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RESEARCH ARTICLE

Seasonal disease: Foamy bark canker of *Citrus maxima* in the delta region of Tamil Nadu

■ **G. Venkatesan and P.S. Sharavanan**

SUMMARY

The *Citrus maxima*, commonly called pummelo, are a Rutaceae family. The Canker disease recently had an issue on *Citrus* species in the Delta region of Tamil Nadu. This disease is appeared by foamy oozes from the bark. The infected plant dies slowly in a short period. This study was identified the microorganism causes of foamy disease from bark and infected area. Totally 19 fungi were isolated. Among these 16 fungi were isolated from uninfected bark, 3 fungal species belonging to Ascomycetes, 2 fungal species belonging to Coelomycetes, and 10 species be classed Hyphomycetes and one sterile form, though 11 fungal species were isolated from infected bark foamy ooze, eight Hyphomycetes, one Oomycete, and two sterile forms were isolated. The RPO statistical analysis resulted, the bark fungi have been separated a group fungus from foamy fungi such a few fungi as the *Fusarium*, *Phytophthora*, and yeast have isolated in the foam. Also, the Jaccard's similarity showed 42.105% and dissimilar among to the bark and foamy fungus. The plant was decay, branch dieback and tree death may induce by fungi also that the Canker disease on *Citrus* may be caused by *Phytophthora* fungal species.

Key Words : : *Citrus maxima*, Bark foamy canker, Phellophyte/endophytes, *Phytophthora*

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The pomelo plant, in scientifically called *Citrus maxima*, is an economically important family Rutaceae. The pomelo common name is derived from the Dutch. The pompelmoes, which is rendered pompelmus in German, Pamplemousse in French and also called Pambalimasu in Tamil. The Pomelo tree is a native plant of Malaya Island and East of India. It is widespread in China, Japan, Philippines, Indonesia, USA and Thailand (Khare, 2007). The *Citrus* of Rutaceae,

an evergreen aromatic shrub and a small tree occupies an important place in the medicine and also in the fruits of India. In India, it is grown in home gardens in all states of India and maximum diversity is reported from Northeast Region (Singh and Singh, 2003 and Ray *et al.,* 2016), Bihar and West Bengal.

The genus *Phytophthora* has a long history. It has been known to cause destructive plant diseases much before its discovery. *P. infestans* as the pathogen causing potato late blight (de Bary, 1876). The across the general term "*Phytophthora* - root rot" refer a complicated disease which is caused by diverse soil-borne species of *Phytophthora* and is recognized as a major fungal disease of *Citrus,* virtually widespread (Boccas and Laville, 1978; Gallegly, 1983 and Tuset, 1990). The *Phytophthora* species are attacked *Citrus* plants at all stages and have been infected all parts of the tree, including roots, stem, branches, twigs, leaves and fruits. It is root rot, stem rot is called "gummosis", or "trunk gummosis" and fruit, brown rot, twig and leaf die-back are called "canopy blight" and rot is called "damping off" of seedlings, all caused by *Phytophthora* spp. may be considered a different symptom of the same disease.

All organisms inhabiting plant organs that at some time in their life can colonize internal plant tissues without causing apparent harm to the host (Petrini, 1991). Many plant parts are colonized by endophytes within the different ecosystem (Brundrett, 2002 and Mandyam and Jumpponen, 2005). Depending on the plant species and theirs interaction, endophytes are colonized in roots, leaves, stem, and bark in tissues (Rodriguez-Cabal *et al.,* 2013 and Verma *et al.,* 2007). Endophytes might grow inter and intracellular as well as endophytic and epiphytically (Schulz and Boyle, 2005 and Zhang *et al.,* 2006). However, endophyte will change their functionally depending on a group of abiotic and biotic factors, as well as the genotypes of plants and microbes, environmental conditions, and also the complex of interactions inside the plant community (Hardoim *et al.,* 2015). A few studies have revealed the influence of seasonal changes on endophyte population in host plants. Marked variations in colonization rates of endophytes have been observed in the dry and wet season for leaves and bark of *Euterpe oleracea* (Rodrigues, 1994). and *Rhizophora apiculata* (Suryanarayanan *et al.*, 1998). One approach to understanding more about these cryptic microorganisms would be to study them at the plant infection and tissues organisms of *Citrus* bark foamy canker disease.

MATERIAL AND METHODS

Study area:

The *Citrus maxima* trees are more than twelve years old, grew in the home garden. These plants were studied in the Delta region (part of the region-Mannargudi) of Tamil Nadu during the period from January to May 2019 and again December 2019 to February 2020. The district receives rainfall under the influence of both southwest and northeast monsoon.

Symptoms of disease:

The primary symptom of white foamy oozes bark disease is the appearance of water-soaked bark and slightly discoloured from the trunk, dull canopy, that very sooner, the entire trunk and branches are the appearances of foamy oozes, cracked bark, and fall down leaves, alcoholic smell or fermentative odor. During the period of time, a few insects, the lizard appeared to the surrounding the area (Fig. A-a, b, c).

Fig. A : Photos are shown on the Symptoms of Foamy Bark Canker. (a) Foamy oozed on bark alcoholic smell liked insects and lizards appeared on the bark. (b) White foamy oozes and appears water-soaked on the bark. (c) Young fruit on hangs down of *Citrus maxima* plant

Collection of samples:

In the investigation was followed by two methods, the first experiment, Oozes "foam" was collected from infected parts and later Incubated in Petri dishes containing PDA medium and oozes foam was smeared each Petri dish. The second experiment, bark endophytes or phellophytes fungi were isolated, also, its experiments may prove the *Citrus* tree was found out uninfected and infected parts of the trunk to be a comparative study (Fig. A). The bark tissue from the healthy tissues (150 Segments) of the infected host was cut into 1 cm^2 (150 Segments) then these segments were surface-sterilized following the method (Fisher *et al.,* 1993). The bark segments were dug in 75% ethanol for 60 sec., immersed in 4% Sodium hypochlorite for 180 sec. and dipped in 75% ethanol for 30 sec. and inoculated. Incubation and isolation of *Citrus* plant tissues, one hundred tissue segment of bark from infected *Citrus* species were distributed in Petri dishes containing PDA medium (with Chloramphenicol 150 $mg1^{-1}$). Ten segments were plated in each Petri dish. The dishes were sealed with Parafilm™ and incubated in a light chamber at 26 ± 1°C for 21 days. The light regimen provided was 12h light: 12h darkness from cool white daylight fluorescent lamps (Bills and Polishook, 1992).

Statistical analysis:

– Colonization frequency (CF %) of an endophyte species was calculated by the method of Hata and Futai (1995).

$$\text{CF \%} = \frac{\text{N colonizes}}{\text{N total}} \text{ x } 100$$

where, N colonizes and N total are the number of segments colonized by each endophyte and the total number of segments observed, respectively.

– Relative percentage of occurrence (RPO) of each group (*viz.,* Ascomycetes, Coelomycetes, Hyphomycetes, Oomycete and sterile forms) of fungal species in each

Plant species was calculated as follows:

$$\text{RPO} = \frac{\text{Total colonization frequency of one group}}{\text{Total colonization frequency for all the groups of fungi}} \text{ x } 100$$

– Jaccard's similarty co-efficient was calculated to compare the similarity between bark (phellophyte) fungi assemblage and foamy fungi (Sneath and Sokal, 1973). This was done as follows:

$$\text{Similarity co - efficient} = \frac{C}{(A + B - C)}$$

where, A and B = The total number of fungal species isolated as bark fungi and from foamy fungi, respectively.

C = The number of fungal species found in common.

RESULTS AND DISCUSSION

In this region of Tamil Nadu are, occurs at the maximum temperature, may be occurring between about 22° C- 38° C. The Southwest winds that set in during April are strongest in June and continue till September. Northeast monsoon starts from October to January. Hence, the season varying from rain and air humidity effect year by year from November to December. The Northeast monsoon which starts in October to December contributes about 60 - 80 % of the total annual rainfall. The Southwest monsoon rains from June to September and summer rains from March to May contributes about 20 to 40% of rainfall. The disease occurs most commonly in *Citrus* plant in this seasonal on last two year.

In the present study, the fungi were isolated from infected and uninfected bark tissues of the same plant. Totally 19 genera were recovered such as *Aspergillus* spp., *Alternaria alternata*, *Botrytis* sp., *Chatomium* sp., *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium* sp., *Nigrospora oryzae*, *Penicillium* spp., *Pestalotiopsis* sp., *Phyllosticta* sp., *Phytophthora* sp., *Sporormiella* sp., *Talaromyces* sp. and also Yeast forms were isolated. However, these fungi consequently varied from Bark and foamy. *Fusarium* sp. 2, *Phytophthora* sp., and two yeast forms were present on only oozes out foamy as well *Botrytis* sp., *Chatomium* sp., *Curvularia lunata*, *Pestalopsis* sp., *Phyllosticta* sp., *Sporormilla* sp., *Talaromyces* sp., were isolated in the bark. A few fungi were isolated very commonly in both of the regions *viz*., *Aspergillus* spp., *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium* spp., *Penicillium* spp. (Table 1).

A total of 19 species of fungi were isolated and were two sterile mycelia. Sterile mycelium was isolated from the bark and bark foamy. Sterile mycelium has the least isolation rate. Hyphomycetes were the abundant group followed by Ascomycetes, Coelomycetes and sterile forms while one fungal species were isolated Oomycete group (Table 1 and Fig. 2a, b). The bark fungi were isolated in a confirmatory investigation, these result

Table 1 : Fungal isolated from uninfected bark (Phellophytes) and infected bark (foamy oozes) in *Citrus maxima* L.

Sr. No.	Name of the fungus	Uninfected Bark CF% (Phellophyte)	Infected Bark (White foamy Ooze)
	Ascomycetes		
1.	*Chatomium* species 1	2.7	-
2.	*Sporormiella* species 1	7.3	-
3.	*Talaromyces* species 1	4.7	-
	Coelomycetes		
4.	*Pestalotiopsis* species 1	5.3	-
5.	*Phyllosticta* species 1	2.7	-
	Hyphomycetes		
6.	*Alternaria alternata*	2.7	+
7.	*Aspergillus flavus*	4.7	+
8.	*Aspergillus niger*	3.3	+
9.	*Botrytis* species 1	1.3	-
10.	*Cladosporium cladosporioides*	5.3	+
11.	*Curvularia lunata*	2.7	-
12.	*Fusarium* species 1	4.0	+
13.	*Fusarium* species 2	0.0	+
14.	*Nigrospora oryzae*	1.3	-
15.	*Penicillium* species 1	4.0	+
16.	*Penicillium* species 2	2.7	+
	Oomycete		
17.	*Phytopthora* species 1	0.0	+
	Yeast forms		
18.	Yeast form 1	0.7	+
19.	Yeast form 2	0.0	+
	Total CF%	**55.3**	---
	No. of Isolates	**83**	---
	No. of Species	**16**	**11**

CF% = Colonization Frequency
(+) = Present (It's considered one or more colonizers) (-) = Absent

of bark fungus may verify that causes of the disease.

The endophytic fungal assemblages of species such as *Guignardia* sp., *Phyllosticta* sp., *Glomerella cingulata*, *Colletotrichum gloeosporioides*, *Phomopsis* spp. and *Pestalotiopsis* spp. All these fungal species have already been isolated as endophytes from tropical regions and colonize leaves, bark, twigs or stems (Petrini *et al.*, 1992; Taylor *et al.*, 1999; Fröhlich *et al.*, 2000; Kumaresan and Suryanarayanan, 2002 and Suryanarayanan *et al.*, 2002). In this investigation, fungal isolates were obtained from bark and foam. A few of the fungal isolates that obtained from the bark were identified to be *Penicillium* species and *Aspergillus* species. Similar endophytes were reported (Khan *et al.*, 2007) in Apocynaceae member of *Calotropis procera* that include *Aspergillus* and *Penicillium* species along with yeast and *Phoma* species from the stem part. The endophytic occurrence of a few yeast species was recorded in an investigation carried out on *Citrus sinensis* in Brazil (Santos *et al.*, 2009). The endophytic fungi occurrence has also been reported for other citrus pathogens, such as the leaf spot agents *Alternaria alternate* (Durán *et al.*, 2005; Peever *et al.*, 1999; Sadeghi *et al.*, 2019) and *Alternaria citri, Fusarium oxysporum* (Juybari *et al.,* 2019). The latter study also introduces new *Fusarium* spp., causing cankers on several citrus species. *Phyllosticta* spp., capable to occupy citrus plants in either symptomatic or latent pathogen reported by several authors (Baldassari *et al.*, 2008 and Wikee *et al.*, 2013) is also present in our study.

In the present study also organs (tissues) preference among pathogen fungus distinct when uninfected bark was compared with infected bark foamy oozes. Assign of the samples by Jaccard similarity showed 42.105% and discloses strong differences between an uninfected and infected region (Fig. 1).

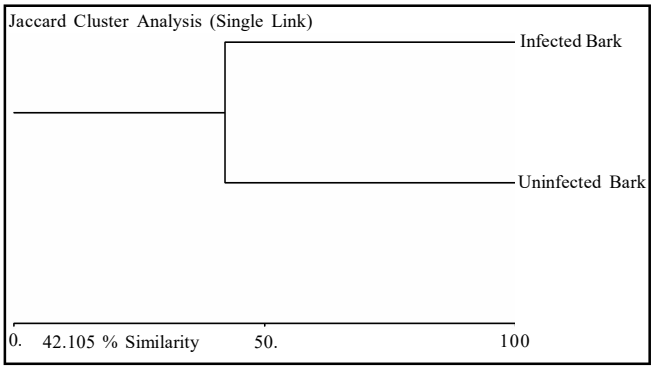

Fig. 1 : Jaccard cluster analysis of infected and uninfected bark

It is known that the disease may have been caused by the presence of fungi of the *Phytophthora,* yeast species distinct group of the according to the Relative Percentage Occurrence (RPO) of each group of fungi (Fig. 2a, b). Here, the document investigating two plants affected in the continuous disease. It is considered a seasonal disease, this disease that in the rainy season the spore germinates and in the spring the symptoms appear and eventually die. We assume that, based on the fungi extracted from the foam and the bark, *Phytophthora* fungus may have caused by foam canker disease and also foamy oozes and alcoholic smell may be produced by Yeast and a few filamentous fungi. So, Insect and lizard come to near found around these foamy oozes of alcoholic or fermentative smell because of the

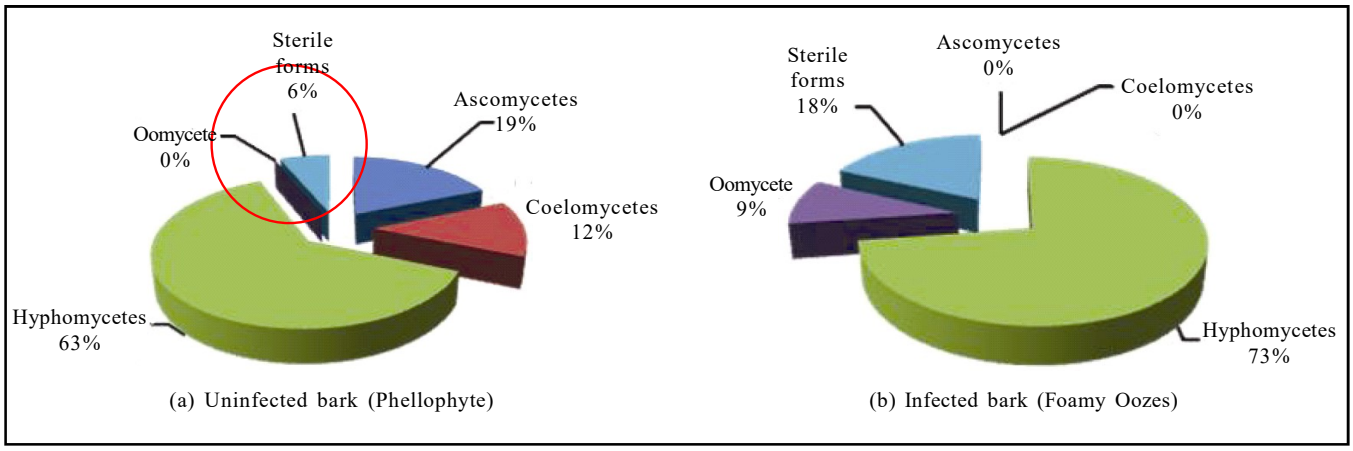

Fig. 2 : Relative Percentage Occurrence (RPO) of endophytes belonging to different groups of fungi. (a). Uninfected (Phellophyte) bark. (b). Infected (Foamy Ooze) bark

yeast fungus which may be fermented in bark tissues. The fungal pathogen may be transformed from infected tissues to non-infected trees branches during movement of water from xylem tissues. Canker can be the definite and localized area that can usually dry and dead, often discoloured, sunken, sometimes split on the stem, trunk, branch, and even small twig (Fig. 3). Although the show of canker symptoms is very variable depending on the plant, pathogen species, environmental conditions and stage of the disease.

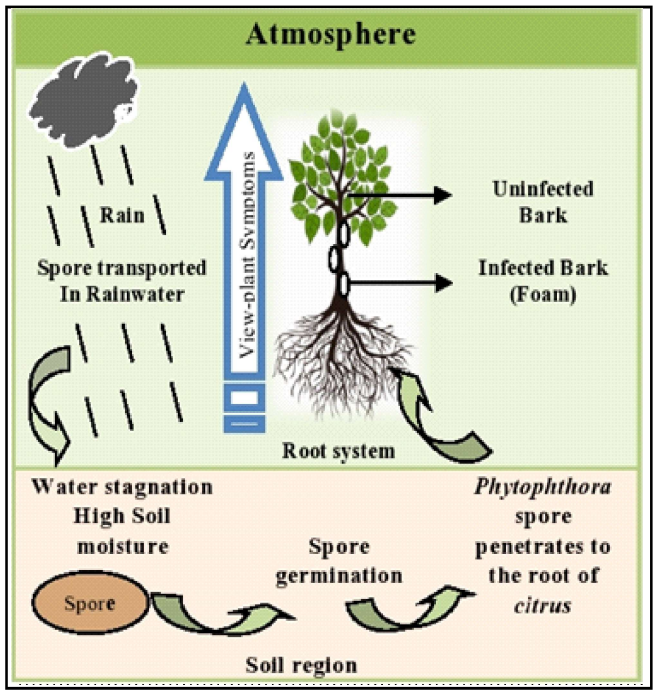

Fig. 3 : A fictitious sketch: *Phytophthora* fungus pattern of disease in citrus canker

These diseases have previously been reported; such species *P. nicotianae*, *P. palmivora* and *P. citrophthora* are the major and widely distributed species causing *Citrus* diseases in India (Lele and Kapoor, 1982 and Naqvi, 2004). *Phytophthora* was a well-known pathogen able to cause stem bleeding on an oak tree as well as many other tree species (Brasier *et al.,* 2004). *Phytophthora infestans*, which caused the great Irish Potato famine during the late 1840s, remains the most destructive pathogen of potatoes and tomatoes. Other prominent species that have appeared in more recent times are *P. ramorum* on oak and *P. kernoviae* on ornamentals, *P. cinnamomi* on forest crops, *P. agathis* on kauri, *P. cactorum* on hardwood trees, *P. capsici* on solanaceous and cucurbitaceous vegetables, *P. fragariae* on strawberries, *P. megakarya* on cocoa, *P. palmivora* on palms. *P. media* which infects mainly cardamom, the present investigation has been isolated that the Pambalimasu tree was affected by the bark foamy canker, caused by the fungus of *Phytophthora anilla* and nutmeg (Bhai, 2016). Chowdappa *et al.* (2014) identified *P. boehmeriae* and *P. capsici* isolates as causal agents of foliar blight disease of pepper in India. *P. nicotine* is also responsible for significant losses on several other economically important species, including fruit, oilseed, vegetable crops ornamental plants and floricultural crops (Erwin and Ribeiro, 1996).

Describe the pathogen:

Phytophthora species is (a water mould fungus) class - Oomycetes belonging to the order Peronosporales and family Peronosporaceae that is found throughout the world. Under favourable conditions of high moisture and

temperature is found it produces large numbers of motile zoospores that may able to swim in water for short distances. These zoospores are the infective agents that may be transported in rain or irrigation to the roots. Rainy, cold, wet environmental conditions with high soil moisture favour disease development. When zoospores contact roots, they encyst, germinate and enter the root tip resulting in the decay of the entire root system (Fig. 3).

Control:

Avoid using lawn tools near the tree to protect damaged the bark and provide sufficient water during the dry months to help prevent foamy oozes from the wound while small infection may be treated by pruning infected branches and removing small areas of bark, these are no complete cure. Stirrer the soil around the roots of the affected plant, then 1 kg of the salt (sodium chloride) with 30 liters water (1: 3) adding in once poured can be prevented the primary stage of an affected plant. However, it's not a complete solution. The agriculture department has recommended for these diseases. It can be reduced the tree strain and increase its resistance to fungi by providing water mix with Copper Oxy chloride fungicide once or twice weekly during the dry season. Copper Oxy chloride is a protective fungicide and also, the Bordeaux mixture or copper-based fungicides can be used against fungal diseases. However, the experience is, too it is better to be protected before to disease come, it is not better to protect after coming to the disease while this disease arises against symptoms extremely delayed.

Conclusion:

This study provides the first description of a new view and seasonal disease of *Citrus maxima* in the delta region of Tamil Nadu. The *Citrus* bark foamy canker, caused by the fungal pathogen *Phytophthora* is considered a serious disease of citrus species. The *Citrus* bark may be initially cracked by fungi in the surface area. These infections have been causes cracked on the stem becoming its tree branch dies and then slowly plant is dead. In the early stage of the infection, the normal appearance may not be visible or identifiable on the bark surface while for day to day the plants may appear many symptoms in the bark split, foamy oozes, water soak, dull stage, leaf fall down, etc. However, some fungi such as *Phytophthora*, *Fusarium* also cause foam oozing, stem split symptoms while alcoholic or fermentation have been producing by yeast fungi. These fungi may continue to be occupying the entire plant, at the time the bark distinct white foamy oozed and appears water-soaked occurs on infected bark. The plant may a result of these symptoms of it may be known to be a deadly disease known to appear at the time of death condition.

The present investigation has been isolated that the Pambalimasu tree was affected by the bark foamy canker, caused by the fungus of *Phytophthora*. *Phytophthora* sp. Is a parasite but is a poor competitive saprophyte in soil. There is more result mention that truth that *Phytophthora* diseases have been and continue to be destructive to plants around the world. *Phytophthora* species are well adapted to the different environments that they organize to varying seasons. In common told concept according to plant pathologist, we assume that disease caused by a micro fungus in this plant in when plenty of microorganisms (bacteria and fungi) disease produce in various plants to adapt to the seasonal conditions.

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