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## CONSERVATION OF *ELEAODENDRON PANICULATUM* WIGHT & ARN. : AN ENDEMIC MEDICINAL TREE SPECIES USING SEED STORAGE TECHNIQUES

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### ABSTRACT

Seed storage of endemic tree species is a crucial conservation method. It serves as a genetic reservoir, safeguarding the unique genetic diversity of these species. These individual trees are exclusively confined to this geographical region. *Elaeodendron paniculatum* Wight & Arn., also known as *Cassine paniculate*, is a recalcitrant seed-bearing tree belonging to the Celastraceae family, which is endemic to the Western Ghats. It is used in traditional medicine to treat cancer and seed oil has antibacterial, analgesic and antioxidant properties. A field experiment was carried out during 2022-2023 at a Nursery in the College of Forestry, Ponnampet, to study the variability in the germination of the seed which is stored for four storage periods S1- Freshly collected seeds, S2- 30 days after collection, S3- 60 days after collection, S4- 90 days after collection in four different storage methods. M1-Control, M2-Carbendazin (1%), M3-Chlorpyrifos 20 % EC, M4- Carbendazin (1%) + Chlorpyrifos 20 % EC. The seeds were sowed in a split plot design with four replications each in different seed storage treatments in four storage periods. Visual observations and data were taken about germination parameters like germination percentage, mean daily germination, peak value, germination energy and germination value in all four storage periods. Results show the statistical difference in germination among different seed storage periods and storage methods under split-plot design. Among them, seeds treated with Carbendazim (1%) in the S1 storage period show the highest germination percentage and other parameters.

**Key words :** Viability, Germination, Storage, Endemic species.

### Introduction

Seeds are a crucial part of a plant's life cycle, containing all the genetic and biochemical attributes necessary to perpetuate the species from one generation to another. However, many tree species produce seeds at long intervals, ranging from a few to many years, making efficient storage of seeds indispensable to ensure a continuous and cost-effective supply of tree seedlings. This is a prerequisite for the success of any massive afforestation program, apart from conserving the tree's genetic resources (Umarani *et al.*, 2015).

Unlike orthodox seeds, recalcitrant seeds cannot be stored at low-moisture and low-temperature conditions.

However, some critical factors have been identified for storing recalcitrant seeds (King and Roberts, 1980) and most of the recalcitrant seeds are big and do not have a hard seed coat. Hence, the seeds are damaged during fall. The damaged seeds are highly vulnerable to fungal infection and lose viability rapidly. Minor damage to the seed coat is an entry point for the fungal attack, a severe case of seed deterioration under storage conditions where the fungi are active, *i.e.*, at seed moisture content above 5.7 per cent (Schmidt, 2000). Most small injuries do not cause an immediate loss in viability but may affect long-term storability (Moore, 1972).

*Elaeodendron paniculatum* Wight & Arn., also known as *Cassine paniculata* (Wight & Arn) Lobreau-

Callen, is a recalcitrant seed-bearing tree belonging to the Celastraceae family. It is commonly known as Mookarathi in Kannada, Thannimaram in Malayalam and Kanneermaram in Tamil. The tree is endemic to the Western Ghats-Anamali and Palakkad hills and is commonly distributed in the deciduous and semi-evergreen forests in coastal and interior Karnataka. It is used in traditional medicine and has various pharmacological activities. From the plants in this genus, flavonoids, terpenoids, cardiac glycosides and cardenolides have been isolated and it is revealed that antiviral, anti-HIV, anticancer, antiproliferative, antioxidant, antifungal, anti-inflammation and anti-diabetic activities in nature (Jennifer *et al.*, 2019). Trace of phytochemicals like triterpene has anticancer and anti-inflammatory activities (Nagase *et al.*, 2003).

The fruit is a drupe,  $2.5 \times 1.5$  cm, globes to ellipsoid, apiculate, freshly yellowish-green, and the seed is dicotyledonous, containing fatty acids like stearic (12.4%), palmitic (14.6%), oleic (41.5%) and linoleic acids (31.5%) (Murali *et al.*, 1965), the oil is commonly known as Celastrus oil used for burning of lamps. The oil content in *C. paniculatum* seeds (45.5%) was higher than that in *Jatropha curcas* (30-40%). Since the seed oil of *J. curcas* is popular in India for biodiesel production, *C. paniculatum* seed oil has a potential application as a biodiesel (Akbar *et al.*, 2009). The bark and root have medicinal properties and are used to treat snake bites (Pullani *et al.*, 2020). The wood of *Elaeodendron paniculatum* is moderately challenging, heavy, even, close-grained and it is helpful for building purposes, cabinet works, furniture, combs and picture frames. It is a moderately good fuel with a high calorific value.

## Materials and Methods

The study was conducted to identify the best storage treatment, *viz.*, storage period to increase the seed storability and storage treatments for prolonging the shelf life of *E. paniculatum* seeds. A field experiment was conducted from July 2022 to late April 2023 at a nursery in the College of Forestry, Ponnampet, Karnataka, India. The experiment was laid out in a Split plot design. Storage treatments were considered the main plot and the storage period was a subplot. There were four main plots and four subplots. All the treatments were replicated four times with 100 seeds per replication.

Seeds of *E. paniculatum* were collected from the Western Ghats of Kodagu district. The mature fruits were ground collected to avoid infected seeds. The seeds were then taken to the laboratory for further studies. Seeds were separated from the damaged seeds and then subjected to shade drying, the seeds were observed for

their seed attributes. After that, the seeds were subjected to the chemical mentioned above for two minutes and stored in polythene bags with a thickness of 100 gauge, which helps to avoid further moisture loss and further damage from external factors. During the experiment the following observations on germination were noted for further calculations:

**Days taken for initiation of germination or Germination initiation period (GIP) :** Number of days taken to initiate germination in each treatment was recorded.

**Germination Percentage (G.P.) :** The germination was recorded when cotyledons emerged from the germination bed and expressed in percentage.

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

## Mean daily germination (MDG)

$$\text{Mean daily germination} = \frac{\text{Cumulative per cent germination}}{\text{Total number of days}}$$

**Peak value (PV) :** Maximum mean daily germination reached at any stage of the germination period.

**Germination Energy (G.E.) :** The percentage of seeds in a given sample that have germinated up to the time of peak germination.

**Germination value (G.V) :** The index of combining seeds and the completeness of seed germination was calculated according to Czabator (1962).

$$\text{Germination value} = \text{Final daily speed of germination} \times \text{Peak Value}$$

The data on all the parameters were subjected to a two-way analysis of variance (ANOVA) based on a Split plot design using the OP Stat. online statistical portal. Data on proportions were arc-sine transformed, and count data were square root altered and subjected to analysis of variance.

## Results and Discussion

The seed attributes like seed colour, shape, size, weight, moisture and numbers of seeds were tabulated in Table 1. The seeds of *E. paniculatum* manifest epigeal germination, characterised by a swift elongation of the hypocotyl that ascends archly, elevating the cotyledons above the soil surface. The occurrence of paired seedlings during this germination process indicates the polyembryonic attribute inherent to these seeds.

### Effect of storage treatments on germination parameters

The study reveals that the seeds stored under carbendazim 1% (M2) show the maximum germination,

**Table 1** : Seed attributes of *Elaeodendron paniculatum*.

Seed parameter	Range	Mean $\pm$ SD
Seed colour	Brown	–
Seed shape	Globes to ellipsoid	–
Seed length (mm)	16.02 - 31.04	23.02 $\pm$ 2.93
Seed diameter (mm)	14.4 - 29.59	19.94 $\pm$ 3.75
Seed weight (100 seeds) (g)	300.17 - 362.48	331.32 $\pm$ 11.12
Moisture content (%)	52.76 - 76.42	64.59 $\pm$ 1.19
Number of seeds kg <sup>-1</sup>	334	–

*oxysporum* growth at 1% concentration among seven fungicides. This improvement may be due to suppression of pathogenic populations and other factors. The study also suggests that toxicants from pesticide application may inhibit protein and carbohydrate synthesis. According to the study, it can be seen that the systemic fungicides, viz., Carbendazim application, gave the best germination, growth, and biomass stimulating effect.

#### Effect of storage periods on germination parameters

The study reveals the decreased germination of seeds with the increased storage period in the present study. i.e., from S1 (Freshly collected seeds) to S4 (90 days

**Table 2** : Germination parameters of *Elaeodendron paniculatum* influenced by seed treatments.

Seed storage chemicals	Days taken for initiation of germination (days)	Germination percentage (%)	Peak value (%)	Mean daily germination (%)	Germination energy (%)	Germination value (%)
M <sub>1</sub>	26.34(5.13) *	23.19(28.79) <sup>b**</sup>	0.46(3.90) <sup>b</sup>	0.31(3.23) <sup>b</sup>	21.23(27.44) <sup>a</sup>	0.26(2.96) <sup>b</sup>
M <sub>2</sub>	26.04(5.10)	32.265(34.61) <sup>c</sup>	0.64(4.60) <sup>c</sup>	0.40(3.65) <sup>c</sup>	27.95(31.92) <sup>b</sup>	0.40(3.64) <sup>c</sup>
M <sub>3</sub>	25.94(5.09)	18.901(25.77) <sup>a</sup>	0.35(3.40) <sup>a</sup>	0.23(2.75) <sup>a</sup>	17.89(25.02) <sup>a</sup>	0.13(2.11) <sup>a</sup>
M <sub>4</sub>	26.02(5.10)	30.39(33.45) <sup>c</sup>	0.58(4.39) <sup>c</sup>	0.39(3.59) <sup>bc</sup>	27.93(31.90) <sup>b</sup>	0.33(3.31) <sup>bc</sup>
S.Em $\pm$	<b>0.010</b>	<b>0.666</b>	<b>0.091</b>	<b>0.115</b>	<b>0.851</b>	<b>0.155</b>
CD (0.05)	NS	<b>2.16</b>	<b>0.294</b>	<b>0.375</b>	<b>2.761</b>	<b>0.503</b>

**Table 3** : Germination parameters of *Elaeodendron paniculatum* influenced by seed storage period.

Seed storage period	Days taken for initiation of germination (days)	Germination percentage (%)	Peak value (%)	Mean daily germination (%)	Germination energy (%)	Germination value (%)
S1	22.92(4.78) <sup>a*</sup>	40.67(39.62) <sup>c</sup>	0.87(5.35) <sup>c***</sup>	0.54(4.24) <sup>c</sup>	39.10(38.70) <sup>c</sup>	0.74(4.94) <sup>c</sup>
S2	26.77(5.07) <sup>b</sup>	27.74(31.78) <sup>b</sup>	0.44(3.83) <sup>b</sup>	0.31(3.19) <sup>b</sup>	22.88(28.58) <sup>b</sup>	0.20(2.59) <sup>b</sup>
S3	26.91(5.18) <sup>bc</sup>	23.57(29.04) <sup>b</sup>	0.46(3.90) <sup>b</sup>	0.30(3.18) <sup>b</sup>	21.22(27.43) <sup>b</sup>	0.21(2.66) <sup>b</sup>
S4	26.97(5.19) <sup>c</sup>	14.24(22.17) <sup>a</sup>	0.31(3.22) <sup>a</sup>	0.20(2.62) <sup>a</sup>	13.51(21.57) <sup>a</sup>	0.10(1.82) <sup>a</sup>
S.Em $\pm$	<b>0.034</b>	<b>0.998</b>	<b>0.0.120</b>	<b>0.120</b>	<b>1.013</b>	<b>0.171</b>
CD (0.05)	<b>0.098</b>	<b>2.874</b>	<b>0.347</b>	<b>0.344</b>	<b>2.917</b>	<b>0.493</b>

which was on par with the treatment combination of carbendazim 1% and chlorpyrifos 20% EC (M4). The least was recorded in chlorpyrifos 20% EC alone. The presence of high moisture content in the seeds possess significant risk of fungal growth during the storage. The use of Carbendazim has eliminated this risk, which has led to maintaining clean and hygienic conditions.

Ergin *et al.* (2021) studied fungicide effectiveness on seedling growth and germination percentage. Results showed no adverse effects and higher germination percentages were obtained with fungicides. Thiram and metalaxyl showed more rapid germination. Carbendazim control pathogens and promotes proper seed emergence.

The study confirms Patil *et al.* (2015) findings that thiram and carbendazim effectively inhibit *Fusarium*

after collection). The highest germination percentage (40.67%) was recorded from freshly collected seeds. The germination was decreased by 25 per cent from S1 to S4 (Table 3). This may be attributed to the fact that the presence of high moisture content in the seeds along with maximum seed stored food and high nutrient concentration in the embryo (Thirtha *et al.*, 2013)

The increase in storage period resulted in a decline in the ability of seeds to germinate. As reported by Walter *et al.* (2005), the length of storage time is strongly influenced by environmental and genetic factors such as storage temperature and seed moisture content.

#### Interaction effects of storage treatments and storage periods on germination parameters

The study revealed a significant difference in the

**Table 4 :** Interaction effect of storage treatments and storage period on germination percentage.

Storage periods \ Storage treatments	M1	M2	M3	M4	Sub factor mean
S1	48.20(43.97) <sup>a**</sup>	50.72(45.41) <sup>a</sup>	29.48(32.88) <sup>a**</sup>	34.93(36.23) <sup>a</sup>	39.62
S2	22.86(28.56) <sup>b</sup>	32.37(34.68) <sup>b</sup>	19.69(26.34) <sup>b</sup>	37.13(37.54) <sup>a</sup>	31.78
S3	17.821(24.97) <sup>b</sup>	25.84(30.55) <sup>b</sup>	17.92(25.04) <sup>b</sup>	33.90(35.61) <sup>a</sup>	29.04
S4	9.2021(17.65) <sup>c</sup>	21.74(27.79) <sup>b</sup>	10.38(18.88) <sup>c</sup>	17.11(24.43) <sup>b</sup>	22.17
<b>Main factor mean</b>	28.79	34.61	25.77	33.45	
<b>S.Em ±</b>	<b>1.33</b>				
<b>CD (0.05)</b>	<b>5.84</b>				

interaction of storage treatments and storage periods. The higher seed germination was recorded in the freshly sown seeds treated with Carbendazim 1% (50.72%) (Table 3). The germination rate was influenced by an interaction between the fungicide application treatment and stratification duration; the results reveal that fungicide application significantly slowed the germination but did not influence germination speed at any other stratification length (Table 3).

### Conclusion

Seed storage and seedling production are crucial for healthy tree species growth. Proper storage ensures the availability of seeds for seedling production, while good seedling production techniques produce high-quality seedlings at a reasonable cost. The study suggests that *E. paniculatum* seeds experience a decline in viability during storage. Nevertheless, these seeds can be stored for a maximum of four months, albeit with a notable decrease in germination percentage. The choice of storage treatment is emphasized and should be determined by the duration of storage. For a storage period of one month, M2 treatment (Carbendazim 1%) is recommended due to the susceptibility of fresh seeds to fungal attack caused by high moisture content. In cases where storage extends beyond a month, the preferred treatment is M4 (Carbendazim 1% + Chlorpyrifos 20% EC) to address both the prolonged storage period and potential insect threats. Consequently, for extended storage, it is advised to use insecticides such as chlorpyrifos. Appropriate seed storage techniques are essential for the conservation of genetic diversity, plant biodiversity and the overall sustainable conservation. They contribute significantly to global efforts in environmental conservation, food security, and scientific research.

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