



## Seed germination study of an endangered species *Myristica beddomei* ssp. *sphaerocarpa* W.J de Wilde by nicking method

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### Abstract

*Myristica beddomei* ssp. *sphaerocarpa* W.J de Wilde is an endangered species in southern Western Ghats of Kerala. This species is included in IUCN 2020 red list category due to its endangered status. The availability of the mature fruits rare from its habitat. The importance of conservation is essential for its sustainable life. Here we are carried out mechanical scarification method for removal of seed dormancy, which is more effective than other chemical and biological methods. Seed without hull in natural condition given best result than seeds without hull in seed germinator after transplanting to the field.

**Keywords:** *Myristica beddomei* ssp. *sphaerocarpa*, endangered species, seed germination study, mechanical scarification

### Introduction

Myristicaceae are medium sized angiosperm tree family with 21 genera and about 500 species. These are pantropical distribution, mostly found in low land rainforest habitats (Sauquet & Thomas, 2003) [10]. The species *Myristica beddomei* King is a one of the wild relatives of commercial nutmeg (*Myristica fragrans*). The Bitter nutmeg is a common name of *Myristica beddomei* King. It is endemic in semi evergreen and evergreen forest of south Western Ghats of Kerala, have three sub species, *Myristica beddomei* ssp. *beddomei*, *Myristica beddomei* subsp. *ustulata* W.J de Wilde, *Myristica beddomei* ssp. *sphaerocarpa* W.J de Wilde. According to IUCN (2020) [5] *Myristica beddomei* ssp. *sphaerocarpa* is under endangered category (Neethu S et al., 2021) [9]. The plant *Myristica beddomei* ssp. *sphaerocarpa* is a high altitude (<900m), evergreen dioecious tree species. The reasons for it endanger status due to its distribution; it is a high-altitude plant so wild attack and wind velocity also affect its existence; the number of mature fruits very low because the fruits will eat by giant squirrels and horn bills; the remaining mature seeds were not naturally germinating in the soil it may be due to the absence of available sunlight or the nature of the soil. The plant *Myristica beddomei* ssp. *sphaerocarpa* is differ from *Myristica beddomei* is due to the unique characters of leaves, fruits and seeds. The leaves are smaller than *Myristica beddomei* have 8-10 pairs of lateral nerves, brown

lower surface, without papillae, globose fruits and seeds (Wilde, 1997) [14]. The female plant of this species was newly collected by Beddome s.n on 1885 from South India (Madras, Kerala), eastern slopes of Western Ghats, Tinnevely District (Wilde, 1997) [14]. The plant *Myristica beddomei* ssp. *sphaerocarpa* is collected from the southern part of Western Ghats from Chemungi hills. The Chemungi hills is a buffer part of Agathyamala Biosphere Reserve in Thiruvananthapuram districts of Kerala at an elevation between 1100- 1430m (Shareef and Santhoshkumar, 2013) [11]. The only myristica species seen in this region is *Myristica beddomei* ssp. *sphaerocarpa* belonging to the family Myristicaceae. The flowering and fruiting phenology of this species since November to April (www.keralaplant.org) [15].

### Materials and Method

#### Collection of seeds

The plants with mature fruits and twigs were collected from Chemungi hills during the fruiting period January to March. Total 120 seeds were collected in a polythene bag included younger, mature and damaged seeds. The flowering and fruiting twigs collected for herbarium preparation (TBGT 97033). The figure 1 showing the *Myristica beddomei* ssp. *sphaerocarpa* fruits (a), nicking method for scarification(b), germination of seeds in artificial and natural condition with and without hull (c, d, e, f).



**Fig 1:** *Myristica beddomei* ssp. *sphaerocarpa* W.J de Wilde (a), Fruits (b), Mechanical scarification by nicking (c), seeds without hull in germinator (d), seeds with hull in germinator (e), seeds without hull in natural condition (f), seeds with hull in natural condition.

**Seed preparation for germination studies**

Seeds were soaked in water (24 h) and grouped as sinkers or floaters. The floating seeds are eliminated for further studies. The mature seeds collected for further regeneration studies by using conventional methods. The moisture content of seeds (%) was determined by oven drying method. The availability of mature fruits very rare so mainly two conditions opted for the studies. The factors as chosen as seeds with hull (hard seed coat), seeds without hull in natural condition and in seed germinator (RH 80, tem.30±2<sup>o</sup>C). The mechanical scarification done for the removal of hull without damaging the kernel by using knife (nicking). The seed germination and seedling growth are very slow. Mainly experiment focused on the time duration for the seed germination and growth in natural and artificial conditions.

Seeds in germinator (artificial)

1. Seeds with hull
2. Seeds without hull

Seeds in natural condition

1. Seeds with hull
2. Seeds without hull

**Germination study**

Germination study of *Myristica beddomei* ssp *sphaerocarpa* was done in two different conditions, seed germinator (Artificial) and Natural condition and the seeds hull removed by mechanical scarification.

Seed germination percentage was calculated by using the following formula (Lazim & Marwan, 2019)<sup>[8]</sup>.

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Germination related parameters were calculated by using following formulas:

**a Speed of germination**

Speed of germination calculated by the formula. (Gairola *et al.*, 2011) [4].

Speed of germination =  $n_1/d_1 + n_2/d_2 + n_3/d_3 + \dots$

Where, n = number of germinated seeds, d = number of days.

**b Mean germination time (MGT)**

The formula is, (Gairola *et al.*, 2011) [4].

$MGT = \frac{n_1 \times d_1 + n_2 \times d_2 + n_3 \times d_3 + \dots}{\text{Total number of days}}$

Where, n = number of germinated seed

d = number of days

**c Mean daily germination (MDG)**

The formula of Mean germination time is, (Gairola *et al.*, 2011) [4].

$MDG = \frac{\text{Total number of germinated seeds}}{\text{Total number of days}}$

**d Peak value (PV)**

The peak value was calculated by the formula, (Gairola *et al.*, 2011) [4].

$PV = \frac{\text{Highest seed germinated}}{\text{Number of days}}$

**e Germination value (GV)**

The germination value was calculated by the given formula, (Gairola *et al.*, 2011) [4].

$GV = PV \times MDG$

**f Germination index (GI)**

The Germination index was calculated by the formula,

$GI = (15 \times n_1) + (9 \times n_2) + \dots + (1 \times n_{15})$

$n_1, n_2, \dots, n_{15}$  = No. of germinated seeds on the first, second and subsequent days until the 15th day; 10, 9... and 1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively. (Kader, 2005) [7].

**g Seedling vigour index (SVI)**

Seedling vigour index was calculated by using the formula as suggested by Abdul-Baki *et al.*, (Abdul-Baki & Anderson, 1973) [1].

Expressed in whole number.

Vigour Index (VI) = Germination (%) x Seedling length (cm)

**Moisture content**

For the moisture content seeds dried in a standard hot air oven at  $105 \pm 1^\circ\text{C}$  for 24 h. The seed moisture content determined by using the following formula: (Gairola *et al.*, 2011) [4].

$\text{Moisture percentage} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$

**Results and Discussion****Germination studies**

*Myristica beddomei* ssp. *sphaerocarpa*, is a wild nut meg, the fruits splits open in its natural suture thereby knowing the seeds covered with yellow-coloured arils. This is an endangered plant. The reasons for its endanger status due to its distribution; it is a high-altitude plant so wild attack and wind velocity also affect its existence; the number of mature fruits very low because the fruits will eat by giant squirrels and horn bills; the remaining mature seeds were not naturally germinating in the soil it may be due to the absence of available sunlight or the nature of the soil. The importance of its ex-situ conservation protects the plants from extinction.

Result showing moisture percentage is  $22.5 \pm 1.5$  %. The final germination percentage of seeds in germinator seed without hull 70 %, seeds with hull 50% and in natural condition seeds without hull 85%, seeds with hull 40%. This observation reveals that final germination percentage is higher in seeds without hull in natural condition and very low percentage in seeds with hull in natural condition. The speed of germination was recorded highest in seeds without hull (4.60) in natural condition and lowest in seed with hull (1.14) in germinator. The mean germination time varied in seed with hull (9.9) in germinator and minimum in seed with hull (4.4) in natural condition. Similarly, the mean daily germination has only slightly variation in seed without hull (1.7) in natural condition and seed with hull (1) in germinator. The germination value was recorded maximum in seed without hull (1.7) in natural condition and minimum in seed with hull (0.3) in germinator. The highest peak value observed in seed without hull (1) in natural condition and minimum in seed with hull (0.3) in g erminator (Table 1).

**Table 1:** Germination characteristics in *Myristica beddomei* ssp. *Sphaerocarpa* W.J de Wilde as influenced by hulling in artificial and natural treatment.

Germination Parameters	Seed Germinator		Natural condition	
	Seeds with hull	Seeds without hull	Seeds with hull	Seeds without hull
Speed of germination	1.14	3.95	4.23	4.60
Mean germination time	9.9	7.75	4.4	6.9
Mean daily germination	1	1.16	1.6	1.7
Peak value	0.3	0.33	0.6	1
Germination value	0.3	0.382	0.96	1.7

The period of seed germination was mostly focused in this germination study because the time period of seed

germination and seedling growth was very slow.

**Table 2:** Seeds germinated in germinator

Observation period	Root length (cm)	Root breadth (cm)	No. of primary roots (cm)	Shoot length (cm)	Shoot breadth (cm)	No. of leaves (cm)	Leaves length (cm)	Leaves breadth (cm)	Seedling length (cm)	SVI (Seedling Vigour Index)
<b>seed without hull</b>										
1 <sup>st</sup> week	1.4±0.213	0.15±0.02	1.6±0.26	0.28±0.04	0.14±0.02	1.1±0.22	0.73±0.15	0.11±0.02	1.68±0.25	117.7±17.8
3 <sup>rd</sup> week	2.36±0.35	0.35±0.05	2.55±0.42	2.42±0.37	0.22±0.03	1.4±0.21	2.26±0.34	1.56±0.23	4.78±0.72	334.9±50.68
5 <sup>th</sup> week	4.47±0.67	0.49±0.07	4.45±0.07	4.12±0.62	0.23±0.03	2.8±0.42	2.95±0.44	2.01±0.30	8.59±1.29	601.65±90.56
7 <sup>th</sup> week	7.8±1.18	0.55±0.08	7.2±1.11	5.66±0.85	0.35±0.05	3.5±0.55	4.39±0.66	2.92±0.44	13.53±2.03	947.45±142.44
9 <sup>th</sup> week	10.67±1.60	0.56±0.08	8.7±1.32	8.58±1.29	0.37±0.05	4.2±0.63	6.82±1.02	3.38±0.50	19.25±2.89	1347.85±202.60
<b>Seed with hull</b>										
1 <sup>st</sup> week	0.65±0.18	0.07±0.02	0.85±0.25	0.13±0.03	0.07±0.02	0.6±0.21	0.43±0.13	0.05±0.02	1.02±0.23	51±11.83
3 <sup>rd</sup> week	1.36±0.32	0.15±0.03	1.5±0.36	1.13±0.26	0.10±0.02	0.9±0.21	1.01±0.24	0.83±0.19	1.58±0.36	79.25±18.22
5 <sup>th</sup> week	1.82±0.41	0.17±0.04	2.55±0.59	1.74±0.40	0.13±0.03	1.65±0.39	1.35±0.31	0.26±0.29	3.21±0.73	160.5±36.89
7 <sup>th</sup> week	3.05±0.71	0.26±0.06	3.3±0.78	2.49±0.57	0.17±0.04	2.4±0.58	1.81±0.41	1.66±0.38	4.11±0.94	205.75±47.29
9 <sup>th</sup> week	4.58±1.05	0.58±0.29	4.65±1.08	3.61±0.83	0.20±0.04	2.7±0.63	2.68±0.61	2.05±0.47	7.52±1.75	376.25±87.70

Values presented in the table are mean ± standard error of mean

**Table 3:** seeds germinated in natural condition

Observation period	Root length (cm)	Root breadth (cm)	No. of primary roots (cm)	Shoot length (cm)	Shoot breadth (cm)	No. of leaves (cm)	Leaves length (cm)	Leaves breadth (cm)	Seedling length (cm)	SVI (Seedling Vigour Index)
<b>seed without hull</b>										
1 <sup>st</sup> week	1.89±0.18	0.13±0.01	2.1±0.26	0.35±0.03	0.26±0.02	1.4±0.21	0.91±0.14	0.21±0.03	2.24±0.22	190.8±18.7
3 <sup>rd</sup> week	2.92±0.28	0.41±0.04	3.1±0.39	4.55±0.44	0.34±0.03	1.7±0.16	4.32±0.42	1.86±0.18	7.47±0.72	635.3±61.63
5 <sup>th</sup> week	3.93±0.38	0.51±0.04	9.05±0.90	9.00±0.87	0.36±0.03	3.4±0.32	5.33±0.51	2.68±0.26	12.94±1.25	1099.9±106.42
7 <sup>th</sup> week	10.22±0.98	0.63±0.06	12.1±1.19	12.43±1.23	0.50±0.04	5.1±0.49	9.63±0.93	3.49±0.34	22.65±2.20	1925.2±187.5
9 <sup>th</sup> week	15.19±1.46	0.69±0.06	14.35±1.40	15.73±1.52	0.53±0.05	5.1±0.49	13.68±1.32	5.07±0.50	30.93±2.98	2629.05±253.7
<b>Seed with hull</b>										
1 <sup>st</sup> week	0.52±0.14	0.07±0.02	0.95±0.27	0.18±0.05	0.08±0.02	0.5±0.19	0.82±0.13	0.05±0.02	0.70±0.20	28.2±8.01
3 <sup>rd</sup> week	1.55±0.15	0.12±0.03	1.45±0.41	1.39±0.39	0.09±0.02	0.8±0.22	1.7±0.47	0.49±0.14	1.94±0.55	77.8±22.06
5 <sup>th</sup> week	1.30±0.30	0.19±0.05	3.25±0.94	2.15±0.60	0.11±0.03	1±0.30	2.47±0.69	0.91±0.25	3.46±0.97	138.4±38.94
7 <sup>th</sup> week	3.18±0.89	0.28±0.08	3.8±1.09	2.83±0.79	0.16±0.04	1.3±0.39	3.26±0.91	1.26±0.35	6.01±1.69	240.6±67.64
9 <sup>th</sup> week	4.19±1.18	0.31±0.08	5.5±1.55	4.28±1.20	0.19±0.05	2.1±0.60	4.12±1.15	1.69±0.47	8.47±2.38	338.8±95.28

Values presented in the table are mean ± standard error of mean

To comparing seed with hull and seed without hull in seed germinator shows that root length in final week highest in seed without hull (10.67±1.60) and lowest in seed with hull (4.58±1.05). The root breadth in final week have least difference between seed with hull (0.58±0.29) and seed without hull (0.56±0.08). The number of primary roots maximum in seed without hull (8.7±1.32) and minimum in seed with hull (4.65±1.08). The shoot length has drastic difference between seed without hull (8.58±1.29) and seed with hull (3.61±0.83). The shoot breadth is maximum in seed without hull (0.37±0.05) and minimum in seed with hull (0.20±0.04). Number of leaves 4.2±0.63 in seed without hull and 2.7±0.63 in seed with hull in the final week. The length of leaves attains maximum in final week of observation of seed without hull (6.82±1.02) and only few numbers in seed with hull (2.68±0.61). The seedling length has drastic changes from 5<sup>th</sup> week to 9<sup>th</sup> week in seed without hull ie. 8.59±1.29 to 19.25±2.89. The seed vigour index highest in week by week and final SVI is 1347.85±202.60 in seed without hull (Table 2).

To comparing seed with hull and without hull in natural condition, the final observations are the highest number of root length (15.19±1.46), root breadth (0.69±0.06), number of primary roots (14.35±1.40), shoot length (15.73±1.52), shoot breadth (0.53±0.05), number of leaves (5.1±0.49), leaves length (13.68±1.32), leaves breadth (5.07±0.50), seedling length (30.93±2.98) and SVI (2629.05±253.7) noticed in seed without hull (Table 3).

To comparing table 2 and 3 between seed without hull in germinator and natural condition, seed without hull in natural condition shows highest number of root length, root breadth, number of primary roots, shoot length, shoot breadth, number of leaves, leaves length, leaves breadth, seedling length and SVI than seed without hull in germinator (Table 2& 3).

Comparing seed with hull in both germinator and in natural condition, highest root length 4.58±1.05, root breadth (0.58±0.29), shoot breadth (0.20±0.04), number of leaves (2.7±0.63), leaves breadth (2.05±0.47), and SVI (376.25±87.70) observed in seed with hull in germinator and highest number of primary roots 5.5±1.55, shoot length 4.28±1.20, leaves length 4.12±1.15, seedling length 8.47±2.38 noted in seed with hull in natural condition (Table 2& 3).

The in-vitro studies of *Myristica beddomei* ssp. *sphaerocarpa* scientifically proved that this species have cytotoxicity against human adenocarcinoma cell line (Neethu S *et al*, 2021) <sup>[9]</sup>. Hard seed coat provides long time protection of seeds, but in the case of *Myristica beddomei* ssp. *sphaerocarpa* hardness of seed coat leads to long term dormancy of seeds. This species has recalcitrant seeds so desiccation leads to loss of viability of the seeds. The viability of the seeds of *Myristica beddomei* King lost after 8 days under normal room temperature (Jose & Chandrasekharapillai, 2016) <sup>[6]</sup>. *Myristica dactyloides* Gaertn. are recalcitrant and also exhibit dormancy (Sivakumar *et al.*, 2006) <sup>[9]</sup>. The juvenile stem cuttings and

ring air layering also was carried for the conservation of some wild nutmegs (Jose & Chandrasekharapillai, 2016) <sup>[6]</sup>. Mechanical scarification by nicking was effective and low-cost method for the breakage of seed dormancy and germination. Chemical and mechanical scarification was used for the dormancy breakage of *mimosa foliolosa* seeds, concluded that mechanical scarification exhibit most reliable effect on dormancy breakage at all temperature (Silveira & Fernades, 2006) <sup>[12]</sup>. The seeds of *Pterocarpus angolensis* treated by gibberilic acid and mechanical scarification (nicking method), nicking method promote high and rapid rates of germination than using gibberilic acid (E Chisha- Kasumu *et al.*, 2010) <sup>[3]</sup>. To compare mechanical chemical and biological scarification of *Prosopis ferox* seeds, conclude that the effects in mechanical treatments obtained highest rates of germination and very low germination rates in biological treatment (Baes *et al.*, 2002) <sup>[2]</sup>.

### Conclusion

The seeds without hull in natural condition is most viable and healthy seedlings than the seeds without hull in germinator after transplanting to the field. The artificially (germinator) growing seeds have much stress to overcome natural condition in the field. The mechanically scarified (nicking) plants shows highest number seed germination. The seed with hull in both germinator and natural condition, almost they showing equal effect of germination. Healthy rooting system developed in seeds without hull in natural condition than seeds without hull in germinator. The highest germination percentage observed in seeds without hull in natural condition. The experiment proved that seed dormancy is the major reason for the germination of seeds, mechanical scarification (nicking) method most effective method for dormancy breakage than any other chemical or biological treatment in the case of *Myristica beddomei* ssp. *sphaerocarpa*.

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