Unveiling theseedstoragebehavior for conservationofIndian olibanum:*Boswelliaserrata*Roxb.ExColeb.

Abstract

The study attempted to understand the storage behavior of *Boswellia serrata*Roxb. seeds; for which seeds were collected from three locationsinthe Shivalik region of the Himalayasduring June 2021.Across the locations, the freshly collected seeds had an average moisture content (MC) of 10.06% and exhibited an average germination of 73%. When the seeds were further desiccated to MC 7%; the average germination was reduced to 51.33%. After this, desiccated seeds were stored hermetically at +5^{o}C temperature for a period of 24 months, and periodic germination trials were conducted at an interval of 4 months. The average germination percentage was reducedfrom 73% to 13.67% during the storage period; the seed deterioration process and low temperature-induced chilling injury could be attributed tothe reduced germination rate.Our results also revealed that the Pinjore seed source outperforms the other two sources in germination rate with an adjusted margin mean of 38.13. The insights derived from the study can aid in designing appropriate protocols for the conservation of the studied species.

Keywords:*Germination, Seed Moisture Content, Storage, Conservation,Boswellia serrataRoxb.Ex Coleb.*

1. Introduction

The vagaries of climate change spread across the living biome of this planet, and kingdom Plantae is no exception to that. The studies reported that climate change will threaten the existence of many members of the biodiversity. In order, to protect biodiversity from the reported ill effects of climate change, the ex-situ conservation is often put forth. However, for proper implementation of the conservation programs, knowledgeofseedstoragebehaviourisessential(WyseandDickie, 2017).Basedonseedstoragebehaviour,threetypesofseedsarerecognizedandoftenused:orthodox,recalcitrant,andintermediateororthodoxwithrestricteddesiccationability(Schmidt, 2000). Baseline data for seed storage behavior is lacking for most of the forest tree species. *Boswelliaserrata* is one such economically important tree species.

*Boswelliaserrata*Roxb.Ex Coleb. (Burseraceae) is a moderate to large-sized deciduous tree endemic to India, Africa, and the Arabian Peninsula. It is the source of a most important gum-resin called ‘salai guggul or ‘Indian olibanum’ obtained from the bark after injury and is used for the treatment of rheumatoid arthritis, osteoarthritis, Crohn’s disease, and asthma (Brendler *et al.*2018; Mishram*et al.*2020). Conventionally, the trees are propagated by seeds. However, poor fruit setting under open pollination (2.6 to 10%), inadequate production of viable seeds (Joshi *et al*.1981), and scanty seed germination (10–20%) restrict the distribution and limits the presence of this species in its natural habitat (Ghorpade *et al.* 2010).Due to the difficulties in seed germination, regeneration, and destruction, the species' natural abundance has decreased.

For conservation biologists, foresters, and small industries, tree species preservation and sustainable exploitation have emerged as major issues. A major constraint for developing effective conservation strategies is the need for more information on their seed storage behavior. To the best of our knowledge, very little information is available on this species' seed storage behavior and germination. Thus, in this study, we aimed to investigate the seed storage behavior of *Boswellia serrata* which can aid in the formulation of strategies for conservation.

2. Materialsandmethods

The fruit is 1.3 cm long, swollen, and trigonous with three valves and 3-heart-shaped, 1-seeded pyrenes which are winged along the margins (Figure 2). Fruits were manually harvested during June 2021 from three sources of Shivalik Himalayas (i.e. Morni hills: N 30^{o}40’50.5” and E 77^{o}04’49.1”;Kalesar National Park:N 30^{o}23’50.91” and E 77^{o}30’23.41”; and Pinjore:N 30^{o}44’51.0” and E 76^{o}58’58.9”) in Haryana, India. A total of 1000 fruits were collected from 8–10 trees (at least 100 m apart) at each location.Fruits were transported in gunny bags to the Forest Tree Seed Laboratory, FRI (Forest Research Institute), Dehradun (N 30^{o}28’11.72” and E 78^{o}03’36.99”). Seeds were extracted from fruits and cleaned. Seed samples without apparent physical damage or insect infestation were selected for the experiment.

Figure 1. Germinated seeds of *Boswelliaserrata*Roxb. Ex Coleb.

Figure 2. De-winged and Winged seeds of *Boswelliaserrata*Roxb. Ex Coleb.

2.1 Morphological analysis

For biometric determination 10 seeds were selected randomly in 4 replications for individual measurement of length and widthusing a digital caliper with a precision of 0.001 mm. The length was measured from the base to the apex, excluding the stalk and the width was measured in the midline of the fruits and seeds (Cheib, 2009). After measurement of length and width, 100 seeds were weighed in 4 replications on an analytical balance accurate to 0.001 g. The quantitative characteristic data were submitted to descriptive analysis, calculated with Excel application, arithmetic mean and standard deviation of seeds.

2.2 Germinationtest

To study the role of Seed Moisture content (SMC) in germination, we germinated seeds at initial moisture content, which varies from 9.25% to 11.60%. For medium to long-term storage of seeds, moisture content should be as low as possible (i.e., below 8-10%). Further periodic germination trials were carried out for two years in laboratory conditions, according to the International Seed Testing Association procedures (ISTA, 2010). Seeds were considered as germinated upon emergence of radical (1 cm in length).

2.3 Desiccationstudy

About2000seeds(eachseedsource)weredesiccatedtosafemoisturecontentof7%frominitialmoisturecontentof9.25%(Pinjore),9.34%(KalesharNational Park)and11.60%(MorniHills)usingsilicageldryingmethod.Thedesiredmoisturecontentofeachsourcesamplewasmonitoredbasedonthebelowgiven equation (HongandEllis, 1996).

$$\text{Weight of seedlot (g)at MCd\%} = \frac{100 - \text{MCi\%}}{100 - \text{MCd\%}} \times \text{ISW(g)}$$

With MCd being the desired moisture content, MCi the initial moisture content, and ISW the initial seed lot weight. After this, whole seeds' Moisture content (MC) was verified following the low constant oven drying method (ISTA, 2010). We randomly selected 1gm seeds each in 4 replications and dried them at 103ºC for 17h in a hot air oven. Seed MC was calculated on a fresh mass basis.

2.4 Storagetrials

Storage trials were conducted with desiccated seeds in an airtight plastic jar kept at +5ºC in a walk in seed storage chamber. The viability of the stored seeds was monitored by germination tests, according to the International Seed Testing Association procedures (ISTA, 2010)**,** at intervals of 4 months for two years.

2.5 Statisticalanalysis

To test the differences between the treatment and the collection source, we employed two factorial ANOVA (Afifi and Azen, 1979). After running the ANOVA, by witnessing the statistical significance, we calculated adjusted marginal means for both treatment and source of seed collection to interpret meaningfully and robustly. The above-mentioned analysis was performed using *STATA 17.0.*

3. Resultsanddiscussion

Table1.Morphological parameters of *Boswellia serrata* seeds across different locations

Particulars	Seed Length (mm)	Seed Width (mm)	100 Seed weight (gm)
Morni Hills	8.6±0.15	7.06±0.06	4.24±0.02
Kalesar National Park	6.63±0.13	5.8±0.04	3.36±0.03
Pinjore	8.42±0.11	6.35±0.10	4.15±0.03
Average	7.88±0.13	6.4±0.07	3.92±0.03

The fruit is swollen and trigonous with three valves, and 3-heartshaped, 1-seeded pyrenes which are winged along the margins. From Table 1 we can observe that seed length, seed width and 100 seed weight was found to be highest for Morni Hills. However, all these values were found to be lowest for Kalesar National Park. We determined that for Kalesar National Park, seed length, seed breadth, and 100 seed weight were 15.86%, 9.37%, and 14.28% less than average values.These seed dimensions can be considered as important traits for early selection of seed sources. Significant positive correlation between seed size and seedling growth has earlier been reported in Sweet Chestnut *(Castanea sativa Mill.)* (Tumpa*et al*. 2021).

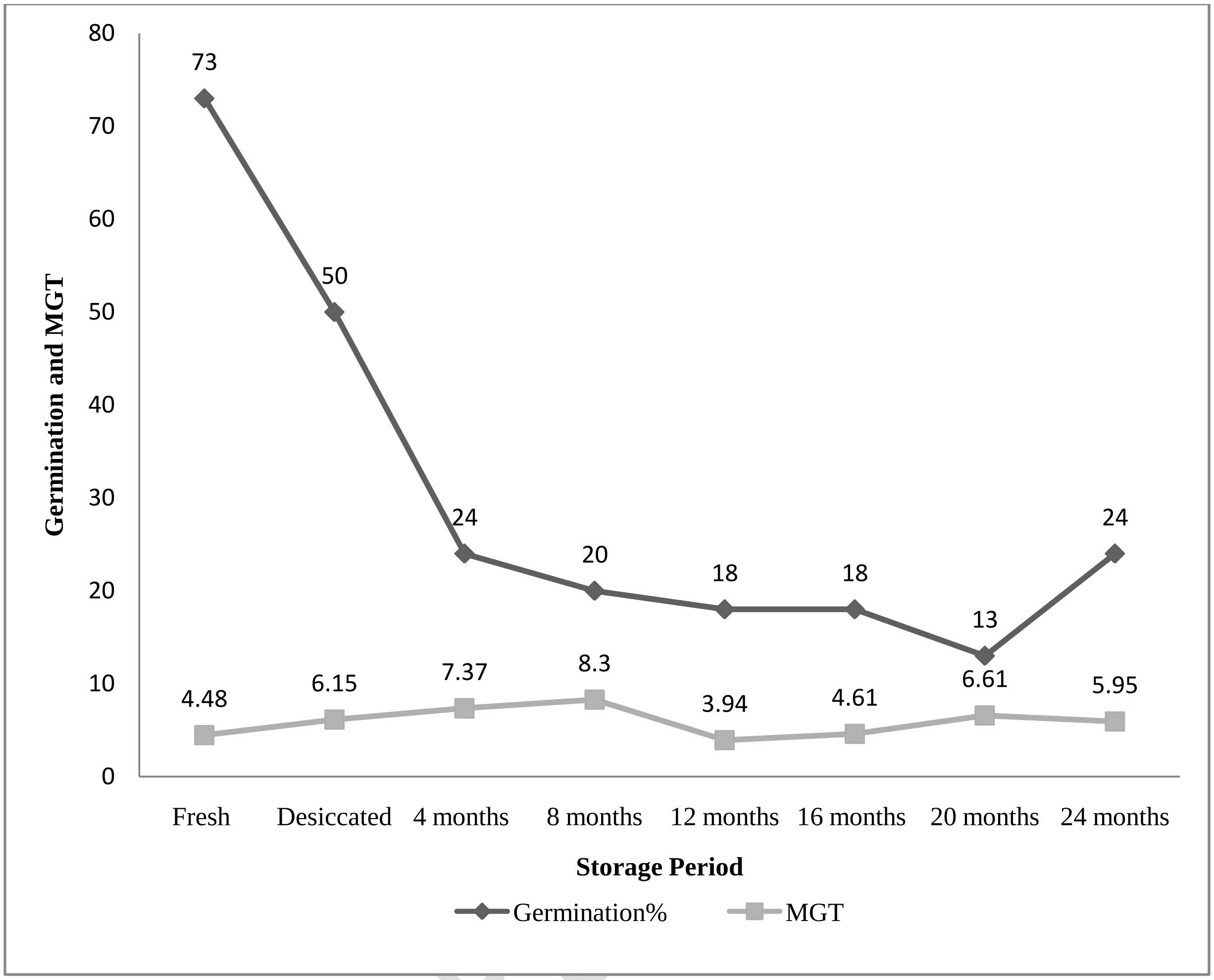

Figure 3. Effect of storage period on Germination and Mean Germination Time (MGT) of *Boswellia serrata* seeds collected from Morni hills, Haryana.

Duringhermeticstorageat+5^{o}Ctemperature,seedmoisturecontentvariedlittle.Whatevertheperiodofstorage,theextremevaluesfoundunderexperimentalconditionsremainedclosetothemoisturecontentmeasuredafterdesiccationi.e.7%.When*Boswelliaserrata*seedsfromMornihillswerestoredhermeticallyat+5^{o}Ctemperature,therateofgerminationwasdecreasedfrom73%to24%andMeanGerminationTime increased from4.48to5.20days over the storage period of 24 months (Figure3).

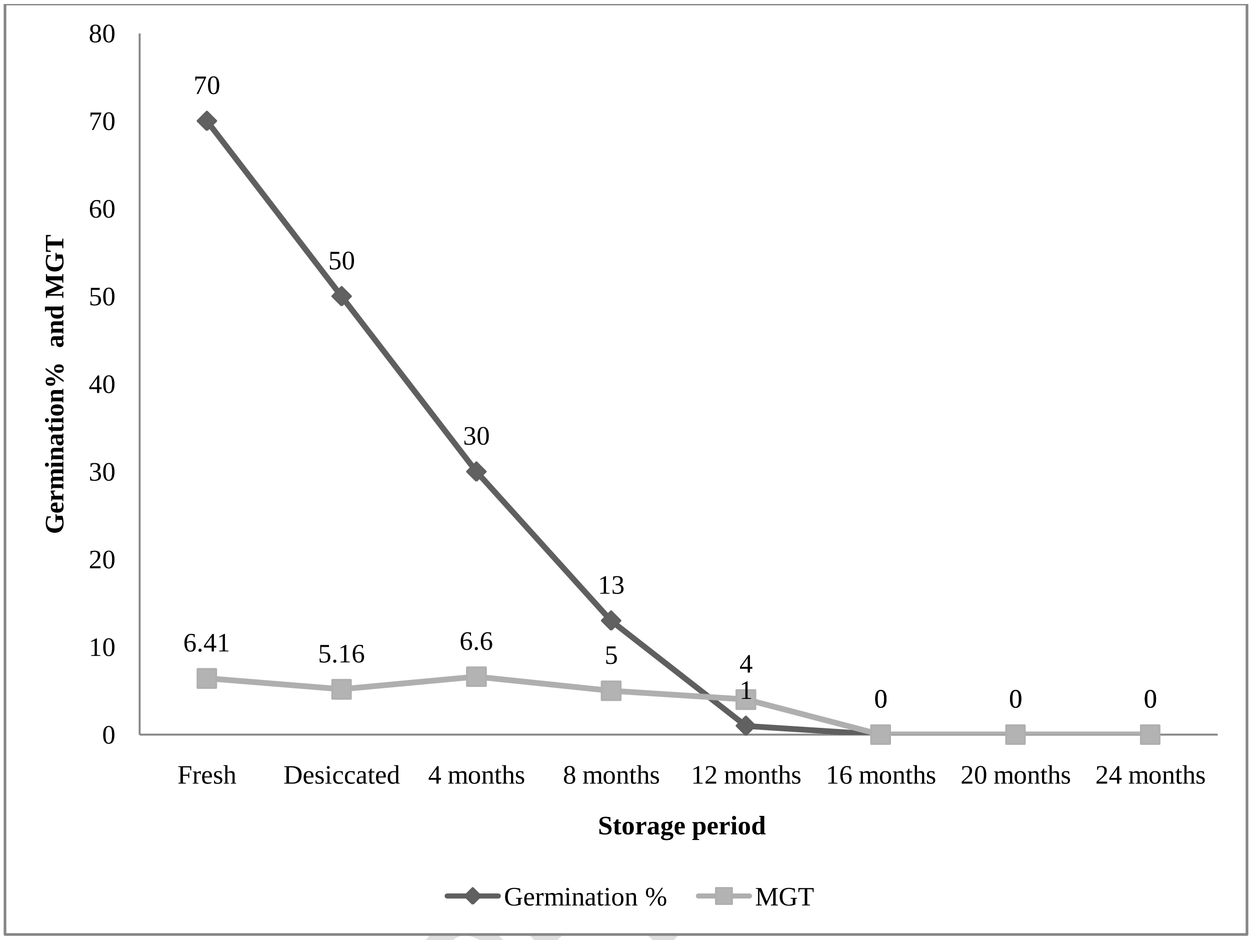

Figure 4. Effect of storage period on Germination and Mean Germination Time (MGT) of *Boswellia serrata* seeds collected from Kalesar NP, Haryana.

WhereasBoswelliaserrataseedsfromKalesarNationalParkdidnotsurviveformorethan12monthsat+5^{o}Ctemperature.Therateofgerminationwasdecreasedfrom70%to1%andMeanGerminationTimealsodecreasedfrom6.41to4days duringstorageperiodof12months.After12monthsthegerminationwasfoundtobezero percent (Figure 4).However,*Boswelliaserrata*seedsfromPinjoresurvivedformorethan24monthsat+5^{o}Ctemperature. During this durationrateofgerminationdecreasedfrom76%to17%andMeanGerminationTimeincreasedfrom3.76to 6.11 days(Figure 5).

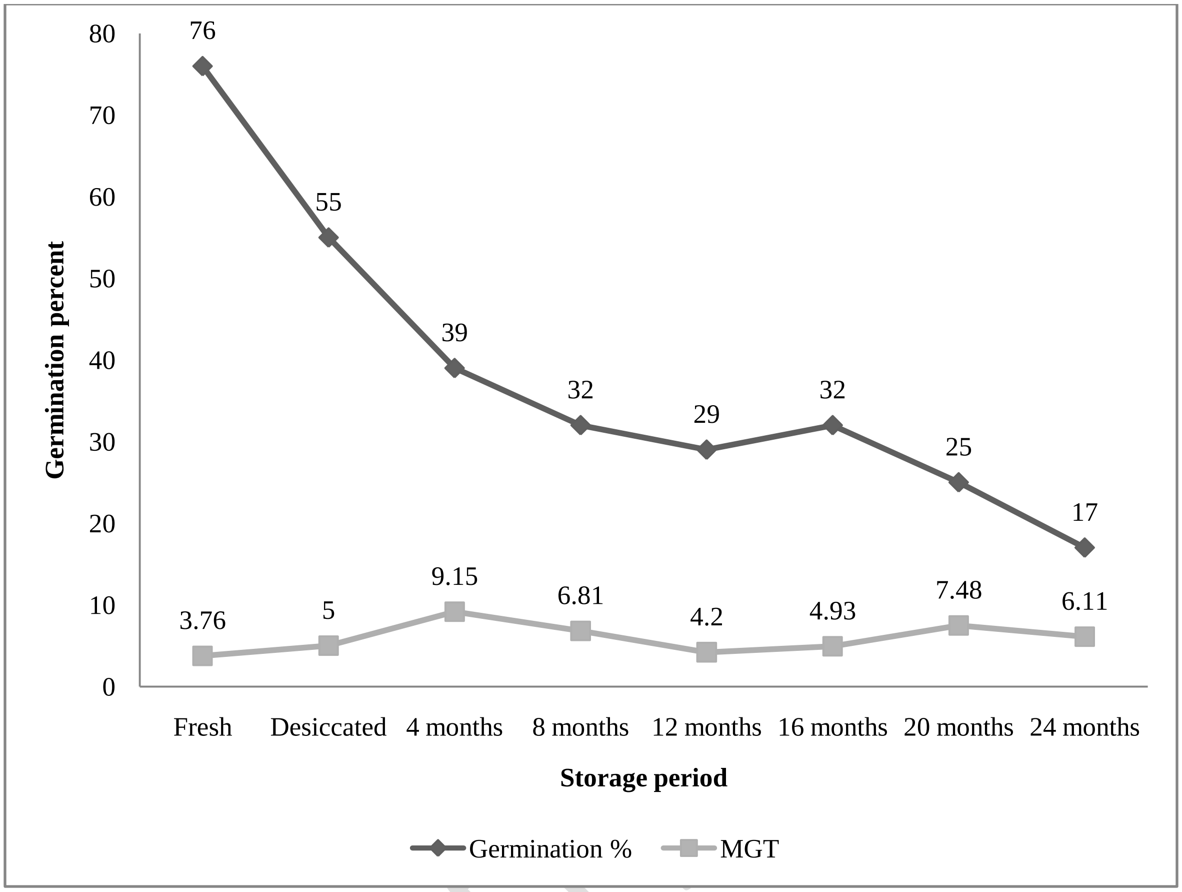

Figure 5. Effect of storage period on Germination and Mean Germination Time (MGT) of *Boswellia serrata* seeds collected from Pinjore, Haryana.

This decrease in germination trend could be attributed to seed deterioration process, which may be accelerated due to the disorganization of cell membranes, affecting the seed germination and vigor, mainly by increasing the leaching of essential constituents for germination, and loss of cell compartmentalization and repair mechanisms (Zhang *et al.* 2021).The current study discovered that there were significant variations in germination for each source as the storage period increased, suggesting that the germination of these seeds is negatively impacted by both seed age and storage duration. These findings also suggest that this species does not have seed dormancy; seeds can germinate as soon as the seed embryos imbibe water. Tropical seeds tend to have a shorterstorage duration, because the higher temperatures of the tropics generates faster chemical reactions, such as respiration and photosynthesis, than in cooler climates. These trials demonstrate that *B. serrata* seeds drastically reduces germination with increase in storage period.

The overall model was found to be statistically significant, which makes us interpret the model. The two-way factorial ANOVA results indicate that there exists a significant difference in germination among the storage period as well as the seed source. To interpret further, the adjusted margin means of germination rate were calculated for both treatments i.e., Storage period and Seed source. We discovered that with an adjusted margin mean of 38.13, the Pinjore seed source is found to be best in terms of germination percentage over the storage period, followed by Morni Hills and Kalesar National Park with an adjusted margin mean of 30 and 20.38 respectively. Interpretation based on marginal means is robust in comparison to actual means.Significant variation was found among the seed sources for germination in different storage periods.This finding agrees with (Garoma*et al.* 2017) demonstrated that seed germination decreased with increase in the seed storage period.It is also reported that genotype strongly influences the seed's vigor (Schmidt, 2000).

4. Conclusions

The present study revealed that *Boswellia serrata* seeds can tolerate desiccation below 10% moisture content, but with the advancement in storage period from 0 months to 24 months the germination percentage decreased drastically and MGT increased. The possible reason for the decrease in germination with an increase in storage period could be attributed to the seed deterioration process and low temperature-induced chilling injury. However further research should be carried out on storage behavior at temperatures above +5^{o}C to ascertain the role of temperature forthe decrease in germination. Our study also revealed that the Pinjore seed source is superior to all other seed sources. Therefore, it is advisable that this seed source should be used for the collection of bulk quantity of seeds to achieve better productivity.

Statements and Declarations

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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