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Fungal pathogens associated with the death of young potted Teak (*Tectona grandis*) stumps in the forest research nursery at Kumbalpola in Sri Lanka

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Abstract

The most common planting material used to establish teak plantations in Sri Lanka is root stumps. However, significant mortality of potted teak stumps was recently observed in the Forest Research Centre nursery at Kumbalpola (Kurunegala district). Three fungal pathogens were consistently isolated from tissues of dead potted teak stumps obtained from the seeds of three local clonal seed orchards as well as stumps obtained from imported seeds of Myanmar origin. Fungal pathogens were identified as two species of *Fusarium* and one species of *Lasiodiplodia* by culture characters of isolates on Potato Dextrose Agar medium and microscopic observations of conidia and mycelia of isolates. Probably this might be the first report of *Fusarium* as well as *Lasiodiplodia* species causing the death of potted stumps of teak in Sri Lanka. Laboratory test results (*in vitro*) indicated that fungicide- Homai (Thiophanate methyl 70WP + Thiram 30 WP) at the concentration of 1000 ppm was the best fungicide controlling both *Fusarium* and *Lasiodiplodia* species followed by Thiram 70WP (1400 ppm) and Captan 50WP (1200ppm).

Key Words: Death of teak stumps, *Fusarium* and *Lasiodiplodia* species

1. Introduction

Teak inhabits naturally, Burma, India, Thailand, and Laos is a tropical deciduous forest tree species, known scientifically as *Tectona grandis* and is classified under the family Lamiaceae [1]. Teak is one of the most expensive timbers in tropical countries. It is widely used in shipbuilding, furniture, carving, and many various purposes. Lightness with strength, stability, durability, lack of cracking and splitting during working, ease to work with, resistance to termites, fungi as well as weather resistance, and non-corrosive properties are the characteristics that fetch a high price for teak. The physical and mechanical properties of the teak timber are comparable to or even superior to other popular timber of the temperate region [1].

About 5.7 million hectares of teak plantations are recorded over 36 countries all over the tropics. Teak constitutes about 75% of the world's high-quality tropical hardwood plantations. India has the highest (43%) amount of teak plantations while Indonesia Thailand, Myanmar, and tropical Africa constitute 31%, 7%, 6%, and 5% respectively [2].

Warm-moist tropical climate together with rainfall between 1250-2500 mm per year with a marked dry period of 3-5 months is ideal for growth teak. The mean day/night temperature range suitable for teak is from 20-27 ^{0}C to 30-36 ^{0}C [1]. Teak prefers deep, well-drained alluvial soils [3], pH between 6.5 – 7.5 is most suitable for teak [4]. Van Rhede, a Dutchman first introduced teak trees to Sri Lanka from the Malabar Coast in 1680 [5].

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Teak is one of the major forest plantation crops in Sri Lanka. It is mainly grown in the Dry and Intermediate lowland areas of the country [6]. Teak timber has high demand and is categorized under the super-luxury class of Timber Classification of the State Timber Corporation, Sri Lanka. Presently, Sri Lanka has nearly 25000 hectares of state-owned teak plantations i.e. 32% of total forest plantations established and managed by the Forest Department for supplying the timber demand of the country (Personal communications with officers of the Forest Department). Furthermore, the main timber species in private timber plantations and home gardens in the dry, as well as intermediate rainfall areas in Sri Lanka is teak [7].

Presently, the Forest Department harvest around 500 ha of teak plantations annually to supply timber, which produces around 60 cubic meters of timber/ha worth around Rs.3.6 million/ha (Personal communications with officers of the Timber Corporation). These harvested lands are again replanted with timber trees as appropriate while considering such factors as ecological conditions, socioeconomic factors, animal damage, etc.

2. Background of the study

Genetically superior and high-quality planting materials are essential for the high productivity of forest plantations. In the year 2020, a study was initiated to compare the performance of teak plants obtained from seeds of 3 local clonal seed orchards (CSO) established in the Kurunegala district (Barigoda, Horakele, and Ethgala) and one exotic source from Myanmar. The most common planting material used to establish teak plantations in Sri Lanka is root stumps. Therefore, well-grown teak seedlings from four different seed sources were uprooted from the mother beds and root stumps were prepared after removing the shoot (about one inch above the collar) and root (about 9 inches below the root collar) of the seedlings. Sprouting of these stumps started two weeks after transplanting in poly tubes, but unexpectedly, mortality of potted stumps was observed in the nursery. Potting media used was topsoil and the size of potted poly bags was 9" X 6". Potted plants displayed symptoms of scorching at the edge of tender leaves of the newly emerged sprouts about four weeks after transplanting followed by wilting appearance and eventually led to the death of whole plants. Further, decaying of root stumps was observed in un-sprouted stumps. Percentage survival of sprouted stumps varied from 18% to 62% and the lowest was observed from Myanmar seed source.

After visual observations of dying seedlings, it was suspected to be bacterial wilt caused *by Ralstonia solanacearum*. However, initial clinical tests revealed the presence of neither vascular discoloration (dark brown) nor ooze (milky-white exudate). Therefore, it was decided that the causal organism was probably not a bacterium.

Further, it had not been found any local research article regarding diseases of teak except bacterial wilt associated with nursery teak plants. However, some research articles published in various countries showed that fungal pathogens caused root rot and tree wilting of teak plants [8, 9].

Therefore, wilted and healthy plotted stumps of all seed sources were collected and sent to Plant Pathology Division at the Horticultural Crops Research and Development Institute-Gannoruwa for laboratory analysis to identify the causal organism/s and effective fungicides to develop control measures for the death of teak stumps.

3. Materials and Method

A. Identification of pathogen/s:

Unstained dead and healthy tissue scrapings obtained from the surface of teak stumps were microscopically observed to identify associated pathogens. Three types of morphologically

different conidia and fungal mycelium were observed in dead tissues. Pieces of rotten tissues were kept on Potato Dextrose Agar (PDA) medium and incubated for 10 days under room temperature to observe the mycelia growth and conidia development of each isolate on PDA. Then single spore inoculants of fungi isolates were made and each isolate was subcultured on PDA and incubated for 10 days under room temperature in continuous light to observe the culture characters and morphological features of conidia microscopically. Conidia suspensions were prepared in sterile distilled water (SDW) and the conidia size of each isolate was determined by measuring the length and width of 50 unstained conidia using an optical microscope.

B. Pathogenicity test:

The pathogenicity was proved as per Koch's postulates by inoculating the healthy potted teak stumps with a fungal spore suspension. Six isolates, two each from a pathogen were collected from three types of pathogens and each isolate was also wound inoculated by pin prink methods [10] using conidia suspension (around 10^4 conidia/ml) on the tip of the shoot of one-month-old healthy potted teak stumps grown in poly-tunnel under 24℃-36℃ and 60-90% relative humidity. Conidia suspensions were obtained from cultures on PDA. Conidia were harvested by adding 10 ml of SDW to the culture plates which were then gently shaken. The density of the conidia in the suspension was measured using a haemocytometer and adjusted number with SDW. Two potted teak stumps were used to inoculate each isolate and un-inoculated potted teak stumps were served as control. Potted teak stumps of each treatment were randomly arranged on a greenhouse bench at a distance of 20 cm from each other. Then inoculated stems were consistently observed for symptom development. Two months after inoculation, fungi were re-isolated on PDA from tissues on inoculated sites of teak stumps to perform Koch's postulate.

C. Identification of effective fungicides (In vitro):

The efficacy of non-systemic fungicides and systemic fungicides against two *Fusarium* spp and *Lasiodiplodia* spp were assessed by *in vitro* test using PDA as basal culture media [11]. The required quantities of each fungicide were calculated and thoroughly mixed with autoclaved and cooled (40-45℃) PDA in conical flasks separately to obtain desired concentrations (Table 1). The conical flask containing the poisoned medium (mixture of PDA + fungicide) was well shaken to facilitate a uniform mixture of fungicides and 20ml of the poisoned medium was poured into 9cm diameter sterile Petri plates and kept for solidifying. The mycelial disc of 5mm diameter of a seven-day-old culture of *Fusarium* and *Lasiodiplodia* species was cut with the help of a sterile cork-borer. Each disc was transferred aseptically to the center of each Petri plate. The PDA plates without fungicide were also inoculated with respective fungi and maintained as control. All PDA plates were incubated at 28℃. The experimental design used was a Completely Randomized Design with three replications. The colony diameter of each fungus was recorded periodically until the Petri plate of the control treatment was fully covered with mycelia growth. Percentage inhibition of mycelia growth (I) of fungi species by different fungicides was calculated by the following equation [11].

$$\mathrm{I} = \frac{\text{Growth of fungus in control plate (mm)} - \text{Growth of fungus on the treated plate (mm)}}{\text{Growth of fungus in control plate (mm)}} x100$$

Table 1. Fungicides and their concentrations were used for the efficacy testing against two species of *Fusarium* and one species of *Lasiodiplodia*.

Treatments	**The common name of fungicides**	**Product concentration used for the study**

T1	Thiophanate methyl 50%WP + Thiram 30% WP (Homai)	1000ppm
T2	Thiram 80% WP	1400ppm
T3	Captan 50%WP	1200ppm
T4	Control	-

4. Results and Discussion

Wilt symptoms started from the tip of the shoot and then continuously spread downward causing defoliation and eventually death of sprouted stumps within a few days. Two *Fusarium* species were identified by comparison of their colony characters on PDA and morphological features of conidia with published data [12].

One *Fusarium* species on PDA was white early and then turns into brown when mycelia become older. The reverse colony colour on PDA was light brown and then turns into dark brown. Microscopic observations of mycelia reveal that there were two types of conidia *i.e*. macro and microconidia. Macro conidia were rare, 5-7 cells, and mean size ranged 44-51 x 5-7 µm. Micro conidia were abundant, cylindrical, 1-2 cells, comparatively smaller than macroconidia, and mean size ranged from 4-6 x 2-3 µm (Figure 1).

The second *Fusarium* spp. on PDA was white early and then turns into cream when mycelia become older. The reverse colony colour on PDA was light brown and then turns into brown. Microscopic observations of mycelia reveal that there were two types of conidia similar to previous *Fusarium* species. However, size, shape, and mean number of septation of conidia were different between both species tested. Microscopic observation of this *Fusarium* species indicated that macro conidia were few, straight, pointed at ends, 5-6 cells, and mean size ranged 42-56 x 3-5 µm. Micro conidia were abundant, cylindrical, mainly single cell, comparatively smaller than macroconidia, and mean size ranged from 2-4 x 1-3 *µm* (Figure 2).

Third fungus species were identified by comparison of their colony characters on PDA and microscopic observations of mycelia, shape, size, and colour of conidia with published data [13]. The colony color was initially white, turning gradually grey on the upper side of the plate and black on the reverse side when grown on PDA. The cultures displayed holoblastic, hyaline conidiogenous cells. Immature conidia were hyaline, ovoid, and unicellular. Mature conidia had thick-walled, dark brown with one septum and conspicuous longitudinal striations. The size of the mature conidia ranged from 25-31 x 14-16 µm (Figure 3). According to these characteristics, the fungus was identified as *the Lasiodiplodia* species.

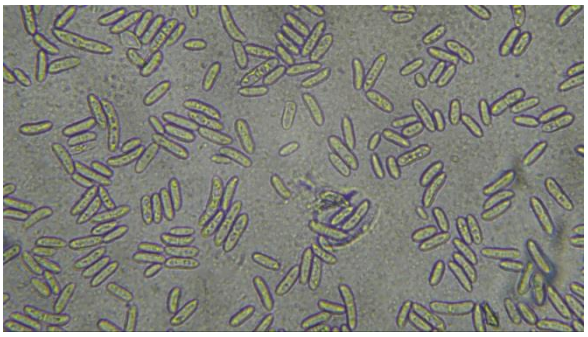

Figure 1. Microscopic view of macro and microconidia of one of the *Fusarium* sp. associated with diseased teak stumps.

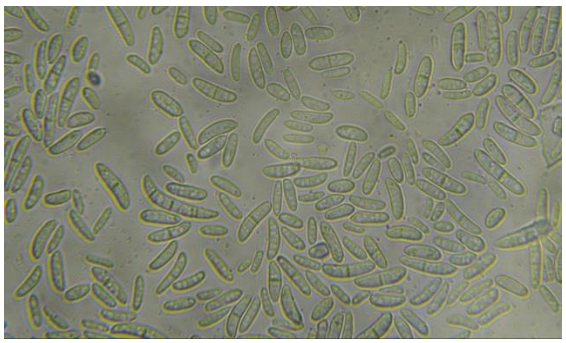

Figure 2. Microscopic view of macro and microconidia of other *Fusarium* sp. associated with diseased teak stumps.

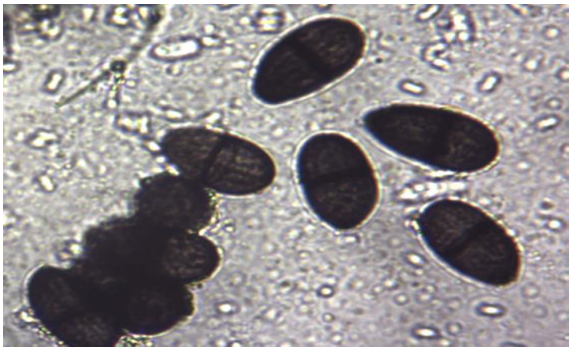

Figure 3. Microscopic view of conidia of *Lasiodiplodia* sp. associated with disease teak stumps

The three fungal pathogens were re-isolated from the disease lesions of the inoculated plants and the re-isolated pathogens *i.e*. two *Fusarium* and one *Lasiodiplodia* species exhibited the same morphological characteristics of mycelia and conidia as those of the originally inoculated isolates. Thus, the isolates of fungal pathogens fulfilled the criteria stipulated by Koch's postulates and all three pathogens were identified as the causal agents.

Tree species are susceptible to pathogen infection when a congenial condition for infection exists. *Fusarium* species have been reported on several tree species and it causes tree and seedling wilt of many forest tree species [14]. Balasundaran and Sankaran, [15] reported that *Fusarium* invaded plants from the roots or caller region of the stem tissues or tip of the plant and subsequently it advanced upward or downward causing defoliation and eventual sudden wilting and drying of trees within a few days. They also reported that association of *Fusarium solani* in the development of stem canker and die-back of teak plants while Jamaluddin et al.,[14] reported that *Fusarium moniliforme* cause wilt of many tree species including *Tectona grandis, Azadirachta Indica*, *Cassia fistula*, etc. and significant damage to forests in many areas of India.

Murali et al., [9] reported that the death of twigs was caused by *the Lasiodiplodia theobromae* of teak plants. Borges et al., [8] reported that vascular tissue discoloration, heartwood rot, and dieback of teak caused by *Lasiodiplodia theobromae* ranged from 5% to 10% across all inspected commercial fields in Brazil.

Table 2. Effect of different fungicides on mycelia growth of two species of *Fusarium* and one species of *Lasiodiplodia* (*In vitro* test)

Treatment	**Percent Inhibition of colony growth by fungicides (%)**		
	Lasiodiplodia spp	***Fusarium spp***	***Fusarium spp***

T1- Homai WP	98.0 (67.0) [a]	84.6 (72.2) [a]	90.6 (85.3) [a]
T2- Thiram 80% WP	89.3 (55.0) [b]	69.0 (54.5) [b]	66.3 (70.9) [b]
T3- Captan 50% WP	84.3 (50.8) [c]	60.0 (51.7) [c]	61.6 (64.2) [b]
T4- Control	0 (2.9) [d]	0 (2.9) [d]	0 (2.9) [c]
CV %	15.3	3.2	0.8

Note: *In each column, values followed by the same letter(s) are not significantly different at p=0.05 according to Duncan's multiple range tests. Values within brackets indicate the arc sign transformation*.

It has been recommended that soil drenching of fungicides namely Homai, Thiram, and Captan for control of fungal diseases in nurseries and roots by the Department of Agriculture, Sri Lanka [16]. Laboratory test results (*in vitro*) indicated that a mixture of systemic fungicide (Thiophanate methyl 70WP) and contact fungicide (Thiram 30 WP) namely Homai at the concentration of 1000 ppm was the best fungicide controlling both *Fusarium* and *Lasiodiplodia* species followed by the contract fungicides Thiram 70WP at 1400 ppm concentration and Captan 50WP at 1200 ppm concentration. The results obtained from the study suggest that the fungicide Homai could be used to control the death of teak stumps.

It has been reported that various environmental factors like drought, high rainfall, hot and moist weather, overcrowding of plants, shading, and stem as well as root damages can cause a tree to be stressed and make a conducive environment for infection by creating a point of entry for pathogens [9, 8,15]. The site inspections of the teak nursery at Kumbalpola, disease symptoms and laboratory testing of diseases stumps and analysis of subsequent results and information, and the environmental conditions at the nursery site made us conclude that the death of teak stumps might be due to the identified fungal infestations, probably due to preparation of root stumps without following proper sanitary procedures and prevailed hot and moist weather, and over the shaded condition in the nursery.

5. Conclusion:

Two species of *Fusarium* and one species of *Lasiodiplodia* caused the death of potted stumps of teak. Laboratory test results (*in vitro*) indicated that fungicide- Homai (Thiophanate methyl 70WP + Thiram 30 WP) at the concentration of 1000 ppm was the best fungicide controlling both *Fusarium* and *Lasiodiplodia* species followed by the Thiram 70WP (1400 ppm) and Captan 50WP (1200ppm) under in vitro conditions. However, further studies are necessary to get conclusive results under field conditions. Further, this may be probably the first report of *Fusarium* as well as *Lasiodiplodia* species causing the death of potted stumps of teak in Sri Lanka.

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