International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 02-07-2025, Accepted: 01-08-2025, Published: 16-08-2025
Volume 10, Issue 8, 2025, Page No. 35-38

Effects of moisture content in developing seeds of *Calophyllum apetalum* Willd., an economically important endemic tree species of Western Ghats

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Abstract
Calophyllum apetalum Willd., an endemic medicinal tree species of Southern Western Ghats belonging to the family Calophyllaceae (Clusiaceae). The seeds are recalcitrant nature and lose their viability within few days after detachment from the mother plant. The mature fruits of *C. apetalum* were collected from the different locations of Alappuzha district, Kerala. Moisture content was noted from seven consecutive developmental stages. November to January (0, 15, 30, 45, 60, 75, 90 days after anthesis) and in each developmental stage the seeds were subjected to moisture content analysis. Moisture content of developing seeds showed an increase, during initial stages then it declines. Thereafter when it attains maturity the moisture content was gradually decreased.

Keywords: Moisture content, recalcitrant, development, calophyllum, anthesis, maturity

Introduction

Calophyllum apetalum Willd., is a species under the genus Calophyllum belonging to the Clusiaceae family. The genus Calophyllum L. (Clusiaceae) includes about 187 species. Of these, 179 are present in the Old World (mainly in the Indo-Malaysian region) and about 8 are in the New World, from Mexico and the Caribbean to Argentina (Chinthu *et al*.,2022) According to the IUCN Red List, *C. apetalum* is classified as 'vulnerable'. Among the species of genus Calophyllum, only three species have been evaluated, while other species remain unevaluated and data-deficient (Chinthu *et al*., 2023) [3]. Fruit development is comprised of a series of complex physiological, biochemical and genetic processes. According to Nitsch (1952) [9] fruit is defined as "the tissues which support the ovules and whose development is dependent on the events occurring in these ovules". Seed development comprises two major phases: embryo development and seed maturation. Seed development commences with the initiation of flowering followed by formation of floral structures and effective pollination. In angiosperms, after fertilization, because of cell division, expansion and histo-differentiation, there is a stage in embryogenesis, in which seed structure primordia are formed and future embryo parts can be envisaged. Seed moisture content increases during the initial part of development after fertilization and later begins to decline until equilibrium is established with environmental factors. Seeds have been categorized into two main groups according to their desiccation response and storage physiology: seeds (desiccation-tolerant) that can be dried, without damage, to low moisture contents, usually much lower than those they would normally achieve in nature and recalcitrant (desiccation sensitive) seeds that do not survive drying to any large degree and are thus not amenable to long term storage (Roberts, 1973 [11]; Berjak, 2005) [1]. The critical moisture level to which mature embryos can be dried without inducing desiccation damage is generally species dependent and serves as a tool to define whether a seed is orthodox, recalcitrant or intermediate (Vertucci and Farrant, 1995) [12]. Recalcitrant seeds on maturation do not undergo drying or undergo drying only to a limited extent, therefore they are shed from the trees with relatively high moisture content of 0.4–4.0 g water/g and often high metabolic activity. The effects of moisture content in *Calophyllum apetalum* (recalcitrant seeds) with respect to the development was depicted in this work.

Materials and methods

Calophyllum apetalum Willd., an endemic medicinal tree species of Southern Western Ghats belonging to the family Calophyllaceae (Clusiaceae). The fruits are recalcitrant nature and lose their viability within few days after detachment from the mother plant. The mature fruits/seeds of *C. apetalum* were collected from the different locations of Alappuzha district (9.4981° N, 76.3388° E), Kerala. Data were subjected to analysis of variance (ANOVA).

Moisture content analysis

Moisture content was determined by the difference between fresh and dry weight.

$$\text{Moisture content} = \frac{\text{Fresh weight} - \text{Dry weight} \times 100}{\text{Fresh weight}}$$

For dry weight determination the seed was taken in a pre-weighed bottle and weighed in an electronic balance, dried in a hot air oven at high constant air oven method, 130^0C ± 2^0C for 1 hour (ISTA 1985) [7]. For each time point, 10 replicates of a sample were made. The dry weight was recorded in every harvest, after cooling to room temperature in desiccators.

Fig1: Fruits

Fig 2: Seeds

Results

Moisture content analysis

Normally moisture content of all developing fruits/seeds showed an increase during initial stages. Because water is the vehicle for transferring nutrients from the parent plant to the developing fruits. Likewise, the moisture content of *Calophyllum apetalum* fruit increases during initial stages. Moreover, enzymes, including those needed to produce the storage compounds, can be more active at high water contents. During 15 DAA, it shows a decline in their moisture content. Then a gradual increase in moisture content was noted till 30 DAA. After this a gradual decrease can be visible till it attains their maturity. Dehydration was observed to be slow during the initial phase and gets accelerated after seed attains maturity. This decrease in moisture content proceeds during the maturation drying phase until hygroscopic equilibrium is attained with the environment.

Table: 1 Moisture content analysis of *C. apetalum* during seed development

Stages (Days after Anthesis)	Fresh weight /10 replicates (gm)	Dry weight/10 replicates (gm)	Moisture content (MC) (%) ± SE
0 DAA	0.86±0.01^{g}	0.22± 0.01^{g}	74.81 ±0.63^{a}
15 DAA	2.23±0.01^{f}	0.65±0.00^{f}	71.00±0.08^{b}
30 DAA	3.89±0.01^{e}	0.98±0.01^{e}	74.78±0.15^{a}
45 DAA	4.12±0.01^{d}	1.21±0.01^{d}	70.65±0.14^{b}
60 DAA	4.60±0.01^{c}	1.52±0.01^{c}	66.95±0.15^{c}
75 DAA	4.76±0.01^{b}	1.92±0.00^{b}	59.62±0.15^{d}
90 DAA	4.91±0.00^{a}	2.63±0.01^{a}	46.47±0.12^{e}
Main effect; Df (n-1) = 6 F value:	26854.67***	22847.40***	1438.18***

±SE- Standard error of the mean, DAA- Day after anthesis, MC-Moisture content

Dry matter accumulation

The length and breadth of the seeds gradually increases during each developmental stage (Fig:1, table:2). The dry weight of the seed gradually increases. The moisture content gradually decreased throughout the development. Dry matter accumulation and moisture content (MC) give a reversible correlation (Fig:2) ie. When the dry matter content increases the moisture content (MC) decreases. The rate of drying depends on initial seed moisture content, atmosphere air temperature, relative humidity and drying temperature.

Table: 2 Dry matter accumulation in seeds of *C. apetalum* during development

Stages (Days after anthesis)	Fruit length (cm)	Fruit breadth (cm)	Fruit weight (gm)	Fresh weight /10 replicates (gm)	Dry weight/10 replicates (gm)
0 DAA	0.34±0.02^{g}	0.25±0.01^{g}	0.56±0.02^{f}	0.86±0.01^{g}	0.22± 0.01^{g}
15 DAA	0.56±0.01^{f}	0.46±0.01^{f}	0.66±0.02^{e}	2.23±0.01^{f}	0.65±0.00^{f}
30 DAA	0.87±0.01^{e}	0.77±0.01^{e}	0.95±0.01^{d}	3.89±0.01^{e}	0.98±0.01^{e}
45 DAA	1.27±0.01^{d}	0.93±0.01^{d}	1.76±0.01^{c}	4.12±0.01^{d}	1.21±0.01^{d}
60 DAA	2.26±0.01^{c}	1.84±0.01^{c}	2.94±0.02^{b}	4.60±0.01^{c}	1.52±0.01^{c}
75 DAA	2.97±0.01^{b}	2.65±0.02^{b}	3.92±0.01^{a}	4.76±0.01^{b}	1.92±0.00^{b}
90 DAA	4.62±0.06a	3.85±0.01^{a}	3.97±0.01^{a}	4.91±0.00^{a}	2.63±0.01^{a}
Main effect; Df (n-1) = 6 F value:	4436.08***	11167.78***	9242.41***	26854.67***	22847.40***

±SE- Standard error of the mean, DAA- Day after anthesis

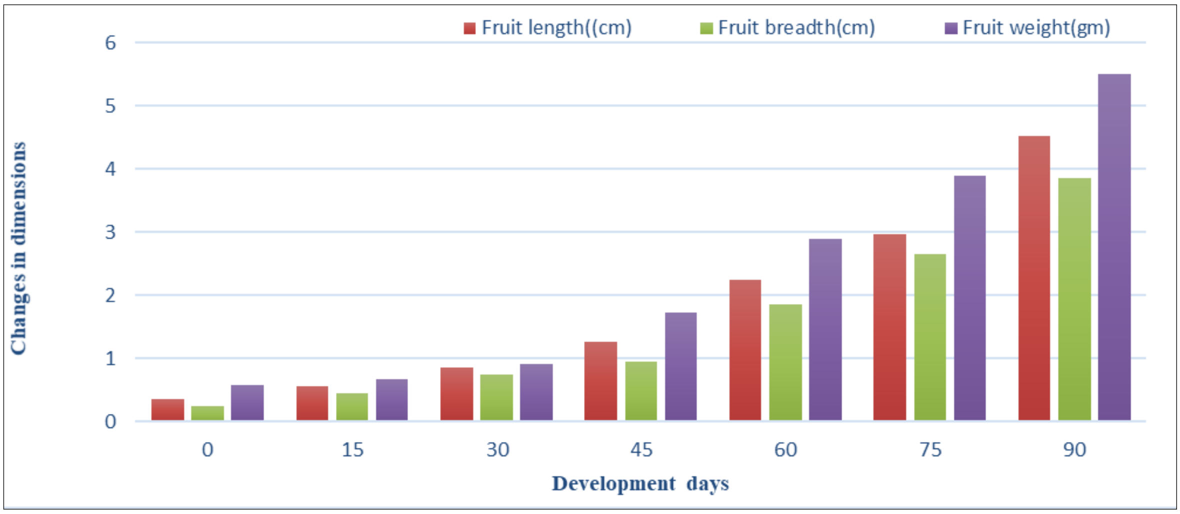

Fig:1 Changes in seed dimensions during development of *C. apetalum* fruits

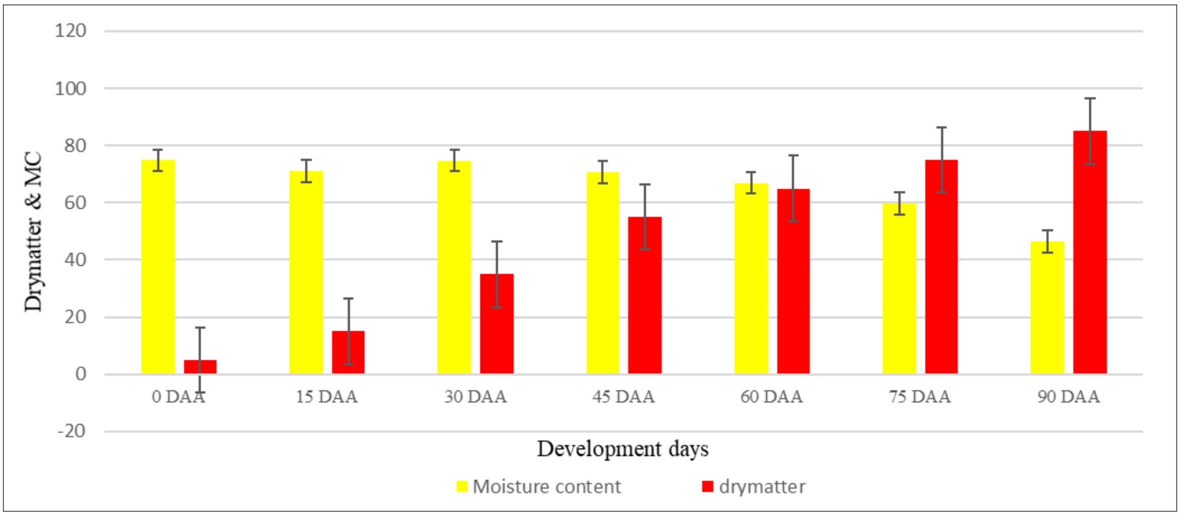

Fig:2 Changes in the dry matter and moisture content during development of *C. apetalum* fruits

Discussion

Induction of flowering and differentiation of flower parts are considered the starting points of seed development. The course of seed development and maturation is controlled genetically and involves an organized sequence of events starting from ovule fertilization to the point in which the seed becomes independent from the parent plant. Seed moisture content, seed size, germination, dry matter accumulation and seed vigour are the best parameters for evaluation of maturity status of developing seeds. Following pollination and fruit set, fruit initiation occurs. In *C. apetalum* fruit matures within 2 to 3 months ie, November to January (Chinthu *et.al*., 2025) [4]. At the beginning of seed development, the water content of *Calophyllum apetalum* fruits/seeds increase. *Ipomea turbinata* (Egley and chandler, 1978) [5] and *Acer pseudoplatanus* (Hong and Ellis, 1990) [6] developing seeds showed similar trends. The process of elimination of moisture from the seed is called drying. Seed drying should reduce the seed moisture content to safe moisture limits to maintain its viability. The present study revealed the moisture content of developing seeds initially high then a gradual reduction can be observed ie, water loss occurs during maturity (Table-1). During the initial stages of seed development, high water content is crucial for maintaining metabolic activity and adequate seed growth (Westgate and Grant, 1989) [13]. The fruits continued to grow both on a fresh and dry weight basis until maturity. It is evident that dry matter accumulation starts immediately after anthesis. There is a lag phase during initial stages of development but increased gradually after 30 days after anthesis. When the seed fully matures, the moisture content loss is most probably due to the accumulation of reserves, displacing water from the storage cells (Kermode, 1990). Seeds were physiologically mature at the end of seed development with maximum dry matter accumulation (Table-2). A similar pattern was observed during the different seed developmental stages in *Helianthus annus* (Renganayaki and Krishnasamy, 2001) [10]. Seed is the basic unit of multiplication, but it should possess quality characteristics in terms of physical, physiological sense, genetic purity and seed health.

Conclusion

The moisture content of *Calophyllum apetalum* seeds was initially high, but as dry matter content increased, some water was lost in the fruit. This water loss is caused by the fruits/seed's metabolic processes. Maturation drying is not possible due to the recalcitrant nature of the fruits/seeds. Thus, fruit requires more studies about their physiology and needs for proper conservation due to its vulnerable nature.

Acknowledgement
I sincerely thanks to The Director, KSCSTE - JNTBGRI for the facility to do the work. Thankful to Kerala University for financial (JRF) assistance.

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