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Lasiodiplodia theobromae infection on *Myristica malabarica seeds:* Threat in artificial regeneration

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Abstract
Lasiodiplodia theobromae (Pat.) Griffon and Maubl. was found associated with seeds of several tropical tree species, which eventually proved decline in regeneration potential in many forest tree species. Germination studies on seeds of *Myristica malabarica* for artificial regeneration recorded incidence of the ascomycetic fungal pathogen in stored *Myristica* seeds. Seeds were collected from the natural stands during the months of February - March for four consecutive years of study 2018 to 2021, from Mullaringad forest range in South India with coordinates of 10°1'4" N and 76°47'10" E. Seeds stored in containers detected fungal infection after one month of storage. Mean incidence percentage of 28.75% was recorded in stored seeds collected during 2018. Infection appeared as fluffy, cottony, grayish white patches. Germination characteristics of fresh and stored seeds were evaluated by ANOVA. Fresh seeds recorded a mean germination percentage of 31% and that of infected seed lot was 4.5%. Seed borne fungi *Lasiodiploida theobromae* is a threat in plantation practices of *Myristica malabarica.* The disease incidence is random in seed lots but has a significant impact in reducing germination. Since *M. malabarica* is listed as vulnerable in IUCN, its propagation requires high consideration.

Keywords: fungal incidence, germination percentage, germination value, peak value, seed dimensions

Introduction

There is a growing need for forest nurturing due to dramatic increase in loss of forest cover. Plantation of native species in reforestation programs has been of great interest in present days [1]. In past decades emerging infective diseases in forest is mainly due to introduction of invasive pathogens to new geographical areas, hybridization of fungal pathogens or hyper virulent strain of an existing pathogen [2]. Pathogenic fungi such as *Puccinia psidii, Cryphonectria cubensis, Phaeophleospora destructans* and more than thirty species of *Mycosphaerella* are a major threat to the *Eucalyptus* plantation in Indonesia [3]. Economic loss to forest owners in Europe were caused by a fungal pathogen *Heterobasidion annosum sensu lato (s.l.)* in 1995 was about € 600 million annually due to wood decay and tree mortality[4]. In northern Europe the frequency of root rot has been increased by 23% in plantation forest [5] *Teratosphaeria zuluensis* causing stem canker in Eucalyptus were observed within the collected seeds and seed capsule [6]. White pines in Northern USA and Eastern Canada which is a crucial forest component are infected with 22 species of fungi including *Lecanosticta acicola, Septorioides sp., Lophophacidium dooksii, Bifusella linearis* affecting pine needles and fruiting bodies [7].

From 100 selected tree species of four forests of Western Ghats, twenty eight *Pestalotiopsis* spp. isolated as foliar endophyte [8]. Seeds collected from forest trees like Teak, Subabul, Gulmohar, Mangium, Ratangunj and Garmalo are frequently infected with *Alternaria*, *Aspergillus* sp., *Fusarium* sp. and *Trichoderama* sp.[9]. Rubber tree plantation is affected by *Rigidoporus microporus* throughout the tropics which is a serious disease causing reduced yield [10]. In tropical forest trees seed storage is a major problem as it is sensitive to desiccation and most of them are recalcitrant [11].(Vázquez-Yanes and Orozco-Segovia, 1990). Fusarium sp. and Cercospora sp. are seed- borne fungi in *Tectona grandis* can cause wrinkling and decay of seeds within stony endocarp [12][13].

Lasiodiploida theobromae, a pathogenic fungi reported to infect host plants like mango, avocado, papaya, cocoa and jatropha [14, 15, 16, 17]. Dieback symptoms of *Mangifera indica* are frequently caused by *Lasiodiploida theobromae* along with four other species of *Lasiodiploida* [16]. Among five species of *Lasiodiploida* identified, *L. theobromae* is the most prominent fungi causing stem-end rot in *Carica papaya* [18].Root rot and collar rot disease reported in *Jatropha curcas* due to *L. theobromae* showed symptoms like yellowing and shedding of leaves, root rotting and blackening and decaying of stem collar[19].

Myristica malabarica Lam. included among top traded twenty medicinal plants of India [20]. Fruit rind is traditionally used to treat gastric disorders, leucorrhea, fever and piles [21]. Methanol extract of plant cures stomach ulcer [22].It is also a component of antitumour drug 'muthu-marunthu'[23].

Seed propagation plays an important role in natural regeneration of forest. Infection and degeneration of fruits and seeds affect restoration of forest cover. Studies based on fungal infection in tropical plants with respect to seeds were scanty. Present study deals with fungal infection in seeds of tropical tree species *Myristica*

malabarica belonging to family Myristicacea. This species is listed as vulnerable in IUCN red list of threatened species.

Materials and methods

Seeds of *Myristica malabarica* locally known as kattujathi were collected from Mullaringad forest range in Idukki district with coordinates of 10°1'4" N and 76°47'10" E. Seeds were collected from the natural stands during the months of February - March for four consecutive years of study 2018 to 2021. Collection of fruits was done with the help of a pole with a net. Split open fruits and mature entire fruits were collected. Plastic sheet were spread under tree prior to collection and freshly fallen seeds were also collected. Seeds were taken out from split open fruits while entire fruits were kept for few days to open. Seeds were covered with leathery aril which was removed carefully. Seed and fruit dimensions were measured (Fig.1).

Seeds were randomly selected and shade dried in open air and stored in containers at room temperature (29 ± 2°C) and humidity between 60%-90%. After one month of storage infection was detected in stored seeds collected during 2018. Region of infection were carefully and critically examined under dissection microscope for symptomatological study. Teased and scratched fragments and hand sections were taken for microscopic observation. Spore ornamentation and morphological characters was carried out. Measurements of microscopic structures were taken using stage micrometer. Microorganism incidence percentage in seeds was calculated using the formula [24].

$$\text{Incidence \%} = \frac{\text{Number of seeds recorded with an organism}}{\text{Total number of seeds examined}} \times 100$$

Fresh seeds, one month stored normal seeds and stored seeds from infected lots were subjected to germination studies. Four lots of 100 seeds each were tested in each case. Seeds were sown in coir pith and soil in 1:4 ratio in root trainers. Germination studies was conducted in poly house located at Dr. T.C Joseph Memorial Botanical Garden, Department of Botany, Union Christian College, Aluva with coordinates 10°7'30"N and 76°20'3"E.

Mean germination time calculated using the formula [25] $MGT = \sum_{i=1}^{n} N_i \; G_i / \sum_{i=1}^{n} G_i$. Germination value [26] GV= MDG x PV were, MDG is the final mean daily germination and Peak value (PV) is maximum MDG. One way ANOVA test was carried out for statistical analysis and significance of the difference between means was determined by Duncan's multiple range tests. Obtained data were presented as means ± SD.

Fig 1: Measurements of *Myristica malabarica* (A) Fruit (B) Seed

Result

Seeds of *Myristica malabarica* were collected from trees of girth 40-55 inch at breast level and height 15-20 meters. Fruit is large, cylindrical, tomentose, brown velvety with a single seed. Aril golden yellow to red completely covers the seed. Pericarp is thick and succulent. Testa is hard. Measurements of fruits and seeds are given in Table 1.

Table 1: Measurements of *Myristica malabarica* fruits and seeds collected

Dimensions of	Mean length (cm)	Mean width (cm)	Mean weight (g)
Fruit	7.72 ± 0.34	4.55 ± 0.18	121.93 ± 10.02
Seed with aril	6.22 ± 0.18	2.27 ± 0.09	19.59 ± 1.80
Seed	5.15 ± 0.24	2.07 ± 0.17	16.07 ± 2.18

Data were represented as mean ± SD, n=15

Seeds stored in containers were detected with fungal infection after one month of storage. Incidence percentage of 28.75% were recorded in stored seeds collected during 2018(Fig 2). Infected seeds were removed from the

bulk of stored seeds. Infection appeared as fluffy, cottony, grayish white patches. Fungus was identified as *Lasiodiploida theobromae* (Pat.) Griffon & Maubl. (Synonym: *Botryodiploida theobromae*, *Diplodia theobromae*) (Fig. 3.).

Pycnidia brownish black about 200 µm diameter formed on a hairy stroma. Conidiophores were septate, cylindrical, hyaline, 52 µm long and 3.6 µm wide. Immature spores were hyaline and aseptate. Mature conidia were dark brown sub ovoid to ellipsoid- ovoid with thick walled, vertically striated and one-septate, 26-30 X 13-15µm.

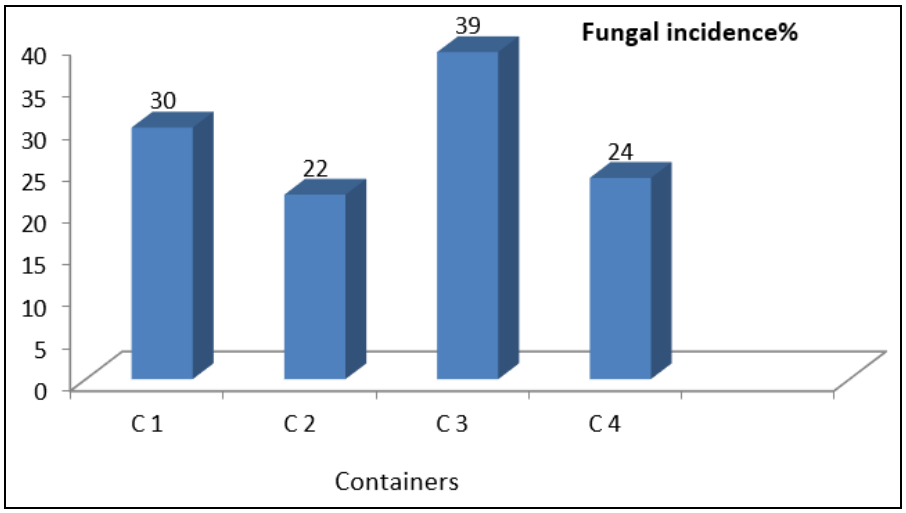

Fig 2: Incidence percentage of *Lasiodiploida theobromae* in stored seeds of *Myristica malabarica*

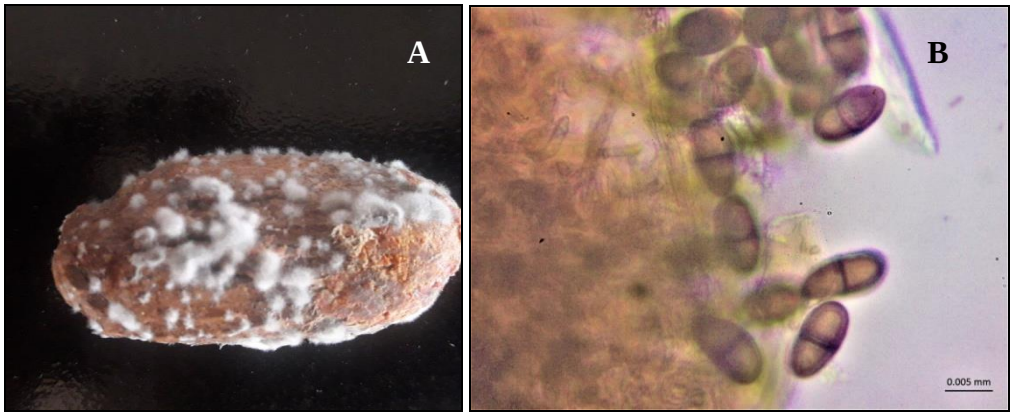

Fig 3: (A) Infected *Myristica malabarica* seeds (B) *Lasiodiploida theobromae* conidia

Fresh seeds, stored seeds and seeds from infected seed lots were germinated in root trainers Germination characteristics like germination percentage, mean germination time, peak value and germination value of fresh seeds, seeds stored for one month and seeds from infected lots were recorded (Table 2). Data were plotted in box plots (Fig. 4).

Table 2: Germination percentage, Mean germination time (MGT), peak value (PV) and Germination value (GV) of fresh seeds (FS), seeds stored for one month (SS) and seeds from infected seed lots (ISS) of *Myristica malabarica* seeds

Seeds	G (%)	MGT ($\bar{t}$) (day)	PV	GV
FS	31.00 ± 2.16 ^{a}	60.04 ± 0.90 ^{b}	0.420 ± 0.031 ^{a}	0.1788 ± 0.024 ^{a}
SS	9.75 ± 1.26 ^{b}	81.89 ± 2.54 ^{a}	0.105 ± 0.014 ^{b}	0.0108 ± 0.003 ^{b}
ISS	4.50 ± 2.65 ^{c}	83.00 ± 4.36 ^{a}	0.052 ± 0.027 ^{c}	0.0031 ± 0.003 ^{c}
P value	<0.001	<0.001	<0.001	<0.001

Data were represented as mean ± SD, n=4. Same superscript letters indicates no significant difference in Duncan multiple range test at $p \leq 0.05$.

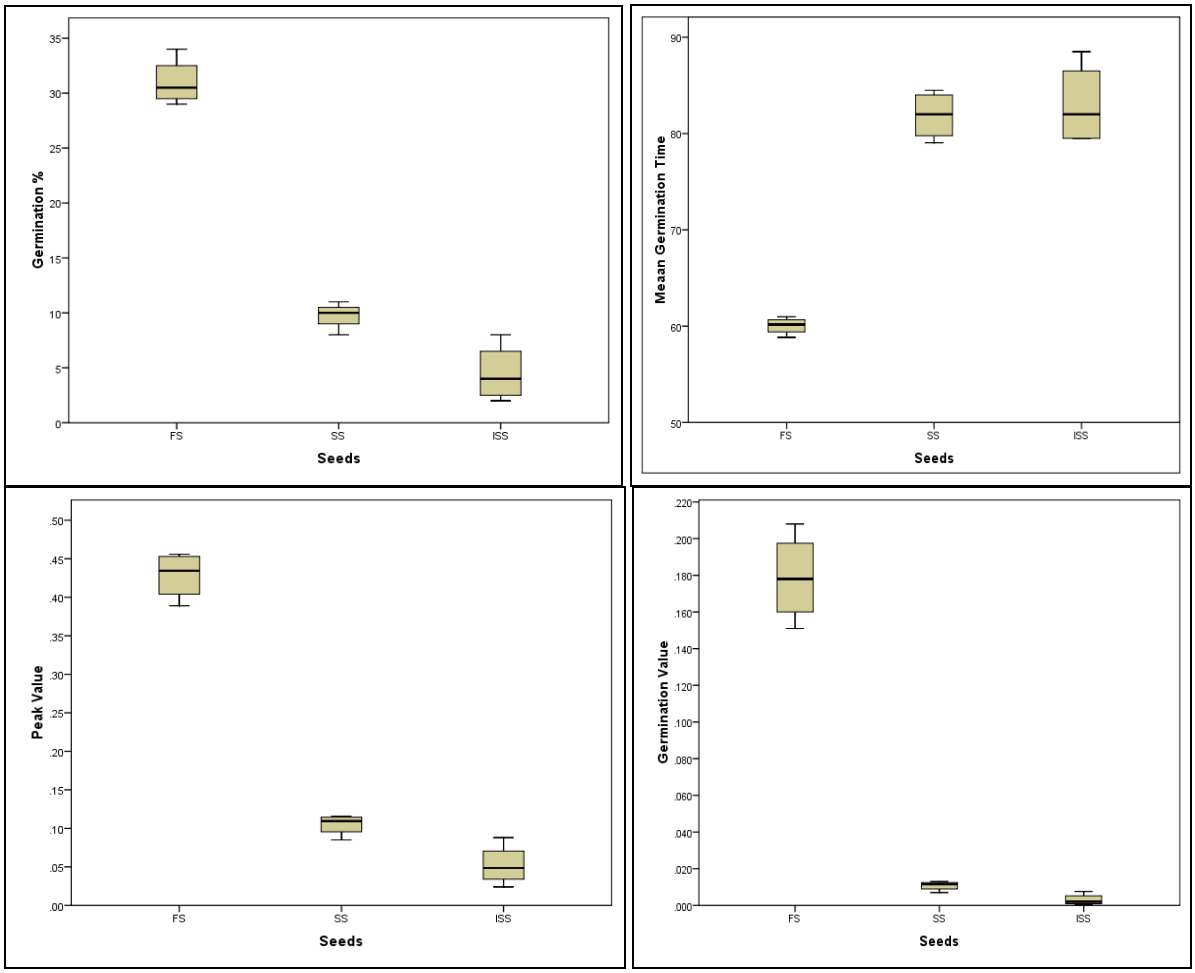

Fig 4: Box plot displaying germination characteristics of *Myristica malabarica* seeds. FS- fresh seed; SS-stored seeds; ISS- infected stored seeds.

Discussion

Seed germination rate of fresh seeds of *Myristica malbarica* exhibited significantly higher germination percentage of 31% compared to one month stored seeds of 9.75%. A decline in germination percentage (4.5%) was found in seeds from *Lasiodiploida theobromae* infected seed lots. Depletion in germination was reported in *Hibiscus cannabinus* seeds infected by *L. theobromae* [27]. Reduced germination potential and damping -off in seedlings recorded in two species of Pinus, *P. elliotii* and *P. taeda* [28]. Lower mean germination time (MGT) of 60.04 days were noted in fresh seeds while higher MGT was recorded in both one month stored normal seeds and seeds from infected lots. Higher peak value of 0.420 and germination value 0.1788 were recorded in fresh seeds and lowest peak value of 0.052 and germination value of 0.0031 in seeds from infected lots. Germination percentage of *M. malbarica* demands a requirement of 30-40% more seeds to be collected than the required number of seedlings. Collection of excess seed is economically advisable [29].

Grayish white patches of fungi on stored seeds of *M. malabarica* were fast growing. *L. theobromae* is an aggressive pathogen affecting seeds, seedlings, leaves, stem and fruit [30]. *L. theobromae* fungal infection starts usually after harvest [31]. Seeds were stored at room temperature was infected. Fungal mycelium grows profusely at optimum temperature 30°C [32]. Fungal incidence of stored seeds was low to moderate (28.75%) as the infected seeds were removed from the lot of stored seeds soon after detected. *L. theobromae* was identified to be transmitted through seeds [30]. This fungus is found in internal structure of seeds reducing germination potential [28]. Thus *L. theobromae* in seeds of *M. malabarica* is of major concern as it affects seed germination.

Conclusion

Seed borne fungi *Lasiodiploida theobromae* were pathogenic to *Myristica malabarica* reducing its germination. Since *M. malabarica* is listed as vulnerable in IUCN list its propagation is of high consideration. Fungi play a critical role in limiting the germination of tropical tree seeds. There is a requirement of further investigation in different pathogenic fungus affecting tropical tree species to maintain biodiversity. Studies are going on to diagnose different fungal pathogen and how to reduce its incidence in seeds of tropical tree species.

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