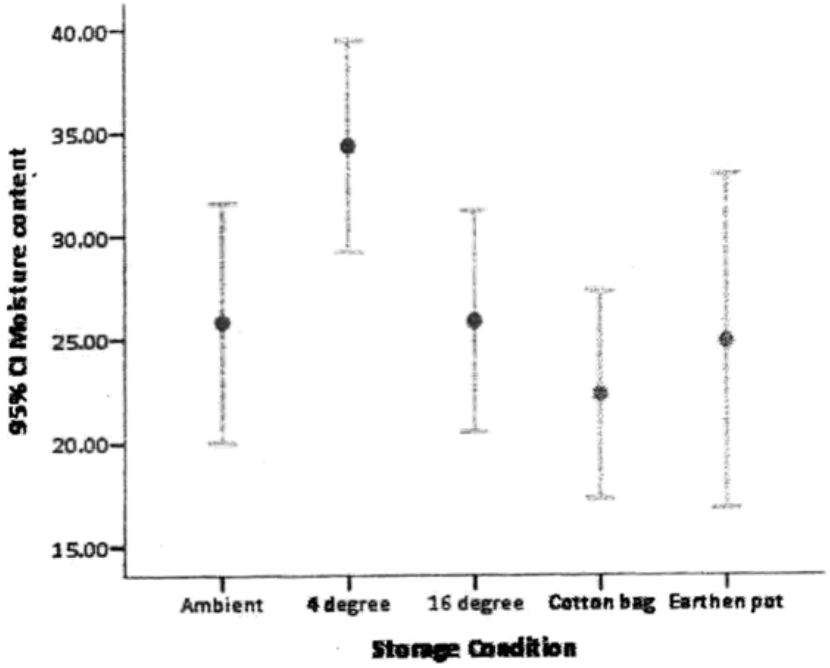


Figure 2: Error bar of G%

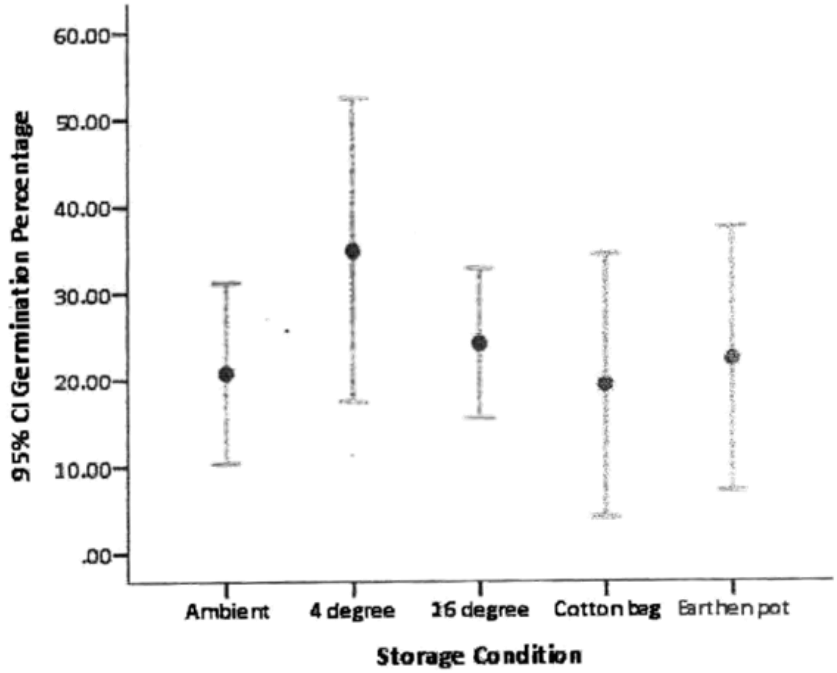
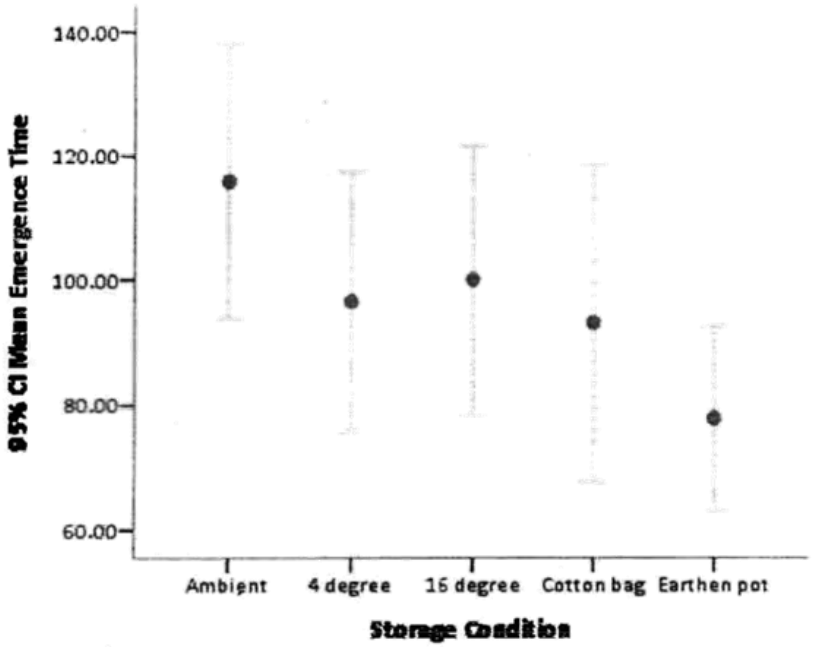


Figure 3: Error bar of MET



Effects of various indigenous pre-sowing treatments on germination of *E. ribes, C. paniculatus, O. indicum, and S. colais* have been investigated. The traditional practices are indigenously developed and have been practiced by our farmers through generations. Techniques such as soaking seeds in powdered rhizomes of *Acorus calamus*, a mixture of milk and ghee, rubbing seeds with a mixture of ashes of the root, bark, fruits, flowers, and leaves of Indian nightshade, for enhancement of germination; use of *Calortopis* spp. against termites and weeds (Subhashini *et. al.*,); Panchagavyam treatment (Jayashree and George, 2006) for growth, etc have been developed for specific plants. Seed propagation is the most common method of reproduction practiced (Purseglove, 1968). Their germination rate, the effectiveness of different treatments, germination duration for seed germination has not been widely reported for these species.

Table 4. Pre sowing treatments of *E. ribes, O. indicum, S.colais,* and *C. paniculatus*

Sl. No	Species	*Embelia ribes*	*Celastrus paniculatus*	*Oroxylum indicum*	*Stereospermum colais*
.	Treatments	Germination Percentage (%)	Germination Percentage (%)	Germination Percentage (%)	Germination Percentage (%)
1	Control	40	12	82	8.88
2	Water soaking 1day	46	14	**95**	11.11
3	Rice water 1day	26	15	88	10.23
4	Rice wash water 1day	23	13.5	86	12.22
5	Cows' milk 1hour	36	14.5	83	7.77
6	Cows' milk 1day	42.5	13.5	91	6.66
7	Cows' urine 1hour	56	13	87	4.44
8	Cow dung slurry 1hour	43	**18.5**	87	3.33
9	Cowdung slurry 1day	**68**	16	89	13.5
10	Panchagavyam 3% 1hour	48	15	88	**18.42**
11	Panchagavyam 3% 1day	44	13	86	15.5

Seed attributes

Table 5. Seed attributes of the plants selected in this study.

Species	**Thousand seed weight (g)**	**No. of seeds /kg**	**Dimension (mm)**	**Thickness (mm)**	**Moisture content (%)**
E.ribes	22.591 ±1.256	9000-9100 ± 5.3665	2.7x2.6 ± 0.05	2.6-2.8 ± 0.091	38.38
C.paniculatus	13.56 ± 0.16	69301-69309 ± 35.7	4.5x1.5 ± 0.054	1.12-1.3 ± 0.03	12.62
O.indicum	0.144 ± 0.0017	9038-9080 ± 5.3	1.6 x1.14 ± 0.009	0.85-0.924 ± 0.005	12.09
S.colais	4.21 ± 0.017	68400-68500 ± 17.88	4.17x6.3 ± 0.012	5.47-5.97 ± 0.05	12.34

Pre-soaking in water improved to some extent. Germination of the decoated seeds of E. *ribes* seeds started 29 days after sowing (DAS).Germination ceased 138 days after sowing. The De-coated seeds soaked in Cow dung slurry for 1 day gave a maximum result (68%) than the control (40%). The seeds were kept in a shady situation for one week and after soaked overnight with 3% Panchagavyam (a mixture of 5:1 cow dung and ghee in a 5:3:3:5 cow's urine, curd, milk and water formulation) Seeds soaked in Panchagavyam 3% for 1 hour (48%) and cow's urine for 1hour (56%) recorded a moderate increase in germination. Soaking seeds in Rice water and Rice wash water for one day had no significant effect on germination (26% and 23% respectively).

Germination of the C. *paniculatus* seeds started 18 DAS. The germination culminated in 90days. The seeds soaked in cow dung slurry for 1 hour give germination reached 18.5% Pre-treating the seeds in various formulations have increased the germination percentage than the control. Germination of the O. *indicum* seeds started 7 DAS. The germination is counted up to 10 days. The seeds soaked in water for 1

day give a maximum result (95%) than the control (82%). All the pre-sowing treatments improved the germination percentage. Germination of the S. *colais* seeds started 20 DAS. The germination is counted up to 10 days. The seeds soaked in 3% Panchagavayam for 1 day give a maximum result (18.42%). No promising results were observed in other treatments. (Table 4).

The effect of fourteen pre-sowing treatments (including control) on seed germination was assessed which included conventionally and traditionally followed methods (Suresh et al., 2013 and Subhashini et al., 2003) (Table 6). All the fourteen treatments were imposed either for the whole seeds with pulp or for the depulped seeds. Seeds were sown in 4 replicates leach replicate containing 30 seeds) in plastic trays containing vermiculate (Willan, 1985) and soil under nursery conditions. During the experiments (July-September) average temperature at this site was 26-36°C, and average humidity and rainfall were 70-90% and 337.3 to 260.7mm respectively.

Each day data was taken by monitoring the experiments. Germination percentage (ISTA, 1999), Mean Emergence Time *(MET- Σ(fx)/Σx, where x is number of newly emerged seedlings on each day, f the number of days after the seedlings were set to germinate and Σx the total number of seedlings emerged at the end of the experiment* (Butola et al., 2004), *Energy Period (EP) Germination Energy (GE) (Paul., 1972), Germination Value* (GV= (Σ DGS/N) x GP/10, Where GP is germination percent at the end of the test, DGS is the daily germination speed obtained by dividing the cumulative germination DGS figures and N, the number of daily counts effective from the date of percent by the number of days since sowing, ΣDGS is the summation of all first germination.(Djavanshir, and Pourbeik, 1976), Germination Index (GI) were statistically analyzed. One-way multiple analysis of variance (MANOVA) was used to evaluate the significance of pulped and de-pulped seeds on seed germination (SPSS version 22.0.0.0).

The results are summarized in table 6. The results prove that traditional pre-sowing treatments show significant results in germination with depulped seeds of E. *ribes* than the conventional methods, MANOVA analysis also indicated a statistically significant difference among pulped and depulped seeds on seed germination of this species (Table7). Seeds with pulp may be one of the reasons for the less seedling emergence and poor seedling establishment at natural conditions of this liana. The use of conventional treatments for germination of this species has been reported

earlier (Gupta, 2003 and Rajanna *et al.,* 2001) The use of traditional methods is safe and recommendable, especially for medicinal plants. The traditional methods suggested by this study for the propagation of E. *ribes* are simple could be adopted by nursery workers and farmers for producing mass planting stock, and it is a green step towards the conservation of threatened liana.

Table. 6. Effect of traditional and conventional treatments on seed germination of *Embelia ribes* Burm. f.

	Germination %		**MET (Days)**		**GI (%)**		**GV**		**GE (Days)**		**EP (Days)**	
Treatments	**Seed.**	**DP*seed**	**Seed**	**DP seed**	**Seed**	**DP seed**	**Seed**	**DP seed**	**Seed**	**DP seed**	**Seed**	**DP seed**
Water -12 h (control)	55.0 (1.4)f	48.3 (2.2)g	86.1 (0.4) d	57.5 (0.4)g	0.025 (0.003)f	0.057 (0.015) d	0.247 (0.039) e	1.282 (0.171) b	7.5	6.6	140	132
GA3 (100 ppm) - 24 h	65.0 (1.6)c	56.0 (2.7)e	61.1 (0.2)g	98.6 (1.9)c	0.059 (0.007) c	0.035 (0.017) e	0.949 (0.110) b	0.878 (0.021) d	28.3	40.8	140	148
GA3 (250 ppm) - 24 h	48.8 (2.0)g	58.3 (1.8)e	72.7 (0.6)f	68.8 (0.3)f	0.034 (0.005) e	0.046 (0.005) d	0.126 (0.022)f	0.606 (0.088) e	28.3	30.0	138	136
GA3 (500 ppm) - 24 h	58.1 (0.2)e	63.1 (1.5)d	54.9 (0.1) h	55.4 (0.1)g	0.066 (0.004) b	0.081 (0.014) b	1.203 (0.233) a	0.465 (0.081)f	22.5	21.6	93	99
GA3 (1000 ppm) - 24 h	65.6 (1.6)c	67.5 (1.2)d	65.6 (0.1)g	63.2 (0.1)f	0.037 (0.005) d	0.050 (0.009) d	1.105 (0.113) b	1.059 (0.112) c	29.1	30.2	150	137
Acid (Conc.	56.6	64.1	102.	99.9	0.023	0.032	1.27	1.261	44.1	48.0	150	149

H2SO4) scarification	(1.1)f	(1.4)d	7 (0.5)c	(0.2)c	(0.002)f	(0.003) e	(0.312) a	(0.043) b				
Weathering at 2 day interval	59.1 (0.8)e	74.1 (1.1)c	93.7 (0.3) d	89.6 (0.2) d	0.029 (0.003) e	0.072 (0.019) b	0.295 (0.048) e	0.362 (0.003)f	40.0	40.0	150	150
Panchyagavyam (3%) - 24 h	41.6 (1.6) h	40.0 (1.1)h	81.7 (0.5)e	103.2 (0.7)c	0.027 (0.003) e	0.021 (0.003)f	0.116 (0.022)f	0.103 (0.018) g	40.0	42.5	116	120
Panchyagavyam (100%) - 24 h	62.5 (1.0) d	79.5 (1.3)b	90.3 (0.4) d	91.6 (0.2) d	0.031 (0.002) e	0.036 (0.003) e	0.215 (0.042) e	0.561 (0.079) e	43.3	40.0	130	136
Cow dung slurry - 1 h	43.3 (0.8) h	54.1 (1.3)f	110.5 (0.6) b	116.0 (0.3) b	0.016 (0.002)f	0.029 (0.002)f	0.505 (0.087) d	0.662 (0.040) e	31.6	27.5	150	149
Cow dung slurry - 24 h	75.8 (1.0) a	83.3 (1.3)a	68.2 (0.2)f	74.7 (1.2)f	0.043 (0.006) d	0.045 (0.005) d	1.127 (0.137) b	1.343 (0.166) b	46.6	52.5	150	142
Cow urine - 1 h	28.3 (0.7)i	30.0 (1.4)i	112.5 (1.5)	106.1 (1.0)c	0.098 (0.001) a	0.174 (0.002) a	0.321 (0.002) e	0.492 (0.063)f	5.5	7.5	150	150

			b									
Cow milk - 1 h	39.1 (1.0) h	47.5 (1.6)g	121.5 (1.3) a	126.8 (0.8) a	0.027 (0.003) e	0.026 (0.014)f	0.050 (0.003) g	0.143 (0.018) g	39.1	47.5	150	152
Cow milk - 24 h	70.0 (1.7) b	71.6 (1.4)c	70.8 (0.4)f	77.9 (0.4)e	0.039 (0.005) d	0.064 (0.003) c	1.07 (0.122) b	1.462 (0.181) a	16.8	23.3	150	149
Rice gruel - 24 h	74.5 (1.0) a	73.3 (1.6)c	78.0 (0.3)e	87.6 (0.5) d	0.037 (0.005) d	0.038 (0.003) e	0.749 (0.113) c	0.875 (0.065) d	40.8	48.3	150	144
									(5.26)	(0.698)	(8.18)	(5.824)

*DP=Depulped

Same letter in the same column indicates insignificant differences and data values in parenthesis are standard error of mean at $p<0.05$.

Table 7. Results of one-way MANOVA of pulped and de-pulped seeds

Effect	Value	F	Hypothesis df	Error df	Sig. (p<0.05)	Effect
Intercept	Wilks' Lambda	.008	489.891	6.000	25.000	.000
Group (Seed and DP seeds)	Wilks' Lambda	.250	12.501	6.000	25.000	.000

CONCLUSION

Based on the results of the storage treatments of E. *ribes*, it confirms that moisture content, storage temperature and storage conditions are generally regarded as the factors governing the seed longevity. The study reveals that E. *ribes* is a recalcitrant one, so it can't keep for a longer time in ambient condition, so it requires better storage conditions. The temperature was an important factor in the seed storage of E. *ribes*. It was observed that seeds stored under 4°C, retained a satisfactory germination capacity after seven months of storage period. Our results provide additional new information that traditional method like the earthen pot with sawdust can also be used to store *Embelia* seeds for longevity, may adopt by the farmers for large-scale propagation of this highly demanded medicinal liana

Traditional pre-sowing treatments were found to be more effective in breaking the seed dormancy of *E. ribes and Oroxylum indicum* though it was not much effective in case of *Stereospermum colias* and *Celastrus paniculatus*. Conventional nursery techniques adopt chemical inputs at various stages, such as storage, preparation of mother beds, increase in germination percentage, better growth, disease, and pest control, better yield, etc., for the production of seedlings. (Anonymous. 1997; NMPB.2004). However, none of these methodologies apply traditional techniques for seedling production. Today, it is becoming evident that whereas chemical inputs show dramatic short-term benefits, in the long run, they adversely impact the soil, water and perhaps the nutritional quality of the plants (Worthington V, 2001). Thus, it is evident that there is a great scope to integrate traditional practices for better productivity of quality planting materials.

EXTENSION PART OF THE PROJECT

A booklet published on

Forest Trees: Seed Treatment and Seedling Production (ISBN 81-85041-84-9)

This illustrated booklet in Malayalam provides description on propagation techniques of 51 forest tree species in detail. This is aimed at increasing the popular awareness and to form a starter guide for those groups and individual who wishes to setup nursery as part of the tree planting and greening programmes. Each and every step involved in explained with the help of simple illustrations.

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Effect of pre-germination treatments and storage conditions on germination of *Embelia ribes* Burm f. *(bidanga)* with special reference to Vrikshayurveda

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Abstract

Effect of pre-germination treatments on freshly harvested seeds and on stored seeds of *Embelia ribes* Burm. f., were investigated in this study integrating with Vrikshayurveda methods. Germination tests were done immediately after the pre-treatments for the freshly harvested seeds after decoating and for the seeds stored in low temperature (4^0C) kept in earthen pot along with the seeds stored in ambient condition after three months of storage. All Seeds including control were subjected to four pre-germination treatments to enhance the germination percentage and maintain its viability. Pre-germination treatments showed significant differences in germination percentage. The highest germination percentage was observed in the seeds soaked in cow dung slurry for the fries (83.33%) and stored (43.33%) compared to control (38.33% and 16.5% respectively). Seeds of *E. ribes* being recalcitrant in nature poses storage problems. From this study it can be concluded that viability of *E. ribes* can be maintained after three months of storage in earthen pots and in low temperature. The versus about *E. ribes* as an important plant recommended in Surapalas Vrikshayurveda for ailing plants, propagation of plants and treatment of seeds was described in this paper.

Keywords: *Pre-germination treatments, Vrikshayurveda, Germination tests, Germination percentage.*

Introduction

Seed storage serves as a safe and relatively inexpensive method of plant genetic resources conservation. Since seed production is seasonal, and usage is continuous, safe storage must be provided for the seed produced until it is needed for successful establishment of plantations and multiplication purposes. Seeds of many species can be stored under cool and dry conditions. Farmers have had to maintain viable seeds from one growing season to the next (i.e. Short term seed storage, typically 3 to 9 months, but occasionally up to18 months). It may also be desirable to maintain 'carry-overstock' for several years (medium-term seed storage, typically 18months to 5 or 6 years). In both these cases, conventional practices have developed from previous experience of problems and successes with seed storage (Hong and Ellis, 1996).

It is estimated that 60-70 per cent of food grains produced in the country is stored at home level in indigenous stuctures ranging from bamboo baskets to mud structures, gunny bags and modern bins (Kanwar and Sharma 2003; Channal *et al.,* 2006). Farmers and traditional grain processors have been evolving number of traditional practices through trial and error method, to avoid huge loss that are occurring in the stored pulse grains due to insect and pest infestation (Pushpamma and Rao, 1980). The present study was carried out with an objective to identify the best method of pre treatment to be adopted for seeds of *Embelia ribes* Burm f., to obtain maximum germination percentage, safe seed storage with special reference to Vrikshayurveda.

E.ribes Burm. f.,a climbing shrub belonging to family Primulaceae is an important medicinal plant used in a number of traditional medicinal preparations. *E.ribes* which is authenticated as *Vidanga* is an essential ingredient of many formulations in Ayurveda (Anon., 2001). *E. ribes*, popularly known as *'Vidanga'* or 'Vavding' in Ayurveda, is a Red-listed species. This species is reported to be vulnerable in the Western Ghats of Tamil Nadu and Karnataka states of India and at a lower risk in Kerala state of peninsular India (Ravikumar *et al.*, 2000). Its great demand in Ayurveda and the pharmaceutical industry (> 100 t/ yr.) has imposed tremendous pressure on natural populations from the Western Ghats of India (Mhaskar *et al.*, 2011)

Vrikshayurveda mainly deals with various species of trees and their healthy growth and productivity. A text on Vrikshayurveda mentions about 170 species of plants, including herbs, shrubs and trees. There are 325 systematically arranged versus, beginning with a salutation to lord Ganesha, followed by glorification of trees, and composition on tree planting and production. Special references are made to procuring, preserving, and treatment of seeds and planting materials. The most noteworthy fact in Vrikshayurveda, however is that it applies the *tridhatu theory* of Ayurveda (the science of life) to plants. *Kapha*, *Pitta* and *Vata* are treated as the basic components of plants, too as of humans and the theory that a balance of the three indicates health and imbalance caused due to vitiation of any one or more of them indicates disease is extended to plants too, justifiying its title 'Vrikshayurveda'.

Materials and Methods

The berries of *E.ribes* were collected from Ponmudi, Thiruvananthapuram district, during June, 2012, depulped by rubbing the fruits on a rough surface after keeping the seeds in water for two days. Decoating is done by grinding the depulped seeds in mortar and pestle to remove the pulp. Then the seeds were sub-divided into two lots. Seed lot A was used to test germination after applying pre-germination treatments, while the seed lot B was used to test germination after three months of storage in cold storage room (4^0C) after applying storage treatment. The seeds were kept in earthen pot, its mouth covered with gunny cloth and stored in the cold storage room.

Storage treatment based on Vrikshayurveda

Sprinkle cow's milk until all the seeds are wet > Mix thoroughly with fresh cow dung, so that a coating is made on all the seeds > Shade dry for 3-5 days

Fig 1: Fruits of *ribes*

Fig 2: Seeds of *ribes* stored in earthen pot

(until completely dry) > Mix profusely with honey and vidhanga powder > Shade dry again for 3-5 days until completely dry > Seeds are ready for storage.

***Control*: (T0)** Seeds are stored without any treatment.

Pre-sowing treatments given to *E. ribes* seeds in this study consists of control (T0-), seeds soaked in cow urine for 1 day (T1), seeds soaked in cow's milk for 1 day (T2), seeds soaked in dung slurry for 1 day (T3). Germination tests of fresh seeds and stored seeds (400 seeds – 4 replications of 100 seeds each) were sown under each treatment following Randomized Block Design. These were compared with control (T0) where no pre-treatment was given. Sowing was done in sterile vermiculite (Willan, 1985) medium taken in plastic trays. The trays were kept in KFRI laboratory at Peechi in Kerala state and the mediums used were maintained moist. Germination trials were conducted periodically for assessing the effect of pre-germination treatments by counting germinated seeds. Germination data was recorded from the date of sowing until germination ceased.

Results and Discussion

Effect of pre-germination treatments on germination:

Germination of the *E. ribes* seeds started 32 days after sowing and culminated after 123 days. Different treatments significantly affected the germination period for the species. Initial germination of fresh seeds (Seed lot A) were highest in T3 (83.33%) followed by T1 (47.5%) which was significantly higher than control (38.33%). The lowest germination percentage was recorded in T2 (30%). The germination is counted upto more than three months (123 days). The germination percentage of stored seeds (Seed lot B) was highest in T3 (43.33%), followed by T1 (35.5%) and T2 (22.2). Lowest germination percentage was recorded in control (16.5) which was similar to that of fresh seeds. In both germination tests (fresh and stored seeds) the seeds soaked in cow dung slurry for 24 hours, gave maximum result than the control. The seeds kept in ambient condition showed poor germination.

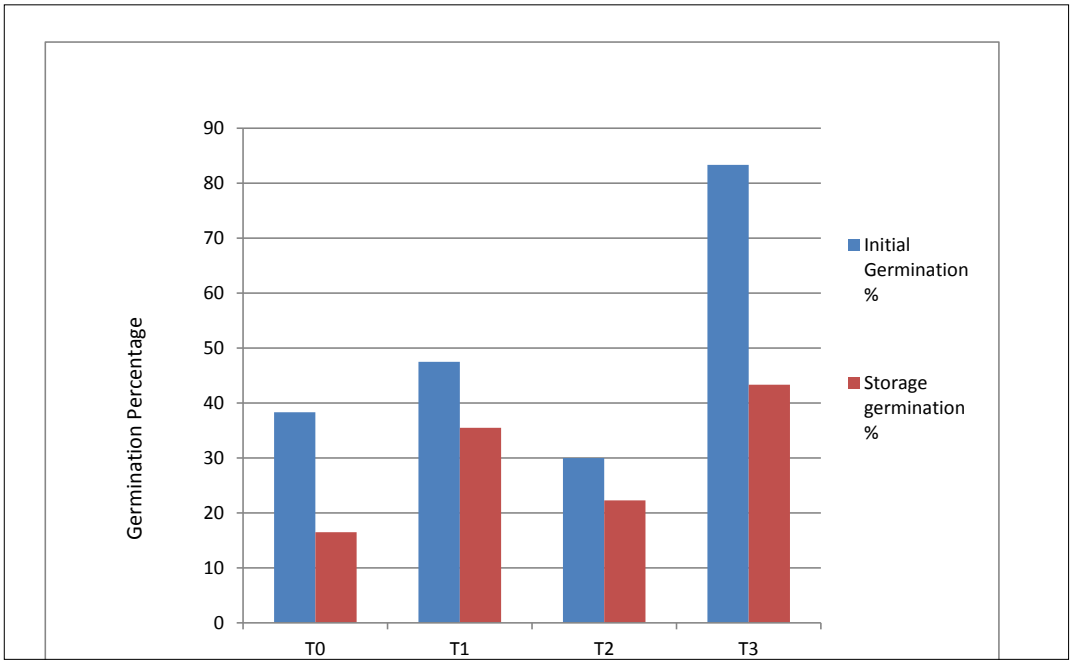

Fig 3: Effect of pre germination and germination % of fresh seeds and stored seeds of *ribes*

Natural regeneration of *E. ribes* is poor due to over-harvesting and exploitation, more fragmented population resulting in inbreeding, development of abortive embryos and the slow germination of fertile seeds which are small in size. On the other hand, artificial regeneration of this species is difficult due to its poor seed viability, low rate of germination and poor rooting from stem cuttings. However, lack of knowledge about its distribution, poor natural regeneration and unknown propagation techniques have resulted in the lack of availability of 'quality planting material' (QPM) for promoting cultivation (Anon, 2008). *E. ribes* is best propagated through seeds, though seed dormancy is the common factor that leads to long period for germination and low germination (Anon, 2011). The seed coats of *E. ribes* are very hard (Ved *et al.*, 2003). The present study investigated the most effective methods of enhancing germination of *E. ribes* through various pre-sowing treatments were examined. The present study explores the response of *E. ribes* seeds to various pre-sowing treatments as an attempt to produce quality planting stock and thus to enhance the cultivation protocol of this important plant. From the above results it can be concluded the viability and longevity can be maintained by storing the seeds in earthen pot and in low temperature.

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