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Effect of Storage Containers and Media on Germination of *Dysoxylum malabaricum* Bedd. In Hilly Zone of Karnataka

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***Abstract*:** The primary objective of seed storage is to preserve viability with minimal loss. However, *ex-situ* storage of recalcitrant seeds is challenging, as they lose viability within days to a few months due to dehydration. This study aimed to extend seed longevity of *Dysoxylum malabaricum*, an endangered evergreen tree, by evaluating different storage containers and treatments. Seeds collected from natural populations were stored in three containers-polythene bags, poly pots and earthen pots-under seven treatments: control, carbendazim (0.5%), chlorpyrifos (2%), abscissic acid (0.005%), carbendazim (0.5%) + chlorpyrifos (2%), carbendazim (0.5%) + chlorpyrifos (2%) + abscissic acid (0.005%), and cow dung + ash. Poly pots were the most effective, and maintained viability for 45 days with 63.18% germination. Among remaining treatments, Chlorpyrifos (2%) was superior, retaining viability for 45 days with 94.81% germination. The highest germination (97.78%) was in seeds stored in polythene bags treated with chlorpyrifos (2%) for 45 days. Appropriate storage treatments and container selection can thus significantly prolong the viability of *D. malabaricum* seeds, aiding the conservation and sustainable use of this endangered species.

***Keywords*:** *Dysoxylum malabaricum*, Recalcitrant seeds, Seed viability, Storage containers, Germination

Dysoxylum malabaricum Bedd. is an endangered tree species belonging to the family Meliaceae. The species is commonly called as “White cedar” and is endemic to Western Ghats, distributed in the evergreen forests from Uttar Kannada southwards up to Kerala at an elevation of 2000 m above mean sea level (Swaminath et al., 2003). The timber of the species is highly valued for its luster, aroma and durability. The species is an important ingredient in Ayurvedic medicines and the leaf extract is used to kill malarial vector (Nathan et al., 2005). The wood oil is remedy for ear, eye diseases and decoction of the wood is used in the treatment of rheumatism. The fruits ripen during May to July are food for many birds including the Malabar grey hornbill (Bachan 2006). The prime objective of storage is to preserve the seeds with their viability as little impaired as possible. Stored seeds are of very little value if on sowing they fail to produce healthy and vigorous plants. Ex-situ storage of recalcitrant seeds poses a major challenge, as they undergo dehydration during storage and rapidly lose viability, often within days to a few months. Unlike orthodox seeds, recalcitrant seeds cannot be stored using conventional methods because their germinability declines once the seed moisture content drops below relatively high critical levels. For *D. malabaricum*, an endangered and highly valued species, information on seed germination and storage is extremely limited. Nair et al. (2002) reported that freshly sown seeds of *D. malabaricum* exhibited only 20% germination without any pre-sowing treatments, and that the seeds lost viability within 7–10 days. Given this knowledge gap, the present study was undertaken to evaluate suitable germination and storage methods for *D. malabaricum* seeds, with the aim of improving their viability and conservation potential.

MATERIAL AND METHODS

The present study was carried out at nursery of College of Forestry, Ponnampet, located in the hilly zone (Zone 9), Kodagu district of Karnataka state. Nursery is situated at 12.142983°N latitude; 75.938617°E longitudes and at an altitude of 856 m above Mean Sea Level (MSL). The study area is under tropical humid climate with monthly mean maximum temperature varied from 31ºC to 37 ºC and mean monthly minimum temperature varying from 24 ºC to 27 ºC. *D. malabaricum* seeds were collected from natural populations of Western Ghats during May to July. The collected fruits were depulped and the depulped seeds were subjected to seven seed storage treatments. The treated seeds were stored in three different containers for four different storage periods (Table 1). The experiment was laid out in split-split plot experimental design by considering seed storage containers as main plot, seed storage treatment as sub plot and storage period as sub-sub plot in three replications (30 seeds per replication). The following observations were recorded in each treatment.

Daily germination count: Daily germination was recorded.

Germination per cent: The germination was recorded when cotyledons emerged out of sand on seed bed and expressed

in percentage. Germination per cent values were subjected to arc sine transformation.

Statistical analysis: The data were analysed in split-split plot design in SPSS ver. 27.

RESULTS AND DISCUSSION

No significant differences were observed among treatments (Table 2). However, the interaction effect of container and treatment indicated that the seeds without treatment (T1) when stored in poly pot (M2) gave maximum germination (87.22%) with a mean germination 56.19 per cent followed by seeds without treatment stored in earthen pot (79.44%) with a mean germination of 48.25 per cent. The interaction effect between storage containers and storage duration was non-significant (Table 3). However, the highest germination (63.18%) was when seeds were stored for 45 days (D4) in poly pots (M2), with a mean germination of 56.19%. Across all storage periods, poly pots consistently maintained higher germination compared to other containers, indicating their effectiveness in reducing seed viability loss. These findings suggest that recalcitrant seeds of *D. malabaricum* can be stored up to 45 days when appropriate pre-sowing treatments and suitable storage containers are adopted. Anilkumar (2009) also observed that freshly sown *D. malabaricum* seeds showed 50.87% germination, while seeds stored for 10 days exhibited only 20% germination. Studies on other recalcitrant species have also emphasized the role of storage conditions in prolonging seed viability. Priyanto et al. (2022) found that *Myristica fragrans* seeds stored for 12 days in plastic standing pouch packaging recorded 56.25% germination. Anandalakshmi et al. (2005) demonstrated that polybags of varying thickness, irrespective of pores, could prolong the viability of *Syzygium cuminii* seeds up to five months at 20°C.

The significant effect of different treatment and storage period and on germination per cent was observed in current study (Table 4). chlorpyrifos 0.5% resulted in maximum germination (94.81%) was recorded under D4 (seeds stored for 45 days) and least (8.89 %) was in D1 (seeds when sown fresh) when treated with carbendazim (0.5 %) + chlorpyriphos (2%). Maximum mean germination (79.44%) observed in control treatment (T1) and minimum mean germination (21.30%) was with cow dung + (T7).

The interaction effect of storage container, storage media and storage period on germination (percentage) of *D. malabaricum* seeds was also non-significant in differentiating the effects (Table 5). However, maximum germination (97.78 %) was observed in seeds stored in polythene bag (M1) and treated chlorpyrifos (2 %)] up to 45 days followed by seeds without treatment sown after 15 days of storage (93.33 %). No germination was recorded in seeds stored for 45 days in earthen pot after treated with carbendazim 0.5 %. Poly pots, in particular, proved to effective for *D. malabaricum*, allowing storage for up to 45 days without severe viability loss. Koppad and Umarbhadsha (2006) reported that carbendazim promoted proper seed emergence by controlling pathogens, while Sharma et al. (2004) observed that carbendazim provided up to 90% control of pre-

Table 1. Details showing different storage treatments

Main plot (storage container)	Sub plot (storage media)	Sub-sub plot (storage periods)
M1: Earthen pots	T1: Control	D1: Freshly collected seeds
M2: Poly pots	T2: Carbendazim (0.5%)	D2: 15 Days after collection
M3: Polythene bags	T3: Chlorpyrifos (2 %)	D3: 30 Days after collection
	T4: Abscissic acid (0.005%)	D4: 45 Days after collection
	T5: Carbendazim (0.5%) + Chlorpyrifos (2 %)	
	T6: Carbendazim (0.5 %) + Chlorpyrifos (2 %) + Abscissic acid (0.005%)	
	T7: Cow dung + Ash	

Table 2. Interaction effect of storage container and storage treatment on germination (percentage) of *D. malabaricum* seeds

Sub plot/ Main plot	T1	T2	T3	T4	T5	T6	T7	Mean T
M1	71.67 (57.84)	36.11 (36.94)	66.11 (54.40)	21.11 (27.35)	33.89 (35.60)	52.22 (46.27)	17.78 (24.94)	42.70 (40.80)
M2	87.22 (69.06)	42.78 (40.85)	61.11 (51.42)	52.22 (46.27)	48.33 (44.04)	70.00 (56.79)	31.67 (34.25)	56.19 (48.56)
M3	79.44 (63.04)	21.11 (27.35)	63.89 (53.06)	52.22 (46.27)	31.11 (33.90)	75.56 (60.37)	14.44 (22.34)	48.25 (44.00)
Mean M	79.44 (63.04)	33.33 (35.26)	63.70 (52.95)	41.85 (40.31)	37.78 (37.93)	65.93 (54.29)	21.30 (27.48)	
CD (p=0.05)				NS				

NS = Not significant at 5% level of significance

emergence mortality and 78% control of post-emergence mortality, contributing to improved germination. Similarly, in *Lophopetalum wightianum*, Maheswarappa et al (2020) demonstrated that a combination of carbendazim (0.25%) and chlorpyrifos (0.89%) treatment in earthen pot storage enhanced germination up to 64.61% after 60 days. In the present study, chlorpyrifos (2%) treatment was particularly effective in preventing pest infestation during storage, thereby enabling higher germination percentages in *D. malabaricum.* Similar results were reported for few recalcitrant seeds, like those of *Myristica malabarica*, remain viable for short periods under natural conditions but can be stored for up to a year in polythene bags at specific temperature and humidity levels (Kumar et al., 2002). Hosur et al. (2023) reported significant variation in seed germination (28.89–98.33%) among seven seed sources of *Dysoxylum binectariferum*, with seeds collected from Jog (Karnataka) exhibiting superior performance across all germination traits. Storage conditions, including container type, temperature, and duration, significantly impact seed germination and viability across different medicinal plant species (Tiwari et al., 2022).

The findings hold significant implications for the conservation of *D. malabaricum*, an endangered and ecologically valuable species. By identifying suitable storage containers and treatments to prolong seed viability, this study provides a practical method for short-term ex-situ conservation and nursery propagation. Such techniques can aid forest departments, and restoration programs in maintaining viable seed stocks for reintroduction and habitat rehabilitation. Moreover, these practices can be extended to other recalcitrant-seeded species of ecological and economic value, thereby strengthening in-situ and ex-situ conservation strategies and contributing to the long-term preservation of tropical forest biodiversity. The study faced certain limitations, including a short storage duration of 45 days and limited control over temperature and humidity under ambient conditions. Seed availability was restricted due to the endangered status and sporadic fruiting of *D. malabaricum*. Additionally, seeds were collected during a

Table 3. Interaction effect of storage container and storage period on germination (percentage) of *D. malabaricum* seeds

Storage period/Storage container	D1	D2	D3	D4	Mean D
M1	45.71 (42.54)	40.32 (39.42)	42.86 (40.89)	41.91 (40.34)	42.70 (40.80)
M2	50.16 (45.09)	56.51 (48.74)	54.92 (47.82)	63.18 (52.64)	56.19 (48.56)
M3	42.86 (40.89)	48.89 (44.36)	47.62 (43.64)	53.65 (47.09)	48.25 (44.00)
Mean M	46.24 (42.84)	48.57 (44.18)	48.47 (44.12)	52.91 (46.67)	
CD (p=0.05)			NS		

NS = Not significant

Table 4. Interaction effect of storage treatment and storage period on germination (percentage) of *D. malabaricum* seeds

Storage period/ Storage media	D1	D2	D3	D4	Mean D
T1	86.66 (68.58)	93.33 (75.03)	77.04 (61.37)	60.74 (51.20)	79.44 (63.04)
T2	50.37 (45.21)	34.07 (35.71)	31.85 (34.36)	17.04 (24.38)	33.33 (35.26)
T3	20.00 (26.56)	60.00 (50.77)	80.00 (63.43)	94.81 (76.84)	63.70 (52.95)
T4	46.67 (43.09)	62.96 (52.51)	23.70 (29.13)	34.08 (35.71)	41.85 (40.31)
T5	8.89 (17.35)	11.85 (20.14)	41.48 (40.10)	88.89 (70.53)	37.78 (37.93)
T6	84.44 (66.77)	60.74 (51.20)	68.15 (55.64)	50.37 (45.21)	65.93 (54.29)
T7	26.67 (31.09)	17.04 (24.38)	17.04 (24.38)	24.45 (29.63)	21.30 (27.48)
Mean T	46.24 (44.18)	48.57 (44.18)	48.47 (44.12)	52.91 (46.67)	
CD (p=0.05)			(23.92)		

Parenthetical values are arc sine transformed values; NS = Not significant

Table 5. Interaction effect of storage container, storage treatment and storage period on germination (percentage) of *D. malabaricum* seeds

Storage period	T1	T2	T3	T4	T5	T6	T7
Polythene bag (M1)							
D1	86.66 (68.58)	66.67 (54.74)	26.67 (31.09)	33.33 (35.26)	0.00 (0.00)	84.44 (66.77)	22.22 (28.12)
D2	93.33 (75.03)	42.22 (40.53)	60.00 (50.77)	26.67 (31.09)	6.67 (14.96)	46.67 (43.09)	6.67 (14.96)
D3	71.11 (57.49)	28.89 (32.51)	80.00 (63.43)	11.11 (19.47)	40.00 (39.23)	48.89 (44.36)	20.00 (26.57)
D4	35.56 (36.61)	6.67 (14.96)	97.78 (81.43)	13.33 (21.42)	88.89 (70.53)	28.89 (32.51)	22.22 (28.13)
Poly pot (M2)							
D1	86.66 (68.58)	42.22 (40.53)	11.11 (19.47)	55.56 (48.19)	26.67 (31.09)	84.44 (66.77)	44.45 (41.81)
D2	93.33 (75.03)	37.78 (37.93)	60.00 (50.77)	88.89 (70.53)	24.44 (29.63)	68.89 (56.10)	22.22 (28.13)
D3	84.45 (66.77)	46.67 (43.09)	80.00 (63.43)	33.33 (35.26)	53.33 (46.91)	64.45 (53.40)	22.22 (28.12)
D4	84.45 (66.77)	44.44 (41.81)	93.33 (75.04)	31.11 (33.90)	88.89 (70.53)	62.22 (52.07)	37.78 (37.93)
Earthen pot (M3)							
D1	86.66 (68.58)	42.22 (40.53)	22.22 (28.12)	51.11 (45.64)	0.00 (0.00)	84.44 (66.77)	13.33 (21.42)
D2	93.33 (75.03)	22.22 (28.13)	60.00 (50.77)	73.34 (58.91)	4.44 (12.17)	66.67 (54.74)	22.22 (28.12)
D3	75.56 (60.37)	20.00 (26.57)	80.00 (63.43)	26.67 (31.09)	31.11 (33.90)	91.11 (72.66)	8.89 (17.35)
D4	62.22 (52.07)	0.00 (0.00)	93.33 (75.04)	57.78 (49.48)	88.89 (70.53)	60.00 (50.77)	13.33 (21.42)
CD (p=0.05)				NS			

single season, and only a few chemical and traditional treatments were tested. Future research should focus on long-term storage conditions, optimization of moisture content and physiological and molecular responses influencing viability loss. Exploring eco-friendly bio preservatives and low-temperature storage methods may further enhance the conservation potential of *D. malabaricum* seeds.

CONCLUSION

This study demonstrated that appropriate storage conditions and treatments can effectively maintain the viability of recalcitrant seeds of *D. malabaricum*. Seeds treated with chlorpyrifos (2%) and stored in polythene bags achieved the highest germination even after 45 days, whereas stored in earthen pots with carbendazim (0.5%)did not germinate. These findings provide valuable insights for developing short-term conservation strategies and enhancing seedling availability for restoration initiatives.

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