

Studies on Soil Pathogens Associated with Citrus Disease

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ABSTRACT: Studies were carried out on soil-borne fungi associated with *Citrus* plant. The common soil fungi such as *Aspergillus spp*, *Rhizopus Botrytis sp* and *Fusarium sp* were isolated. In addition, some uncommon soil fungi like *Phytophthora spp* and *Fusarium verticilliodes* were isolated within the rhizospores of the *Citrus* plants. The frequency of occurrence and the percentage incidence of the soil fungi, both the common and the uncommon ones, from old *Citrus* plant and relatively young *Citrus* plants respectively were determined. The result also showed that the *Phytophthora spp* was always found associated with old *Citrus* plants while *Fusarium verticilliodes* was found in *Citrus* plants of all ages.

KEYWORDS: Soil fungi, Pathogens, Associated, *Citrus*, Plant diseases.

INTRODUCTION

The number of diseases affecting *Citrus* is not so large as in many other groups of fruits, but it is sufficiently important to attract serious attention. These diseases at times make it impossible for the grower to realize profits from his crops (Harold, 1957; Dutta, 1979; Jose, 2002). Based on materials originally written by Olsen *et al*, (2000) and Timmer and Menge (2000), *Citrus* diseases are classified into two major groups, parasitic and non-parasitic diseases. Interestingly, this work centered on soil fungal pathogens associated with *Citrus* plants. Many *Citrus* diseases are due to the attack of fungi. These diseases are caused by *Phytophthora spp*, *Pythium spp*, *Fusarium spp*, *Hendersonula torulordea*, *Anthrodia sinuosa*, *Coniphora eremosphila*, *Penicillium spp* etc (Olsen *et al*, 2000; Timmer and Menge, 2000). According to Graham and Timmer (2002), *Phytophthora spp* also infect the root and cause fibrous root rot disease of *Citrus*. Ohazuruike and Obi (2000), implicated *Pythium Phytophthora*, *Corticium* and *Fusarium spp* as specific pathogens causing damping off diseases of *Citrus*. Brown rot of fruits and blue and green fruit molds diseases of *Citrus* are caused by *Phytophthora spp* and *Penicillium spp* (Joffe, 1972; Olsen *et al*, 2000; Mohammed *et al*, 1995). Agostini *et al* (2002) recorded *Candida krusei* as a primary pathogen causing lemon fruit rot in California. But, in South Western Nigeria, Adisa and Fajola (1982) implicated *Botryodiplodia theobromae*, *Aspergillus spp* and *Penicillium spp* as the most important fruit rot pathogens of *Citrus*.

According to Dean (2000), many phytopathogenic fungi are found to be parasitic but are poorly competitive saprophyte in soil. These pathogens grow well on nutrient obtained from the living plants and under favourable condition, undergo repeated life cycles of mycelia to sporangia and zoospores which significantly shows that the environment of an organism is a major factor in determining how well or otherwise the organism thrives. It is therefore, the aim of this study to determine the concentration of fungi within the rhizosphere of the *Citrus* plant, which could be pathogenic to the plants and extrapolate from literatures the fungi that are pathogens of *Citrus* in both the aerial and soil environments.

MATERIALS AND METHODS

Soil Samples:

The soil samples were collected aseptically from the Rhizosphere of *Citrus* plants and from far distance outside the *Citrus* orchard in National Horticultural research Institute (NIHORT) Okigwe, Imo State, Nigeria.

Indian hoe and trowel were used in collecting the soil samples which was done from a depth of 18cm as proposed by (Nyle and Ray, 1972). Each of the soil samples was collected with a clean polyethylene bag and later transferred into an aseptic calico bag and labeled. The samples from the orchard were collected according to the age of the plants. Samples labeled A and B were obtained from the rhizospheres of the oldest *Citrus* plant of 1977 section in the orchard. Samples labeled C and D were obtained from the rhizosphere of *Citrus* plant of 1980 section in the orchard. Samples labeled E and F were obtained from the youngest *Citrus* plants of 1983 section in the orchard. Each soil sample collected to be analyzed is a mixture of three different samples from different areas of the same section. Then soil sample labeled G,H,I and J (served as control) were collected from far distance outside the *Citrus* orchard, and each sample collected is a mixture of three different samples from different areas of close distance.

The soil samples were taken to Plant Science and Biotechnology Laboratory of Imo State University for analysis.

Processing of Soil Samples

Each of the soil samples inside the calico bags were dried in a dehumidification chamber for two days. The "air-dried" soil samples were ground with wood enroller and sieved with 2mm test sieve to remove plant debris and gravels. The resulting samples were used for mycoflora analysis.

Mycoflora Analysis

The planting technique described by Fawole and Oso (1988) was adopted. One gramme (1g) of each of the soil sample were diluted serially to forth diluents. One milliliter (1.0ml) of the diluents were introduced aseptically into sterile Petri-dish containing Potatoe Dextrose Agar (PDA).

The inoculated and labeled plates were incubated at $27^{\circ}\pm 2^{\circ}\text{C}$ for five days. The plates were replicated three times, and pure cultures of the isolates were obtained. Lacto phenol cotton blue were used for wet mount under the microscope for the identification and characterization of the isolates using Barnett and Hunter. (1972)

RESULTS AND DISCUSSION

The following fungi were isolated within the rhizosphere of the *Citrus* and soil outside the *Citrus* orchard as shown in table 1.

Table 1: Fungi isolated within and outside the Rhizosphere of the *Citrus* orchard.

Fungi isolated within the rhizosphere of the *Citrus* plants

- Aspergillus niger*
- Aspergillus flavus*
- Phytophthora spp*
- Rhizopus spp*
- Botrytis spp*
- Fusarium verticillioides*
- Fusarium oxysporum*

Fungi isolated from soil outside the *Citrus* orchard

- Aspergillus niger*
- Aspergillus flavus*
- Rhizopus spp*
- Botrytis spp*
- Fusarium oxysporum*

The same fungi were isolated from the rhizosphere of the *Citrus* and the control (soil from outside the *Citrus* orchard) though with exceptions of *Fusarium verticillioides* and *Phytophthora spp* which were present only in the rhizosphere of the *Citrus* plants, as shown in table 1. The pure culture of some of the fungi isolates are shown in plates 1, 2, 3,4,5,6, and 7 below.

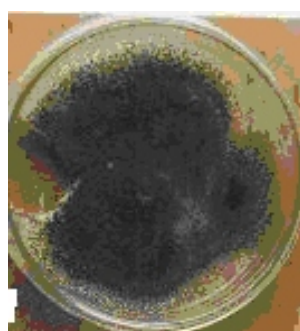


Plate 1: Culture of *Aspergillus niger*



Plate 2: Culture of *Aspergillus flavus*



Plate 3: Culture of *Rhizopus spp*



Plate 4: Culture of *Phytophthora spp*



Plate 5: Culture of *Botrytis spp*



Plate 6: Culture of *F. Verticilloides*



Plate 7: Culture of *Fusarium oxysporum*

However, the frequency of occurrence of the fungi isolated from both samples of the rhizospheres of the *Citrus* and that of the control analyzed varies as shown in tables 2 and 3.

The rhizospheres of both old (1977) and relatively young *Citrus* plants (1983) contained *Aspergillus niger* and *A. flavus* in the same frequency but *Phytophthora spp* and *Fusarium oxysporum* were more frequent in the rhizospheres of older plants (1977) than those of (1983) as shown in table 2.

Table 2: Isolates from the rhizospheres of *Citrus* orchard

Isolates (organisms)	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Pytophthora spp</i>	<i>Rhizopus spp</i>	<i>Botrytis spp</i>	<i>Fusarium verticilliodes</i>	<i>Fusarium oxyporium</i>
Soil Samples(years)							
1977 A	+	+	+	+	-	+	+
B	+	+	+	+	-	+	+
1980 A	+	+	-	+	+	+	+
B	+	+	-	+	+	+	+
1983 A	+	+	-	+	-	+	+
B	+	+	-	+	-	+	+
Frequency of Occurrence	6	6	4	5	3	4	5
Percentage Incidence (%)	100.0	100.0	66.7	83.3	50.0	66.7	83.3

Key:

+ = Present (counted as 1)

- = Absent (counted as 0)

Aspergillus spp and *Rhizopus spp* were also found to be of frequent occurrence in the soil samples collected from outside the orchard. *Phytophthora spp* and *Fusarium verticilliodes* were not isolated. This is shown in table 3.

Table 3: Isolates from soil outside the *Citrus* orchard (serving as control).

Isolates (organisms)	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Pytophthora spp</i>	<i>Rhizopus spp</i>	<i>Botrytis spp</i>	<i>Fusarium verticilliodes</i>	<i>Fusarium oxyporium</i>
Soil Samples(years)							
G	+	+	-	+	+	-	+
H	+	+	-	+	-	-	-
I	+	+	-	+	+	-	+
J	+	+	-	+	+	-	-
Frequency of Occurrence	4	4	0	4	3	0	2
Percentage Incidence (%)	100.0	100.0	0	100.3	75.0	0	50.0

Key:

+ = Present (counted as 1)

- = Absent (counted as 0)

This study established the presence of eight fungi, *Aspergillus niger*, *A. flavus*, *Rhizopus spp*, *Botrytis spp*, *Phytophthora spp*, *Fusarium verticilloides*, *Fusarium oxysporum* isolated and are among fungi associated with the rhizospores of *Citrus* plants. Among the eight fungi, *Fusarium verticilloides* and *Phytophthoras spp* were not isolated from the soil samples obtained from the soil outside the citrus orchard. Although many of these fungi isolated are either sabrobes or weak parasites, some of them have however been reported to cause serious diseases of *Citrus* and majority of them originated from different parts of the world other than Nigeria and their activities are universal (Harold, 1957). Timmer and Menge (2000) listed *Phytophthora spp*, *Fusarium spp*, *Aspergillus spp*, *Rhizopus spp*, *Pythium spp* and *Penicillium digitatum* as

specific common pathogenic fungi in the rhizospheres of diseased *Citrus*. Mohammed *et al* (1995) implicated *Geotrichum candidum* (found in the soil) as the major pathogen causing sour rot of *Citrus*. Harold (1957), observed that *Fusarium*, *Rhizoctonia* and *Sclerotium* are pathogens causing damping-off of *Citrus*. Olsen *et al* (2000) implicated *Phytophthora spp* as the major pathogen causing foot rot and Gummosis of *Citrus* and *Antrodia conioophora* causing Brown-heart wood rot of *Citrus*.

Phytophthora spp and *Fusarium spp* were not isolated from the soil outside the *Citrus* orchard, while in the rhizospheres of *Citrus* plant both organisms were frequently isolated. Their frequency of occurrence within the rhizosphere of *Citrus* suggests that they could have feeding relationship with the plant.

The plant flavonoids may stimulate spore germination and this could lead to development of plant free culture of these endomycorrhizal fungi which in turn enhance uptake of mineral element by *Citrus* plants. Both organisms could be examples of endomycorrhizal fungi (Precolt *et al*, 1999; Sylvia, 1998).

Again, while *Phytophthora spp* was frequently associated with the old trees (1977 trees, table 2), *Fusarium verticilloides* was closely associated with trees of all ages (1977-1983). This suggests that there may be exudates secreted by the older trees that attracts *Phytophthora spp* particularly.

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