



Seasonal Variations of Floral Biology and Identification of Floral visitors of *Santalum album* Linn in Mid-Hill Zone of Himachal Pradesh

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Abstract: The current study investigates the Seasonal variations of floral biology, and the identification of floral visitors in *Santalum album* within the mid-hill region of Himachal Pradesh. Five phenotypically superior genotypes were selected from fields for two flowering seasons in 2020. The study revealed that *S. album* flowers bloom twice in a year in the same trees but there is variation in flowering intensity, with a moderate level during the season 1 (March- April) period and an intense level during season 2 (August-September) period. Seasonal variations in 2020 significantly influenced bud, inflorescence, flower, and seed morphology. Inflorescence morphological parameters and flower size increased in season 2, indicating favourable reproductive conditions, while bud count and flower numbers were higher in season 1. Fruit and seed traits, such as weight and germination rate, showed higher growth in season 2. Additionally, both seed setting and seed germination percentages were higher in the second season (August to September). Pollen viability decreased with increased storage time and among seasons was maximum for season 2. Floral visitors; black ants (*Lasius niger*) and Italian honey bees (*Apis mellifera*) were the most frequent and consistent visitors, particularly in the morning. In contrast, blue bottle flies (*Calliphora vomitoria*), dragonflies (*Anisoptera spp.*), and beetles (*Coleoptera spp.*) showed lower and more sporadic visit patterns, and beetles were more active during the evening hours.

Keywords: *Santalum album* Linn, Floral biology, mid hills zone, seed setting, floral visitors

The most common and widely accepted fragrant tree, known as the Sandalwood, is a moderately-sized evergreen belonging to the genus *Santalum* in the Santalaceae family. Its primary distribution is in the drier tropical regions of India and the Indonesian islands of Timor and Sumba. (Subasinghe 2022). The first sandalwood survey conducted in India during 1977-78 revealed that sandalwood is distributed throughout the country, with the natural habitats primarily located in the southern part of Karnataka and the northern part of Tamil Nadu. (Rocha et al 2017). It was estimated that approximately 90% of the sandalwood population was in these two states, covering an area of around 8,300km². It adapts well to various soil types, including sand, clay, red soils, laterite loam, and even black cotton soils, although it prefers red ferruginous loam with varying fertility. However, it does not grow well in waterlogged or icy areas. Trees growing in stony or gravelly soils are known to produce wood with a higher scent (Prasath et al 2019).

Santalum album is a small evergreen tree that reaches a height of 15-20 meters and a girth of up to 2.4 meters, characterized by slender, drooping branchlets. The flowers are small, measuring about 4-6 mm long, and can be purplish-brown, straw-colored, reddish, green, or violet (Garg et al 2016). They appear in small terminal or axillary

clusters of up to six and are unscented, arranged in axillary or terminal panicle cymes that are shorter than the leaves, with floral organs developing in acropetal succession. Floral biology is considered a pillar for systematic, evolutionary, and conservation studies. Understanding the reproductive biology of species, coupled with an analysis of their genetic variation, provides essential data for conservation and various tree improvement programs. This is particularly crucial for endangered species, where limited populations must supply propagules for future generations. Knowledge of floral biology is a prerequisite for both evolutionary and conservation studies (Pullaiah et al 2021).

Flowers of the *Santalum album* are self-incompatible and are specifically adapted for cross-pollination by various flower visitors (Bhasker 1993). This process enhances genetic diversity and ensures the production of viable, high-quality seeds. Effective transfer of pollen through flower visitors between flowers enhances the chances of successful fertilization, resulting in improved seed development and overall reproductive success of the tree (Akbar 2020). The demand for sandalwood is increasing, but forest supplies have nearly ceased, creating a significant gap between demand and availability. Given its vulnerable status and dwindling availability, this species is under threat and requires conservation. Effective conservation and large-

scale afforestation efforts hinge on the production of high-quality seeds. To achieve this, it is essential to understand its floral biology. Therefore, there is an urgent need to identify the flower visitors of the *Santalum album*. This study aims to study flower biology and identify effective flower visitors for high-quality seed production of the *Santalum album*.

MATERIAL AND METHODS

The present study was carried out in the field and laboratory of Tree Improvement and Genetic Resources, Dr YSPUHF, Nauni Solan. Five phenotypically superior genotypes were selected from fields for two flowering seasons in 2020. The first flowering season occurs in March to April and the second in August to September. Nine inflorescences were tagged for the study of floral biology. Furthermore, the flower visitor's activity was also observed during the first season (Ghazoul 1997).

Bud and inflorescence morphology: The number of buds, rachis per inflorescence, and flowers per inflorescence were recorded. Lengths and widths of buds and inflorescences were measured.

Flower morphology: Flower length and width were determined. Another length and width, ovary length, and width were measured using the image analyzer.

Fruit and seed morphology: Number of fruits was calculated and fruit diameter (mm) was recorded by digital vernier calliper. Fresh fruit weight (g) and dry seed weight (g) were recorded and seed setting and seed germination percentage were also calculated.

Pollen Studies

Pollen collection: Pollens were collected in isolation to prevent contamination, and then stored in glass vials. These vials were kept in a refrigerator and pollen viability of stored pollens was observed after 30 days.

Pollen viability: Pollen viability of freshly collected as well as previously stored pollens for 30 days from five genotypes was recorded. Pollen viability was assessed using a 2% acetocarmine solution. Freshly dehisced pollen was placed on a clean hemocytometer, and 1 to 2 drops of the acetocarmine solution were added to the pollen mass. This mixture was allowed to settle for 10 to 15 minutes to enable the pollen grains to absorb the stain. Within the five squares of the hemocytometer, pollen grains that were deeply stained and appeared normal were counted as viable, while those that were shrivelled and weakly stained were categorized as non-viable (Monga 2021).

Floral visitor's visit: Floral visitors' visit and their activities were observed during season 2 of 2020. The pollination dynamics of the primary pollinators were observed twice daily: during the morning from 9-11 am and in the evening

from 5-7 pm at the time of flowering and the photograph was used to identification of each flower visitor (Talwar and Bhatnagar 2014). Insect visiting time was recorded in the morning and evening with the help of a stopwatch.

Statistical analysis: Data analysis was done through R software and graphs were also generated with R studio (Posit team 2024).

RESULTS AND DISCUSSION

Bud and inflorescence morphology: The morphology of bud and inflorescence across two distinct seasons in 2020 revealed several notable variations. The inflorescence length showed a slight seasonal increase, ranging from 7.00-7.77 cm in season 1 to 7.12-7.99 cm in season 2. Similarly, the inflorescence width expanded from 3.13-4.12 cm in season 1 to 3.26-4.54 cm in season 2. The number of rachises per inflorescence remained relatively consistent, ranging from 6.69-7.78 in season 1 and showing a minor increase to 7.34-7.58 in season 2. The reduction in the number of flowers per inflorescence was observed, with counts decreasing from 49.00-56 in season 1 to 40.79-45.00 in season 2. Bud parameters also varied between seasons; the bud count per inflorescence was higher in season 1, ranging from 77.7-88.9, while in season 2, it was 74.35-77.45. Bud length exhibited a slight decline from 0.67-0.72 cm in Season 1 to 0.56-0.67 cm in Season 2, though bud width remained fairly stable across both seasons, ranging from 0.33-0.45 cm in season 1 and 0.3-0.45 cm in season 2 (Table 1). Krishnakumar and Parthiban (2017) also reported that morphological parameters of buds and inflorescence were maximum in season 2 as compared to season 1.

Flower morphology: Flower length increased from 0.33-0.49 cm in season 1 to 0.43-0.57 cm in season 2, while flower width also increased from 0.65-0.77 cm to 0.78-0.89 cm. The number of fruits was maximum in season 1 ranging from 8.23- 12.00 and 11.22-18.78 in season 2. Fruit diameter increased from 8.17-9.22 mm to 9.56-9.95 mm, and fresh fruit weight increased from 1.45-2.12 g to 1.95-2.89 g. Anther length ranged from 0.43-0.50 mm in season 1 and increased to 0.44-0.53 mm in season 2. Anther width ranged from 0.65-0.69 mm in season 1 and from 0.60-0.67 mm in season 2 (Table 1). However, the decrease in anther width suggests that not all parameters are uniformly affected by seasonal changes. Ovary length and width both showed similar patterns of slight seasonal variation. The observed seasonal increases in flower length, flower width, and ovary width indicate that external environmental conditions may favor reproductive growth during the second season, highlighting the dynamic nature of plant development (Ratnaningrum et al 2017, Krishnakumar and Partiban 2017). Seasonal

variability of floral traits may be useful for understanding plant reproductive strategies.

Seed morphology: The number of fruits increased from Season 1, with a range of 8.23-12.00, to season 2 (11.22-18.78 fruits). Fruit diameter also showed an increase, from 8.17-9.22 mm in season 1 to 9.56-9.95 mm in season 2, and fresh fruit weight followed this trend, increasing from 1.45-2.12 to 1.95-2.89 g. In terms of seed morphology, the seed diameter ranged from 6.12-6.78 mm in season 1 and slightly varied to 5.99-7.11 mm in season 2, while dry seed weight showed greater variability between the seasons, ranged between 0.13-0.22 g in season 1 to 0.11-0.32 g in season 2. Seed setting percentage increased from 16.14-23.08% in season 1 to 27.52-43.00% in season 2. Seed germination percentage (%) also increased from 45.88-55.3% in Season 1 to 56.78-73.12% in Season 2. Seed and seedling traits play a crucial role in determining plant fitness and long-term

survival, with these traits being shaped by the dynamic interaction between the plant and its surrounding environment (Saini et al 2022, Madhuvanti et al 2024). The high fruit set rate observed during the August-September flowering season can be attributed to the nutrient-rich soil conditions resulting from the occurrence of rains.

Pollen studies: Storage time affects the pollen viability of the *Santalum album*. Freshly collected pollens were more viable as compared to stored pollen for 30 days. Pollen viability showed a decreasing trend with increasing time (Fig. 1). Pollens of season 2 were more viable than season 1. In season 1, maximum viability of freshly collected pollens was for T₃ (86.15%) followed by T₅ and T₁ whereas for stored pollens pollen viability follows the trend viz., T₃ (81.15%) followed by T₁, T₅. The maximum pollen viability was for T₃ (87.98%) followed by T₁, T₂, T₄ whereas for stored pollens pollen viability was T₃(79.63%) followed by T₅ and T₁ (Fig. 1).

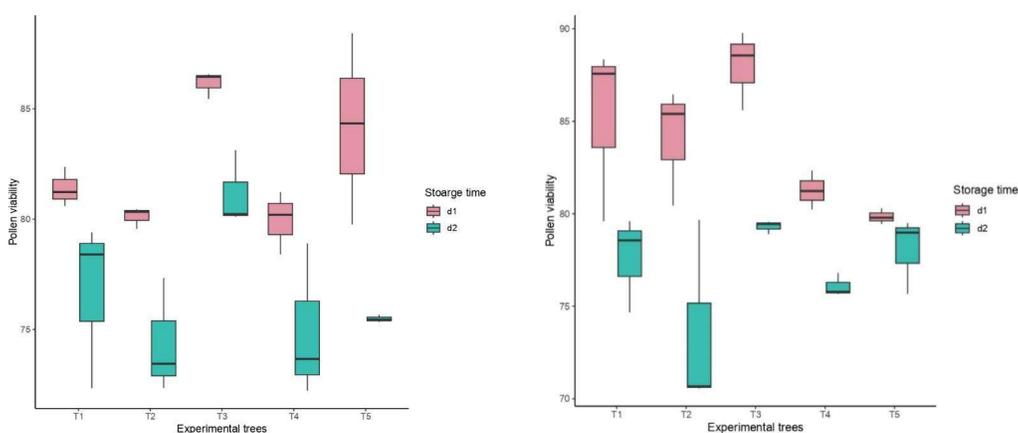


Fig. 1. Effect on storage duration (days) on pollen viability (%) of *Santalum album* Linn (A) Season 1 and (B) Season 2

Table 1. Morphological variations in buds, inflorescence, flowers, fruits and seeds of *Santalum album*

| Parameters | 2020 (Season 1) | 2020 (Season 2) | Parameters | 2020 (Season 1) | 2020 (Season 2) | Parameters | 2020 (Season 1) | 2020 (Season 2) |
|---|-----------------|-----------------|-------------------------------------|-----------------|-----------------|---|-----------------|-----------------|
| Morphological variations of bud and inflorescence | | | Morphological variations of flowers | | | Morphological Variation of Fruit and Seed | | |
| Inflorescence length (cm) | 7.00-7.77 | 7.12-7.99 | Flower length (cm) | 0.33-0.49 | 0.43-0.57 | No of fruits | 8.23-12.00 | 11.22-18.78 |
| Inflorescence width (cm) | 3.13-4.12 | 3.26-4.54 | Flower width (cm) | 0.65-0.77 | 0.78-0.89 | Fruit diameter (mm) | 8.17-9.22 | 9.56-9.95 |
| No. rachis/inflorescence | 6.69-7.78 | 7.34-7.58 | Anther length (mm) | 0.43-0.50 | 0.44-0.53 | Fresh fruit weight (g) | 1.45-2.12 | 1.95-2.89 |
| No. of flower/inflorescence | 49.00-56 | 40.79-45.00 | Anther width (mm) | 0.65-0.69 | 0.60-0.67 | Seed diameter(mm) | 6.12-6.78 | 5.99-7.11 |
| No. of bud | 77.7-88.9 | 74.35-77.45 | Ovary length (mm) | 1.29-1.46 | 1.33-1.40 | Dry Seed weight (g) | 0.13-0.22 | 0.11-0.32 |
| Bud length (cm) | 0.67-0.72 | 0.56-0.67 | Ovary width (mm) | 0.60-0.66 | 0.70-0.77 | Seed setting percentage (%) | 16.14-23.08 | 27.52-43.00 |
| Bud width (cm) | 0.33-0.45 | 0.3-0.45 | - | - | - | Seed germination percentage (%) | 45.88-55.3 | 56.78-73.12 |

Pollen visitors: Black ants (*Lasius niger*) and Italian honey bees (*Apis mellifera*) were the most frequent and consistent flower visitors with 80 % and 100 % of their visit, particularly in the morning. In contrast, blue bottle flies (*Calliphora vomitoria*), dragonflies (*Anisoptera* spp.) and beetles (*Coleoptera* spp) showed lower and more sporadic visitation patterns with 40, 30 and 10% flower visit and beetles being more active during the evening with 20% of visit (Table 2). Hareesha et al (2022) also observed that ants and bees are the primary pollinators in *Santalum album*.

Time spent on flower: Black ants were consistent visitors across most days, spending between 5 and 30 seconds on

flowers. The highest was on day 8 (20-30 seconds), while the shortest was on day 1 (5-20 seconds). Black ants did not visit the flowers on Day 3. Italian honey bees visited flowers each day, spending between 0 and 30 seconds. The longest time was observed on day 8 (20-30 seconds), while the shortest was on day 7 (0-15 seconds). These flies were observed on six days, with a duration range of 5-20 seconds. The highest time spent was on days 1 and 2 (10-20 seconds), and it did not appear on 4, 5, 6, 7, 9, and 10 day. Dragonflies were observed for three days only, with times ranging from 0 to 15 seconds longest on flowers on day 9 (10-15 seconds) and the least on day 2 (0-5 seconds). Beetles visited on three occasions (2, 8,

Table 2. Pollinators visiting time in *Santalum album* Linn.

| Visiting days | Pollen vectors | | | | | | | | | |
|---------------|---------------------|----------------------|-----------------------|----------------------|-----------------------|-----------------------|-----------------------|----------------------|-----------------------|----------------------|
| | Black ant | | Italian honey bee | | Blue bottle fly | | Dragon fly | | Beetle | |
| | Morning (9 am-11am) | Evening (5 pm - 7pm) | Morning (9 am - 11am) | Evening (5 pm - 7pm) | Morning (9 am - 11am) | Evening (9 am - 11am) | Morning (9 am - 11am) | Evening (5 pm - 7pm) | Morning (9 am - 11am) | Evening (5 pm - 7pm) |
| Day1 | √ | - | √ | - | √ | - | √ | - | - | - |
| Day2 | √ | - | √ | - | √ | - | √ | - | √ | - |
| Day3 | - | - | √ | - | √ | - | - | - | - | - |
| Day4 | √ | - | √ | - | - | - | - | - | - | - |
| Day5 | √ | - | √ | - | - | - | - | - | - | - |
| Day6 | √ | - | √ | - | - | - | - | - | - | - |
| Day7 | √ | - | √ | - | - | - | - | - | - | - |
| Day8 | √ | - | √ | - | √ | - | - | - | - | √ |
| Day9 | √ | - | √ | - | - | - | √ | - | - | √ |
| Day10 | √ | √ | √ | - | - | - | - | - | - | - |
| Morning (%) | 80 | | 100 | | 40 | | 30 | | 10 | |
| Evening (%) | 10 | | - | | - | | - | | 20 | |
| No visit (%) | 10 | | 100 | | 60 | | 70 | | 70 | |

Table 3. Variation for pollinators' time spent / flower in *Santalum album* L. at different days of flowering

| Day fatter flowering | Pollinators' time spent on flowers (seconds) | | | | |
|----------------------|--|--|---|------------------------------------|--------------------------------|
| | <i>Lasiusniger</i> (Black ants) | <i>Apis mellifera</i> (Italian honey bees) | <i>Calliphora vomitoria</i> (Blue bottle fly) | <i>Anisoptera spp.</i> (Dragonfly) | <i>Coleoptera spp</i> (Beetal) |
| 1 | 5-20 | 10-20 | 10-20 | 5-10 | - |
| 2 | 15-20 | 5-15 | 5-10 | 0-5 | 30-40 |
| 3 | - | 10-15 | 10 -15 | - | - |
| 4 | 10-15 | 15-20 | - | - | - |
| 5 | 15-25 | 10-15 | - | - | - |
| 6 | 10- 20 | 5-15 | - | - | - |
| 7 | 15-20 | 0-15 | - | - | - |
| 8 | 20-30 | 20-30 | 5-15 | - | 25-35 |
| 9 | 15-20 | 5-20 | - | 10-15 | 25-40 |
| 10 | 5-15 | 10-20 | - | - | - |

and 9). They spent the most time on flowers compared to other pollinators, with a duration range of 25-40 seconds. Their longest time spent was on day 9 (25-40 seconds) (Table 3). Some pollinators like black ants and Italian honey bees visit more consistently, while others like dragonflies and beetles visit less frequently but spend longer durations on certain days (Baskorowati 2011, Krishnakumar et al 2018).

CONCLUSIONS

The seasonal variations in 2020 markedly affected bud, inflorescence, flower, and seed morphology. Season two displayed enhanced reproductive success, as evidenced by increased inflorescence size, flower dimensions, and improved fruit and seed traits, including weight and germination rate. Seed setting and germination percentages were highest in season two. These findings suggest that season two (August to September) provided optimal conditions for floral, fruit, and seed development, underscoring its significance for future reproductive and morphological studies. Black ants (*Lasius niger*) and Italian honey bees (*Apis mellifera*) were observed as the most frequent and consistent visitors, particularly in the morning hours, suggesting their role as primary pollinators. In contrast, blue bottle flies (*Calliphora vomitoria*), dragonflies (*Anisoptera* spp.), and beetles (*Coleoptera* spp.) demonstrated lower and more sporadic visitation rates, with beetles exhibiting peak activity in the evening.

REFERENCES

- Akbar S 2020. *Santalum album* L. (Santalaceae). In: *Handbook of 200 Medicinal Plants*. Springer, Cham. https://doi.org/10.1007/978-3-030-16807-0_165
- Garg A, Sharma D and Shiwani K 2022. Floristic Diversity of L. Populations in Mid Hill *Santalum album* Zone of Himachal Pradesh. *Indian Journal of Ecology* **49**(5): 1885-1890.
- Ghazoul J 1997. *Field Studies of Forest Tree Reproductive Ecology*. ASEAN-Canada Forest Tree Seed Centre. Muak-lek, Saraburi 18180, Thailand.
- Hareesha AS, Belavadi VV and Tharin KB 2022. Pollination Biology of Sandalwood. *Indian Sandalwood: A Compendium*, 213-227.
- Krishnakumar N and Parthiban KT 2017. Flowering phenology and seed production of *Santalum album* L. *International Journal of Current Microbiology and Applied Sciences* **6**(5): 963-974.
- Krishnakumar N, Parthiban KT and Umesh Kanna S 2018. Identification of pollinators for good quality seed productions in sandal wood (*Santalum album* L.). *Journal of Entomology and Zoology Studies* **6**(3): 1467-1470.
- Madhuvanathi CK, Eswaran M, Karthick T, Balasubramanian A and Ghosh Dasgupta M 2024. Assessment of seed-and seedling-related traits in *Santalum album* (Indian sandalwood) reveals high adaptive potential. *Journal of Biosciences* **49**(1): 13.
- Monga 2021. *Crossability studies in Bauhinia species*. Ph.D. Thesis Department of Tree Improvement and Genetic Resources, YSP University of Horticulture & Forestry, Solan, 53p.
- Posit Team 2024. RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL <http://www.posit.co/>
- Prasath CH, Balasubramanian A and Radhakrishnan S 2019. Sandalwood-expensive and medicinal tree. *Van Sangyan* **6**: 4-9.
- Pullaiah T, Ramulu DR, Murthy KSR, Reddy VD, Saradamma B and Swamy MK 2021. Phytochemistry and Pharmacological Properties of *Santalum album* L.. In: Pullaiah T, Das SC, Bapat VA, Swamy MK, Reddy VD, Murthy KSR (eds) *Sandalwood: Silviculture, Conservation and Applications*. Springer, Singapore. https://doi.org/10.1007/978-16-0780-6_5
- Ratnaningrum YW, Faridah E, Indrioko S and Syahbudin A 2016. Flowering and seed production of sandalwood (*Santalum album*; Santalaceae) along environmental gradients in Gunung Sewu Geopark, Indonesia. *Nusantara Bioscience* **8**(2). <https://doi.org/10.13057/nusbiosci/n080209>.
- Rocha D, Ashokan PK, Santoshkumar AV, Anoop EV and Sureshkumar P 2017. Anatomy and functional status of haustoria in field grown sandalwood tree (*Santalum album* L.). *Current Science* **113**(1): 130-133.
- Saini P, Kumari A, Chauhan S, Jha S and Rawale GB 2023. Variation studies in fruit characteristics, seed germination and seedling growth of *Diospyros montana* (Roxb.) in Himachal Pradesh. *Indian Journal of Ecology* **50**(3): 650-657.
- Sindhuveerendra HC and Sujatha M 1989. Pollination studies in *Santalum album* L. *Current Science* **58**: 629-630.
- Subasinghe SMCUP 2022. *Santalum album*: Current Status, Research and Future Perspectives in Sri Lanka. In: Arunkumar, AN, Joshi G, Warriar RR, Karaba NN (eds) *Indian Sandalwood. Materials Horizons: From Nature to Nanomaterials*. Springer, Singapore. https://doi.org/10.1007/978-981-16-6565-3_8
- Talwar S and Bhatnagar AK 2014. Pollination biology of *Terminalia chebula* Retz in Delhi and western Ghats. *The International Journal of Plant Reproductive Biology* **6**(2): 181-194.

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