

Fruit and Seed Phenological studies of *Rauvolfia* species distributed in the Southern Western Ghats

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Abstract

Fruiting pattern of five *Rauvolfia* species in the southern Western Ghats were observed to get an insight and limitations impacting their fruiting cycles. These species displayed parallel seasonal occurrences in their flowering and fruiting. Over two continuous years, from January to December in 2011 and 2012, monthly observations highlighted discrepancies in the duration of flowering and fruiting, despite the shared seasonal aspects. The peaks and patterns of fruiting varied among these species, demonstrating differences in key factors like fruiting duration, peak times, single/double fruit ratio, and seed purity. Moreover, it was observed that annual precipitation influences the flowering and fruiting behaviors of *Rauvolfia*.

Keywords: Phenology; Flowering; Fruiting; Western Ghats; Rainfall

1 Introduction

Flowering patterns vary across species, closely tied to the ecosystems they inhabit, suggesting that specific flowering behaviors might align with particular ecosystem types (Pojar, 1974; Heinrich, 1976). Lokho and Kumar (2012) elaborated that plant phenology encompasses both vegetative and reproductive aspects, including bud formation, flowering, fruiting, seed germination, and vegetative phases like leaf budding and shedding. Three environmental factors—photoperiod, temperature, and moisture—have been recognized as triggers for plant phenological progression (Rathcke and Lacey, 1985). In tropical species, various environmental shifts linked to elevation, such as differences in rainfall, temperature, and exposure, notably influence not only species distribution but also individual seasonal patterns of leafing, flowering, and fruiting (Koptur *et al.*, 1988).

Due to phylogenetic constraints, plants within the same family or genus are anticipated to exhibit similar reproductive phenology, occurring during comparable times or seasons of the year (Rathcke and Lacey, 1985). As phenological patterns vary among species across diverse ecosystems, climatic and flowering data aid in the determination of triggers for flowering and the specific developmental needs different species.

The duration of distinct phenophases encompasses fruit and seed development, aspects of fruit setting, and seed purity. This study delves into the pertinent habitat aspects and analyzes the patterns of flowering, fruiting, the formation of single and double fruits, as well as seed purity—comprising viable seeds and chaff—across all five *Rauvolfia* species found in the Southern Western Ghats.

Rauvolfia, a genus within the Apocynaceae family, comprises approximately 80 species (Mabberley, 2008), predominantly consisting of shrubs. Hooker (1882) described seven species in the Flora of British India, five of which were later documented by Chakravarthy (1955) and Sulochana (1959). In the Flora of Madras Presidency, Gamble

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(1921) recognized five species: *Rauvolfia serpentina* (L.) Benth. Ex Kurtz, *Rauvolfia hookeri* Srinivas. et Chithra (also known as *R. beddomei* Hook. f.), *Rauvolfia micrantha* Hook.f. (commonly referred to as 'Malabar *Rauvolfia*'), and *Rauvolfia verticillata* (Lour.) Baill. (also recognized as *R. densiflora* (Wall.) Benth. ex Hook. f.), alongside *Rauvolfia tetraphylla* L. (*R. canescens* Linn.), a West Indies species introduced to India over a century ago must be naturalized.

Rauvolfia serpentina thrives in the Western Ghats region, while *R. verticillata* is distributed in India and neighbouring countries (Sahu, 1979). *R. hookeri* and *R. micrantha* are indigenous to the Southern Western Ghats (Ahmedullah and Nayar, 1986), both being rare and endangered shrubs in the Western Ghats (Nayar, 1996, 1997).

All *Rauvolfia* species boast a rich composition of alkaloids and various chemicals. The extraction of 'reserpine' from the plant's roots has led to its extensive use, resulting in overexploitation and endangerment within its natural habitat (Swarup and Arora, 2000). Singh (1999) included *R. serpentina* on the list of plants on the brink of extinction or those that have become rare in India due to this extensive utilization. Among all Indian species, *R. tetraphylla* contains the highest quantity of reserpine, surpassing the well-known *R. serpentina*, while significant amounts also have been reported in the endemic Indian *R. micrantha* (Bindu *et al.*, 2014).

The captivating appearance and red-colored fruits of *R. tetraphylla*, even at a young stage, have prompted its cultivation as a garden plant (Anonymous, 1969), with the fruit juice employed as a fabric dye (Kar and Borthakur, 2008). Kumar *et al.* (1998) highlighted the striking similarities between *R. hookeri* and *R. micrantha*, except in their flowering habits. In the Apocynaceae family, drupes are exclusively found within the genus *Rauvolfia* (Thomas and Dave, 1990). Anilkumar *et al.* (2011) extensively described fruit morphology, including scanning electron microscope (SEM) features of seeds, encompassing characteristics like color, size, shape, surface texture, weight, and embryo shape.

Sihag and Wadhwa (2011) delved into the reproductive biology of *R. serpentina*, while Thomas and Dave (1990) studied cellular development in *R. tetraphylla* during drupe development. Despite their significant importance and numerous chemical studies, adequate conservation methods for these species have yet to be established.

Preserving crucial medicinal plants on the brink of extinction requires conservation efforts in their natural habitats and within homegrown environments. Fruit maturity plays a pivotal role for farmers seeking seeds for cultivation. According to Ramírez and Briceño (2011), fruits were deemed ripe when their development reached completion. In instances where fruits showed no evident changes, the seeds were closely examined.

In India, these five species face significant threats, particularly *R. serpentina*, which is experiencing local extinction due to exploitation by drug collectors. Reports indicate varying seed purity ranging from 21.57% to 95%. A notable disparity in fruit availability was observed during carpology data collection, differing among the species in fruiting periods, double-single fruit ratios, and more. *R. hookeri* displayed a scarcity of pollen grains, negatively impacting fruit setting and seed purity (Bindu and Anilkumar, 2014). Thus, this study aimed to investigate the fruiting phenology and related aspects among the five South Indian *Rauvolfia* species.

The observed fruit maturation periods were reported as follows: *R. hookeri* 40 ± 1.79 days, *R. micrantha* 36.6 ± 1.63 days, *R. serpentina* 40.8 ± 1.24 days, *R. tetraphylla* 70.2 ± 0.86 days, and *R. verticillate* 68.6 ± 2.66 days. Detailed documentation over two consecutive years included data on fruit availability, the count of double-seeded and single-seeded fruits, the number of viable seeds, and chaff across the five selected species. The objectives of this study aimed to compare the fruit and seed production behaviours among *Rauvolfia* species through focus on total fruit count, double-seeded and single-seeded fruits, seed purity and viability.

2 Materials and methods

The research took place within the premises of the Jawaharlal Nehru Tropical Botanic Garden and Research Institute, located in Palode, Thiruvananthapuram, positioned in the southernmost region of the Southern Western Ghats (Latitude - $8^{\circ}45'$ to $8^{\circ}47'N$, Longitude - $77^{\circ}1'$ to $77^{\circ}4'E$). The area typically receives an average actual and normal rainfall of approximately 2163.3 mm (source: [https://sdma.kerala.gov.in/wp-content/uploads/2018/11/\(2.1\)2014-Memorandum-Monsoon-Rainfall.pdf](https://sdma.kerala.gov.in/wp-content/uploads/2018/11/(2.1)2014-Memorandum-Monsoon-Rainfall.pdf)). The plants grew in an environment with partial sunlight under the canopy of trees. Days in December were the shortest, while June witnessed the longest daylight hours.

Five plants from each species in the Southern Western Ghats were selected for data collection. These plants were left to undergo natural pollination. Observations on fruiting phenology were conducted monthly, spanning from January to December in both 2011 and 2012. Fruiting phenology was determined based on the presence of both unripe and ripe fruits, following the definition by San Martín-Gujardo and Morellato (2003).

Data collection involved documenting the flowering and fruiting patterns, along with the formation and availability of double or single-seeded seeds, as well as the count of good or chaff seeds each month. All species fell under the shrub category and were situated within an altitude range of 300 to 1000 meters. This comprehensive data collection spanned two consecutive years.

2.1. Flowering and fruiting

Over the span of two continuous years, monthly sampling was conducted to gather data on the timing and quantity of flowering and fruiting. This involved considering the number of flowers per peduncle, including buds, and counting the fruits produced from a half-matured stage. During this period, unfertilized fruits dropped prematurely, while the remaining ones were tallied. Fruit ripening was determined by a colour change to dark purple, accompanied by softening and juiciness in the pulp. At this stage, birds and monkeys consumed the fruit pulp, and ripe fruits either fell naturally or could be plucked from the plant. The count of chaff and viable seeds was established based on their weight using the floating method. Monthly averages were used for assessment purposes.

The genus *Rauvolfia*, part of the Apocynaceae family comprising 80 species, was cultivated at the Jawaharlal Nehru Tropical Botanic Garden and Research Institute. Voucher specimens for all species were deposited at the Jawaharlal Nehru Tropical Botanic Garden and Research Institute in Palode, Thiruvananthapuram, Kerala, India, with respective reference numbers: *R. hookeri* – 66449, *R. micrantha* – 66450, *R. serpentina* – 66451, *R. tetraphylla* – 66452, *R. verticillata* – 66453.

3 Results

The plants grew under a canopy at the JNTBGRI Campus, receiving less than 50% open sunlight. Data regarding the average rainfall in India for 2011 and 2012 were gathered from the India Meteorological Department's records ([http://hydro.imd.gov.in/hydrometweb/\(S\(eibinu55lyexxhqecj1iapn2\)\)/PRODUCTS/Publications/Rainfall%20Statistics%20of%20India%20-%202012/Rainfall%20Statistics%20of%20India%20-%202012.pdf](http://hydro.imd.gov.in/hydrometweb/(S(eibinu55lyexxhqecj1iapn2))/PRODUCTS/Publications/Rainfall%20Statistics%20of%20India%20-%202012/Rainfall%20Statistics%20of%20India%20-%202012.pdf)) and plotted (Fig.1). These data revealed similar rainfall patterns during both years.

Notably, the fruit set from available flowers within a season or across the year was significantly low in most species. For instance, in *R. hookeri*, only 7.52% of flowers resulted in mature fruits (Table 1). Conversely, *R. tetraphylla* exhibited the highest fruit setting at 70.36%. Various factors, such as weather conditions and lack of fertilization, led to the withering of remaining flowers. Furthermore, the limited light availability under the canopy directly impacted fruit production in relation to leaf phenology, as observed by Maeno and Hiura (2000).

3.1. Flowering and fruiting

In the selected five species of *Rauvolfia*, the data on flowering were plotted in Table 1. The highest number of flowers per peduncle was observed in *R. serpentina* (37.29 ± 3.04), whereas the lowest count occurred in *R. micrantha* (17.7 ± 0.92). Remarkably, *R. tetraphylla* exhibited the maximum fruit set at $70.36 \pm 3.47\%$, while *R. hookeri* displayed the minimum fruit set at $7.06 \pm 0.44\%$.

Table 1 Flowering/fruiting details of *Rauvolfia* species in the Southern Western Ghats

Trait/Species	<i>R. hookeri</i>	<i>R. micrantha</i>	<i>R. serpentina</i>	<i>R. tetraphylla</i>	<i>R. verticillata</i>
Flower/peduncle (nos.)	26.62±1.97	17.7±0.92	37.29±3.04	29.06±3.33	20.9±1.66
Fruit set (%)	7.06±0.44	59.81±3.20	22.63±1.85	70.36±3.47	50.04±2.54

3.1.1 *Rauvolfia hookeri*

In *R. hookeri* (as shown in Table 1), the lowest fruit set was observed. Fruit setting occurred consistently throughout the year, with the highest observed in August (refer to Fig. 2). Among the total fruits, the count of double and single-seeded fruits was recorded. August exhibited the maximum count of both double (6.4 ± 1.69) and single-seeded fruits (45 ± 14.41). The peak count of good seeds was observed in July (60.33 ± 31.49), while the highest count of chaff was noted in August (18.67 ± 5.52). December marked the period with the least availability of fruits.

3.1.2 *Rauvolfia micrantha*

In *R. micrantha*, fruits were consistently available throughout the entire year, spanning from January to December. The highest abundance was observed in the months of May and June, with counts of 47.5 ± 28.5 and 64.33 ± 22.29 , respectively. Interestingly, June revealed the presence of both double and single fruits, as well as chaff seeds (as depicted in Fig. 3). The maximum counts of double and single fruits were recorded in June, while the peak count of good seeds was observed in May. Surprisingly, June also marked the period with the highest count of chaff seeds. Conversely, the lowest availability of fruits was noticed in January.

3.1.3 *Rauvolfia serpentina*

In *R. serpentina*, fruit setting was observed solely during May and June, followed by a subsequent occurrence after a five-month interval, specifically in December. Among the total collected fruits, approximately 51% were identified as double-seeded. However, in December, only 60% of the collected fruits were double-seeded (Fig.2). Remarkably, a high percentage, 99% of the total collected seeds, were categorized as good quality.

3.1.4 *Rauvolfia tetraphylla*

In the case of *R. tetraphylla*, fruits were present year-round except during December-January and March. The highest fruit availability, accounting for 70%, was observed in April (refer to Table 1). Among the total collected fruits, a significant proportion, 72.87%, were classified as double-fruited. Moreover, the overall purity level of the seeds stood at an impressive 94.89%, as depicted in Figure 5.

3.1.5 *Rauvolfia verticillata*

In the case of *R. verticillata*, fruits were accessible from June to November, with the highest count recorded in July at 66.67 ± 30.91 . Similarly, the numbers of double fruits, single fruits, good seeds, and chaff seeds peaked during July. Subsequently, after the peak fruit set in July, there was a sudden reduction in fruit count, as illustrated in Figure 6. Out of the total available fruits, 43.99% were identified as double-seeded fruits, and the seed purity was measured at 79.66%.

4 Discussion

Rauvolfia species span semi-evergreen forests across tropical regions in Asia, Africa, and the Americas. In India, they're predominantly found in moist deciduous forests and plains. Flowering initiation and anthesis in these species, as noted by Borchert (1983), are influenced by distinct climate changes separated by considerable rest periods. Unlike flowering, fruit production displays less seasonality, typically coinciding with the peak of the rainy season.

Annual precipitation, persisting for approximately five months dominates the region's climate. Except for *R. tetraphylla*, most species exhibit maximum fruiting during this rainy period. Rainfall patterns, as correlated by Opler, *et al.* (1976), are associated with flowering in plants. Reproductive phenology within a family or genus tends to synchronize during the same time or season, as observed by Rathcke and Lacy (1985), Kochmer and Handel (1986), and Herrera (1992).

Garwood and Horvitz (1985) observed lower fruit set rates in open-pollinated flowers, noting that the number of ovaries and ovules often exceeds the number of resulting fruits and seeds. This study, relying on natural pollination, identified limitations in various reproductive stages, such as seed-to-fruit and fruit-to-flower ratios and ovule development. Challenges in pollination, limited pollinator availability or post-pollination environmental factors may lead to the development of only one ovule out of two in a flower. These limitations offer insights valuable to collectors like farmers and raw drug collectors.

Morphological continuity among flowers, fruits, and trees, highlighted by Primack, *et al.* (1987) is observed in *Rauvolfia*. Each species follows specific flowering and fruiting seasons, occasionally overlapping with others. A dry period followed by a rainy season may serve as a trigger for both flowering and fruiting in some species as reported by Opler, *et al.* (1976).

Juicy fruit production peaks during the wet season in tropical Montane cloud forests (William-Linera, 2003), akin to the predominantly rainy season fruit production in *Rauvolfia*. Fruit development and maturation time as per Primack (1985), correlate with fruit size, larger fruits requiring longer maturation periods.

Studies by Bazzaz *et al.* (1979) and Watson and Casper (1984) suggested that factors like the photosynthetic contribution of fruits may limit fruit set and seed production per fruit.

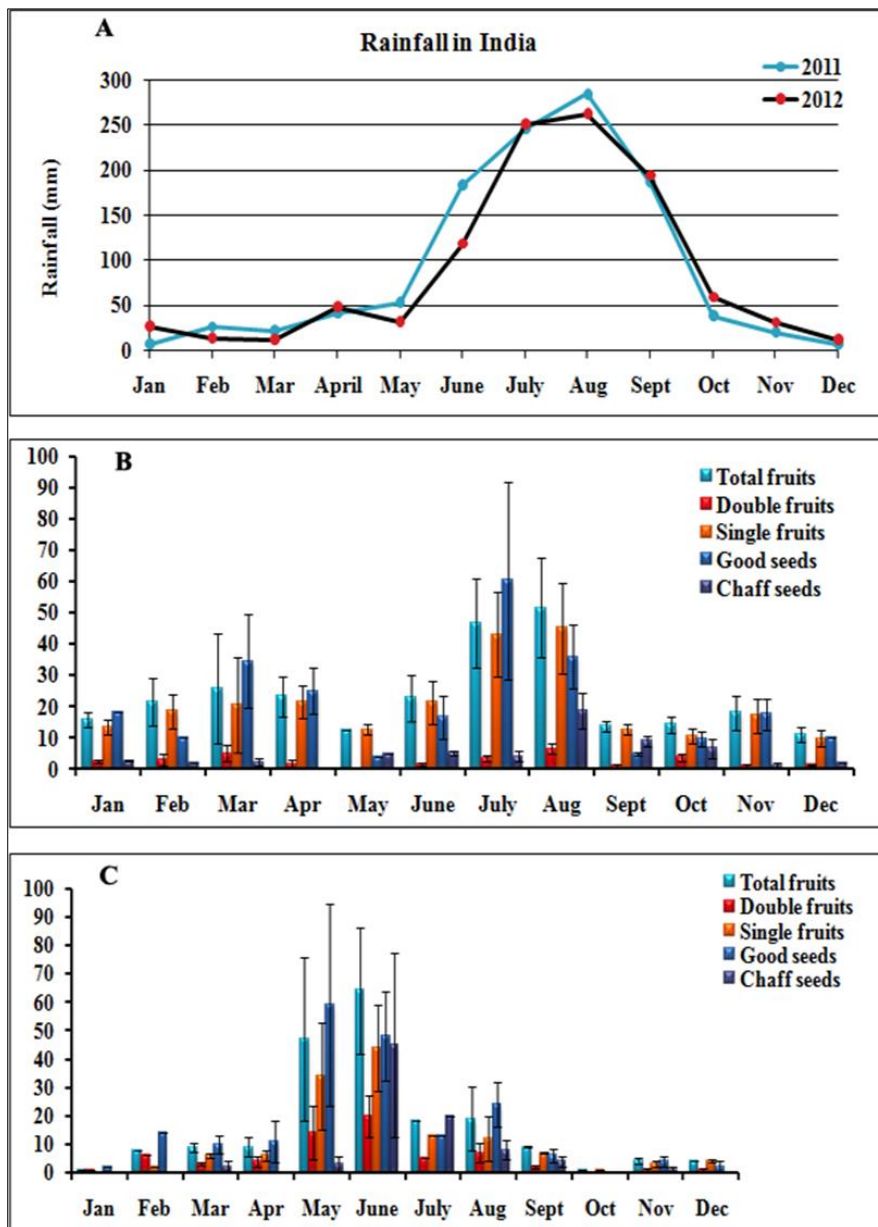


Figure 1 A. Monthly rainfall during 2011 and 2012, B, C. Monthly variation in the number of total fruits, double and single fruits, number of good and chaff seeds in *R. hookeri* and *R. micrantha* respectively

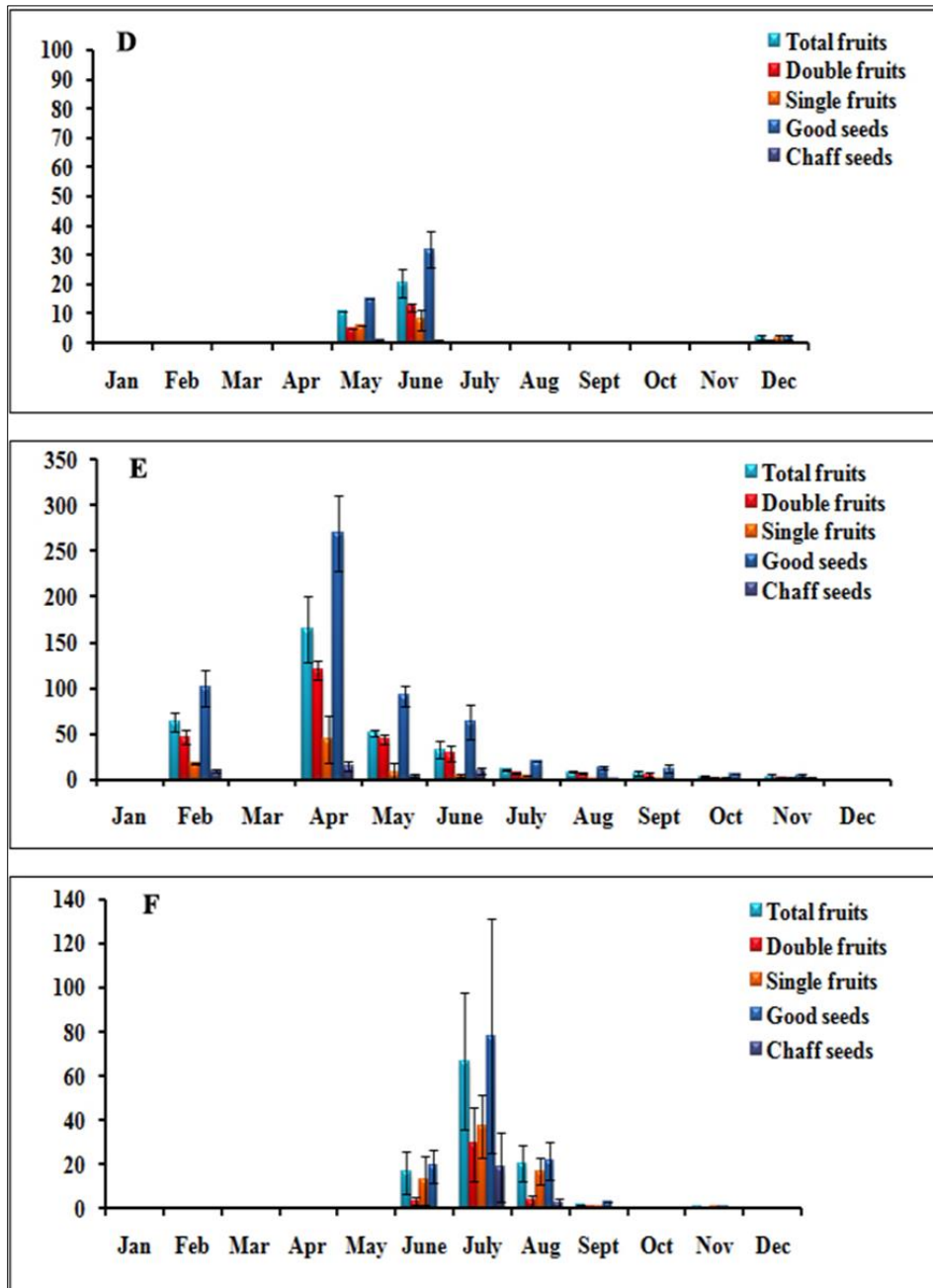


Figure 2 Monthly variation in the number of total fruits, double and single fruits, number of good and chaff seeds: D - *R. serpentina*; E - *R. tetraphylla* & F - *R. verticillate*

5 Conclusion

Rauvolfia species exhibit a finely tuned reproductive cycle linked to environmental factors, particularly rainfall and climatic patterns. This understanding of how these factors influence the timing of flowering and fruiting is crucial not only for conserving these species but also for optimizing agricultural practices involving them.

By recognizing and deciphering these relationships, conservationists and agricultural experts can better manage these plants. Implementing strategies that leverage the natural triggers for optimal flowering and fruiting can aid in both conservation efforts to protect these species and agricultural practices aimed at cultivating them. This knowledge enables more informed decisions in terms of planting, harvesting, and overall management, contributing to the sustainability of *Rauvolfia* species in their natural habitats and agricultural settings alike.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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