Studies on the Etiology of a Leaf Spot Disease of Rough Lemon (*Citrus jambhiri* - B. Jamir; H. Jhambhiri)

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Abstract

Studies were carried out on a striking leaf spotting observed on the leaves of rough lemon (Citrus jambhiri) plant at the permanent site of the University of Ilorin, Nigeria. Disease survey revealed that the disease was prevalent within the area of the University in the months of June-July, 2004. Symptoms begin as tiny brown spots with yellow halos on the adaxial surface of the leaf. Isolation from infected leaf materials yielded Aspergillus niger and Rhizoctonia sp. However, only Rhizoctonia sp. reproduced similar foliar lesions when artificially inoculated on healthy citrus leaves. The fungus was also re-isolated. A. niger did not elicite any disease under the conditions of this study. Rhizoctonia sp. is therefore, the causal organism of the disease under study.

Keywords: Etiology, Leaf spot disease, Rough lemon, *Citrus jambhiri, Aspergillus niger, Rhizoctonia sp.*

Introduction

The genus *Citrus* is a member of the large dicotyledonous family Rutaceae comprising about 130 genera. The number of species in this family is about 1,500 most of them being evergreen aromatic trees and shrubs of the tropics. *Citrus* is a very large genus with several species and varieties with most of the species being characterized among other features by their large juicy fruits. Some known species include sour lime (*Citrus aurantifolia*), sweet lime (*C. limetta*), lemon (*C. limon*), rough lemon (*C. jambhiri*), citron (*C. medica*), pummelo or shaddock (*C. grandis*), mandarin orange and tangerine (*C. reticulata*), sour or bitter orange (*C. aurantium*), sweet orange (*C. sinensis*), grape fruit (*C. paradisi*) and wild orange (*C. indica*) (Dutta, 1979).

The origin of the genus *Citrus* is not certain but the history of cultivated species suggests that they may have been domesticated in the drier tropics of South-east Asia (Cobley and Steele, 1976). Though the crop originated in the tropics it is now cultivated most extensively in the sub-tropics with a Mediterranean climate. The United States is the leading producer of citrus fruits. The world annual production of all kinds of citrus is above 40 metric tonnes. The plant thrives best in warm, sunny climates with 900mm or more annual rainfall or with irrigation.

Citrus fruits are berries but are called hesperidiums because of their unusual structure. The exocarp and mesocarp are leathery and protect the juicy inner tissues derived from the endocarp from damage and desiccation. The exocarp and mesocarp together form the rind of the fruit. The change in colour, which occurs as the fruits ripen, is due to the gradual breakdown of chlorophyll in the plastids of the exocarp, which continues until the predominant pigments are xanthophyll and carotene giving the fruit its yellow or orange colour. All of the cultivated species are diploid (2n=18), and though they are described as distinct species, their taxonomy has been confused because they readily hybridize.

Citrus plants constitute a ready source of mineral nutrients such as vitamins for humans and animals. The fruits are used for the production of different types of juice drinks. *Citrus* trees are aromatic because the leaves and the rind of the fruits are dotted with glands, which produce different essential oils extracted as an important subsidiary industry of citrus production. *C. jambhiri* is useful in the manufacture of preservatives and for desired flavour in baking industries ((Cobley and Steele, 1976).

Citrus plants have an array of diseases that afflict their different parts. Timmer *et al.* (2000) have reported several diseases of citrus both in the nurseries, orchards and in transit/storage. Some of these diseases include citrus canker, fruit rot, yellows and leaf spots. Leaf spot disease of citrus is particularly important because of the role the leaves play in food manufacture in a process known as photosynthesis. Leaf spot disease destroys the leaf surfaces thereby reducing the photosynthetic area of leaves. This reduction consequently limits the efficiency of the plant to manufacture its own food.

This study is therefore important and aims at isolating and identifying the causal organism(s) of leaf spot disease of rough lemon (*Citrus jambhiri*) plants at the permanent site of the University of Ilorin. Knowledge of the causal agent(s) of this disease will facilitate effective control measures.

Materials and Methods

Survey and sample collection: A survey was carried out for a period of two months (June-July, 2006) to determine the incidence and severity of *Citrus* leaf spot disease at the permanent site of the University of Ilorin, Nigeria. Four areas within the University were surveyed namely, the Jalala village, the senior staff quarters, parks and garden and the main campus. Disease incidence and severity in these areas were assessed and recorded using a disease rating scale (Table 1). Disease incidence was determined by the presence or absence of leaf spotting on selected plants while the severity was

determined by the intensity of spotting on individual leaves on the selected plants. Percentage areas of infected leaves covered with spots were calculated to arrive at the severity index.

Table 1: Rating scale for the assessment of citrus leaf spot disease severity

Score	Description	Inference
0	No Symptoms observed	No infection
1	1-25% leaf area covered with spots	Slight infection
2	26-50%leaf area covered with spots	Moderate
3	51-75%leaf area covered with spots	Severe
4	75% and above leaf area covered with spots	Very Severe

Isolation and identification: Leaf samples of *Citrus* showing leaf spot symptoms were collected in separate sterile polyethylene bags from the surveyed locations. The samples were taken to the laboratory for isolation of the associated organism(s). The diseased portions were cut into small discs of about 1-2mm diameter using sterile scapel. The pieces were surface-sterilized in 2% $NaOCl_3$ for 3 minutes and then rinsed in two changes of sterile distilled water. The infected pieces were dried in-between sterile Whatman No. 1filter paper and then plated on sterile potato dextrose agar medium. Fifty (50) diseased leaf discs were plated altogether. The plates were incubated at room temperature (25 ± 1 ^{o}C) and observed for colony development. Emerging colonies were sub-cultured to obtain pure cultures. Isolates were identified with the aid of laboratory manuals.

Pathogenicity test: Pathogenicity test was carried out following standard techniques (Agrios, 1978). All the isolates in this study were used for pathogenicity test by artificially inoculating the isolates individually unto sterile healthy *Citrus* leaves. Inoculum was obtained from 2-week-old culture plates by washing off spores from the plates using sterile distilled water. The spore suspension of each test organism was rubbed separately on the surface of citrus leaves using sterile cotton wool. Inoculated leaves were incubated at room temperature (25±1^{o}C) on wet sterile Whatman No. 1 filter paper in sterile Petri dishes. Inoculated leaves were monitored for symptom development. Mature ripe fruits were also inoculated separately with the two isolates to determine if there is any relationship between leaf and fruit infection in *Citrus jambhiri.*

Results

Leaf spot of rough lemon (*C. jambhiri*) was first observed at Quarter No. 50 at the Senior Staff Quarters of the University of Ilorin in May, 2006. Subsequent survey in June-July, 2006 revealed that lemon trees on other parts of the University campus also showed spot symptoms. Though the disease occurred in all the areas surveyed, the severity differed from one location to another. The disease was most severe at the parks and garden citrus plantation (Table 2) while the severity was least at the Jalala village. Leaf spot of rough lemon is characterized by tiny spots on the adaxial surface of the leaf. The initial diameter of spots ranged from 0.5-1.0mm surrounded by a yellow margin (Plate 1). Some of the spots coalesced and enlarged with time reaching 2-5mm diameter with a wide band of surrounding yellow margin. Fully enlarged spots were silvery-brown with faint concentric ring pattern. Over time, the enlarged spots were shot out from the leaves leaving shot holes on infected leaves.

Table 2: The incidence and severity of leaf spot in rough lemon (*Citrus jambhiri)*

Location	Incidence	Severity
Snr. Staff Qtrs.	P	++
Jalala Village	P	+
Parks & Garden	p	+++
Main Campus	p	++

P = Present; + = Mild; ++ = Moderate; +++ = Severe.

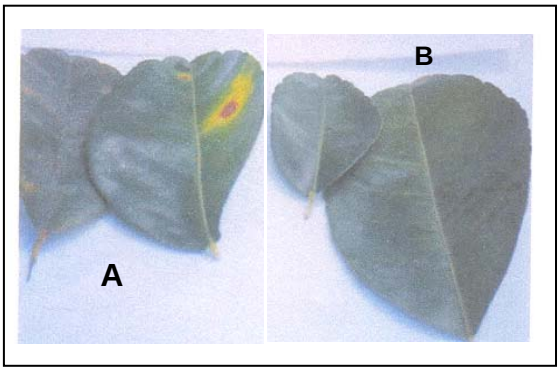

Plate 1: *Citrus jambhiri* leaves showing brown spots with yellow margin (A) and healthy leaves (B)

Isolation and pathogenicity: Two organisms were isolated from infected *C. jambhiri* leaves in this study. These isolates were identified as *Aspergillus niger* and *Rhizoctonia solani*. Eighty five percent (85%) of fifty (50) infected leaf discs plated gave rise to *R. solani* colonies, 12% yielded *A. niger* while there was no growth from the remaining 3%. Subsequent pathogenicity test revealed that *R. solani* was the cause of leaf spots in *C. jambhiri* under the conditions of this study. *R. solani* forms a whitish cottony mass of mycelium (Plate 2) in culture turning brown-deep brown with age. No fruit bodies are produced but characteristic branching pattern and septation are peculiar features of this organism. The hyphae are 4-5µm wide and tend to branch at right angles with a septum near each branch.

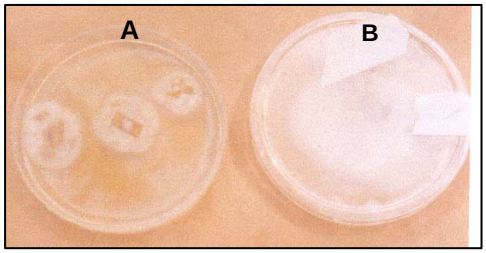

Plate 2: Emerging colonies of *Rhizoctonia solani* from leaf discs of *Citrus jambhiri* (A), 1-week-old culture of *R. solani* (B)

Slight spot symptoms appeared on artificially inoculated leaves 72 hr after inoculation. The size of the spots increased slightly with time. After 1 wk of incubation, the spots produced were very typical of the spots observed in naturally infected citrus plants. Both the control leaves and those inoculated with *A. niger* did not show any spot symptoms.

Artificial inoculation of *C. jambhiri* fruits with the two isolates revealed that *A. niger* is the cause of fruit rot. Stem end rot symptoms consisting of black discoloration at the fruit attachment end (Plate 3) and softening were observed on inoculated fruit. No symptoms were observed on fruits inoculated with *R. solani.*

Plate 3: Fruits of rough lemon *Citrus jambhiri* (L) Healthy (R) Inoculated

Discussion

R. solani was shown as the causal agent of leaf spot of *C. jambhiri* in this study. This organism has been described as the anamorph of *Thanatephorus cucumeris* = *Pellicularia filamentosa*, the causal agent of areolate leaf spot of citrus (Anon 2007). *R. solani* is a plant pathogen with a wide host range and world wide distribution. It also causes damping off in citrus and kills seedlings in horticulture. Foliar diseases such as leaf spots are generally important because of the limiting capacity of the disease on food manufacture due to the damage on the photosynthetic pigments of the plant. According to Agrios (1978), leaf spots and anthracnoses often have a prolonged initial stage in fruit infection and may also cause twig or branch dieback.

It was observed in this study that *A. niger* isolated from the leaf caused citrus fruit rot when it was artificially inoculated on healthy fruit. This finding is significant because it shows that both pathogens can be associated with one disease condition and so provides ready inoculum for the next stage of infection. Moss (1994) had reported that *Aspergillus* and *Penicillium* species produce their toxins mostly in stored seeds, hay or commercially processed foods and feeds although infection of seeds usually takes place in the field. The infection of *C. jambhiri* fruits in this study may also have taken place in the field. Amador (2005) has reported that *Mycosphaerella horii*, the causal organism of greasy leaf spot in citrus can also cause rind blemishes on some varieties.

Citrus is susceptible to a large number of diseases caused by plant pathogens and economic losses due to these diseases can be severe (Seif, 2000). Other important fungal diseases of citrus include anthracnose, greasy spot, black spot, *Septoria* spot, powdery mildew, scab (*Elsinoe australis* and *E. fawcetti*), albinism (*Alternaria alternata, Aspergillus flavus*), damping-off (*Pythium* spp.), black root rot, *Fusarium* wilt, *Ganoderma* root rot, greening (Huanglongbing), postbloom fruit drop and mushroom root rot. Postharvest loses in citrus are caused by *Alternaria* rot, *Aspergillus* rot, brown rot, *Fusarium* rot, gray and blue molds, *Penicillium* decays and stem-end rot (Timmer and Menge, 1994). *Phaeoramularia* fruit and leaf spot (PFLS) caused by *Pseudocercospora angolensis* (formerly *Phaeoramularia angolensis*) is a damaging fungal disease that affects citrus in Florida (Seif, 2000) and like any other leaf spot disease has a potential to become a significant problem in citrus production.

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