Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2015; 3(12): 1063-1067 **ISSN 2347-9515 (Print)**
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(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI :** 10.36347/sajb.2015.v03i12.014

Research Article

Reproductive Biology and Seed Storage Physiology of *Oroxylum Indicum* (L.) Kurz: Implications for Conservation

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Abstract: *Oroxylum indicum* (L.) Kurz is a medicinally vital, nocturnally blooming tree species facing significant threats from habitat destruction and unsustainable harvesting. This investigation explores key aspects of its reproductive biology and seed physiology to inform conservation strategies. Pollen viability and longevity were assessed under three storage conditions: room temperature, 4°C, and -4°C. Pollen stored at -4°C demonstrated maximum longevity, remaining viable for up to 300 hours (13 days), whereas viability was lost within 120 hours at 4°C and 100 hours at room temperature. Seeds were stored at room temperature to evaluate changes in moisture content and germination potential over time. Freshly collected seeds exhibited 100% germination. After 11 months of storage, germination remained high at 94.67%, but declined significantly to 37.33% after 16 months. This decline correlated with a reduction in seed moisture content, confirming the orthodox nature of *O. indicum* seeds. These findings provide a crucial baseline for developing effective *ex situ* conservation protocols, including pollen cryopreservation and seed banking, for this endangered medicinal tree.
Keywords: Oroxylum indicum, Bignoniaceae, Pollen Longevity, Seed Storage, Orthodox Seeds, Conservation, Medicinal Plant

INTRODUCTION

Oroxylum indicum (L.) Kurz is a medium-sized, nocturnal blooming tree belonging to the family Bignoniaceae (Fig. 1). Native to tropical regions of India, China, Japan, Sri Lanka, and Malaysia, this species is distinguished by its racemose inflorescence and large, sword-shaped capsular fruits containing numerous winged seeds (Harminder *et al.,* 2011). The tree holds significant cultural and economic value due to its extensive use in traditional medicine. It is a principal component of the *Ayurvedic* formulation '*Dasamula'* (ten roots) and is used in well-known preparations such as Chyawanprasham and Narayana taila (Deka *et al.,* 2013; Harminder *et al.,* 2011). However, the increasing demand for its medicinal parts has led to destructive and non-sustainable harvesting practices. This pressure, combined with low natural regeneration and widespread habitat destruction, has resulted in *O. indicum* being classified as an endangered species on the IUCN Red List (Harminder *et al.,* 2011).

Effective conservation of this threatened species requires a thorough understanding of its reproductive biology. Factors such as pollen viability and seed storage behavior are fundamental for developing successful *ex situ* conservation programs like seed banks and cryopreservation protocols. While studies have focused on its phenology and pollination ecology (Mayank Gautam *et al.,* 2009; Srithongchuay *et al.,* 2008; Sritongchuay *et al.,* 2010), quantitative data on pollen longevity and long-term seed viability under controlled conditions remain limited. This study aims to address this knowledge gap by focusing on the reproductive and seed storage physiology of *O. indicum*. The specific objectives were to: (i) determine pollen viability and longevity under room temperature, 4°C, and -4°C storage conditions; (ii) assess the germination percentage of seeds stored at room temperature at regular intervals to establish their storage potential; and (iii) investigate the relationship between seed moisture content and germination percentage during storage.

MATERIALS AND METHODS

Pollen viability and longevity

Freshly opened flowers of *O. indicum* were collected for pollen analysis. To determine longevity, flowers were divided into three batches and stored under distinct conditions: ambient room temperature, refrigeration at 4°C, and freezing at -4°C. Pollen germination was assessed at regular intervals using the hanging drop method in a standard Brewbaker and Kwak (BK) medium. The medium consisted of 10% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulfate, and 100 ppm potassium nitrate in distilled water, as described by Taylor & Hepler (1997). Pollen grains were dusted onto a drop of BK medium in a cavity slide and incubated for one hour

at room temperature. Germination was observed under a compound microscope, and a pollen grain was considered germinated if the pollen tube length exceeded the grain's diameter (Fig. 2). The percentage of germination was calculated from three replicates for each storage condition and time interval using the following formula and Pollen was monitored until the germination percentage reached zero.

$$\text{Pollen germination percentage} = \frac{\text{Number of germinated pollen grains} \times 100}{\text{Total number of pollen grains}}$$

Seed collection and storage

Mature, dehisced fruits were collected from trees in Ettumanoor, Kerala, and seeds were extracted and stored at room temperature. Additionally, one-year-old seeds previously stored at room temperature were procured from the Kerala Forest Research Institute (KFRI), Peechi, Thrissur. All seeds for the experiment were stored in airtight polyethylene bags at ambient room temperature.

Determination of seed moisture content (MC)

The moisture content (MC%) of the seeds was determined prior to each germination test using an electronic moisture analyser. For each measurement, three replicates of approximately one gram of seeds were used, and the mean MC% was recorded.

Seed germination assays

Germination tests were conducted monthly for the one-year-old seeds obtained from KFRI and every 15 days for the freshly collected seeds. Each test consisted of three replicates of 25 seeds each, sown in appropriate germination trays (Fig. 3). Germination was recorded starting from day seven after sowing. The final germination percentage was calculated after no further germination was observed, using the formula:

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

RESULTS

Pollen longevity

Pollen viability was significantly influenced by storage temperature (Table 1). At room temperature, viability declined sharply, with germination dropping from 84.03% to 18.54% within 25 hours and reaching zero by 100 hours. Storage at 4°C extended longevity, maintaining over 50% germination for 50 hours, with viability lost after 120 hours (5 days). The most effective condition was storage at -4°C, where pollen remained viable for up to 300 hours (13 days), showing 39.89% germination at 100 hours and only declining to zero after the 300-hour mark.

Table 1: Pollen longevity of *O. indicum* at different storage conditions

Pollen storage conditions	Mean germination percentage against time in hours*						
	15	25	50	100	120	200	300
Room temp.	84.03 ± 2.06	18.54 ± 3.26	2.25 ± 0.76	0	-	-	-
At 4^0 C	93.19 ± 4.18	72.33 ±1.80	53.39 ± 5.97	2.04 ± 1.28	0	-	-
At -4^0 C	90.49 ± 1.89	70.73 ± 4.30	52.74 ± 2.41	39.89 ± 5.28	19.68 ± 3.20	3.76 ± 0.44	0

*Value indicated as mean ± SD of 3 samples

Seed moisture content and germination

Freshly collected seeds had an initial moisture content (MC) of 8.82 ± 0.61%. During open storage at room temperature, the MC of the seeds gradually decreased. After 11 months, the MC had reduced to 5.07 ± 0.64%, and by 16 months, it had further declined to 4.25 ± 0.21% (Table 2).

Table 2: Moisture content (%) of *O. indicum* seeds affected by open storage

Storage period (Months after storage)	Storage conditions
	Open storage*
11	5.07 ± 0.64
12	5.33 ± 0.71
13	4.68 ± 0.87
14	4.39 ± 0.03
15	4.37 ± 0.02
16	4.25 ± 0.21

*Value indicated as mean ± SD of 3 samples.

The germination potential of the seeds was directly related to the storage duration and corresponding moisture content. Freshly collected seeds exhibited 100% germination. After 11 months of storage, the germination percentage remained high at 94.66 ± 2.30%. However, as storage progressed, viability declined more rapidly. After 16 months, the germination percentage had dropped to 37.33 ± 2.30% (Table 3). Although there was a general downward trend, some fluctuations in germination were observed, potentially due to environmental factors influencing seed batches.

Table 3: Germination percentage of *O. indicum* seeds affected by open storage conditions

Storage period (Months after storage)	Storage conditions
	Open storage*
11	94.66 ± 2.30
12	66.66 ± 10.06
13	53.33 ± 6.11
14	73.33 ± 12.86
15	69.33 ± 2.30
16	37.33 ± 2.30

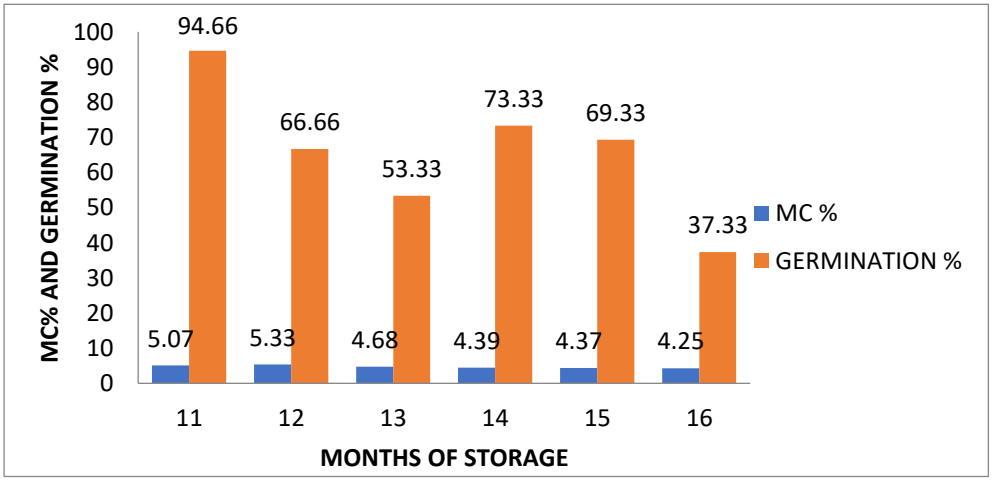
* Value indicated as mean ± SD of 3 samples.

DISCUSSION

The findings of this study provide critical insights into the reproductive physiology of the endangered medicinal tree *Oroxylum indicum*, with direct implications for its conservation. The extended pollen longevity at -4°C (13 days) compared to 4°C (5 days) and room temperature (<4 days) is a significant finding. This demonstrates that low temperatures drastically reduce pollen metabolic activity, thereby preserving viability. This principle is fundamental to cryopreservation (Knowlton, 1922) and suggests that *O. indicum* pollen is an excellent candidate for long-term storage in pollen banks. Such banks can support controlled pollination programs, genetic diversity conservation, and breeding efforts for species restoration.

The seed storage experiment confirms the orthodox nature of *O. indicum* seeds. Orthodox seeds are characterized by their ability to tolerate desiccation to low moisture levels and remain viable for extended periods, especially under cold storage (Koornneef *et al.,* 2002). In this study, seeds stored at room temperature maintained over 94% viability for 11 months, but viability dropped to 37% by 16 months. This decline correlated directly with the decrease in seed moisture content from an initial 8.82% to 4.25% (Graph 1). This behaviour is highly advantageous for *ex situ* conservation, as orthodox seeds can be successfully stored in conventional seed banks for decades at low temperature and humidity, a cornerstone of global plant conservation efforts. The ability to store seeds for approximately two years at room temperature provides a practical and low-cost option for local conservation and nursery initiatives.

The conservation implications of these results are substantial. Given its endangered status due to over-harvesting and habitat loss, establishing *ex situ* collections is a priority. Our data confirms that seed banking is a viable and efficient strategy for *O. indicum*. Furthermore, the successful short-term storage of pollen facilitates assisted pollination, which can help overcome reproductive barriers in fragmented populations and enhance genetic exchange.



Graph 1: Effect of MC% and storage period on Germination

Figure 1: Habit of *Oroxylum indicum*

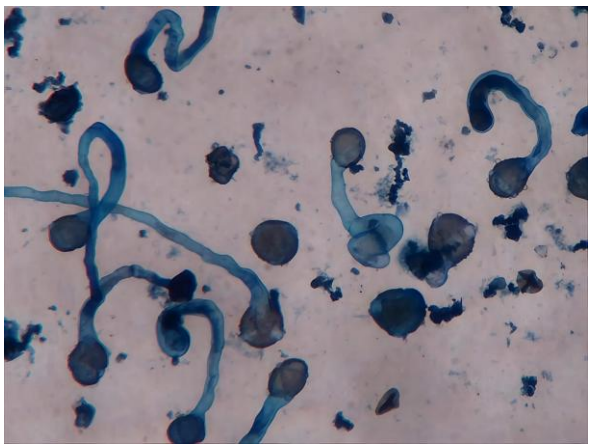


Figure 2: *Oroxylum indicum* Pollen germination under light microscope

Figure 3: *Oroxylum indicum* Seed germination

CONCLUSION

This study demonstrates that *Oroxylum indicum* pollen longevity is significantly enhanced by low-temperature storage , and its seeds exhibit classic orthodox storage behavior. Viability can be maintained for up to 13 days for pollen at -4°C and for over a year for seeds at room temperature. These physiological traits are highly favorable for conservation. The data presented here provides an essential foundation for developing robust *ex situ* conservation protocols, including seed banking and pollen cryopreservation, which are crucial for safeguarding the genetic diversity and ensuring the long-term survival of this valuable medicinal plant.

Acknowledgement

The authors are thankful to the Principal, St. Berchmans College, Changanassery for support and providing necessary facilities.

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