

Plant sciences or botany is the study of plants in all their forms and interactions using a scientific approach. This book comprises of recent and review papers written by professors and researchers on recent advances in plant sciences such as study of green alga Pithophora oedogonia, Soil Fungi isolation and their biological and mechanical role, Mycoflora From Rhizospheric soil, Comparative anatomical study of two Cordia species, Post-Harvest Infected Fruits Diseases, In vitro evaluation of different Biocontrol Agents, Vegetation Cover for Abatement of Noise, Diversity of Scenedesmus, Ethnobotanical Investigation on Wild Edible Vegetables, Insectivorous and Parasitic plants, Morphology and Anatomical Study of important plants, Weed plants as Valuable Resource etc. This book is useful for researchers, academicians, students and common people.

Recent Advances in Plant Sciences



Pratap Naikwade

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Dr. Pratap V. Naikwade, well known botanist is editor of this book. He has completed post doc research from USA. He is author of several research papers and books, worked as invited speaker in many international conferences. His research work got international recognition and received Young Scientist, Outstanding Researcher and many other Awards.

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## CHAPTER 7

### Identification and Characterization of Cotton Plant Post-Harvest Fungal Infections in The Siricilla District

T. Srinivas<sup>1</sup> and S. Vijaya<sup>2</sup>

<sup>1</sup>Department of Botany, Govt. Degree College Agraharam, (T.S.)

<sup>2</sup>Department of Botany, Tara Govt. College (A), Sangareddy, (T.S.)

#### Abstract

The current study was carried out in the Department of Botany, Govt Degree College Agraharam in order to determine the fungal diversity in the Cotton (*Gossypium hirsutum* L.) Plant's leaves, roots, and fruits, among other things. According to research, the cotton plant has been recognised as one of the most vulnerable to fungi that may be discovered on its leaves, roots, and in the surrounding soil. During a survey of cotton fields in the villages of Siricilla and Agraharam-Rajanna siricilla District in the state of Telanaga, samples of cotton leaves, fruits, and roots were collected, and the results were examined. It was completed at the pathological laboratory during the experimental phase, when it was carried out in the Pathogenicity laboratory. Growing fungus in artificial mycological medium was accomplished using the techniques of leaf and root plating, and the results were quite positive in nature. A total of nine isolates were generated from cotton leaves and roots throughout the course of the experiment. All of the isolates were identified by a pathologist with substantial experience using a standard key and a standard procedure. *Rhizoctonia solani*, *Fusarium solani*, *Fusarium oxysporum*, *Fusarium Chlamydosporum*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rizopus spp.*, *Aspergillus niger*, and *Penicillium notatum* were among the fungi that were discovered. As a result, *Rhizoctonia solani* and *Fusarium solani* are present. According to our findings, *R. solani* and *F. solani* had much higher percentages of leaves, fruits, and root colonisation than the other species studied. Upon examination of symptomatic plant samples, researchers discovered that the *R. solani* had a root colonisation proportion of more than 50 percent. In this study, *F. solani* had 30 percent



RC, which was followed by *F. oxysporum*, *S. rolfsii*, and *S. sclerotiorum*, all of which had 20% RC in their respective studies. As a result of the research, it has been determined that the most infamous soil-borne fungus is found in the cotton crop field of the screening region in Rajanna siricilla District, Telangana State, and that the frequency of these fungi is significant.

**Key Words:** Leaves, fruits and Root borne fungi, Cotton, *F. solani*, *F. oxysporum* and *Rhizoctonia* spp

## Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most strategically valuable fibers such as cotton on the world, responsible for about a quarter of all world production. It also is a significant cash crop, contributing for a major portion of the world's total output of agricultural products. It is commercially grown in more than 90 countries, with the majority of the crop Chinese - made, India, the United States, Pakistan, and Uzbekistan, according to the International Food Information Council. Pakistan is the India's third exporter of cotton after the United States and China (Khan *et al.*, 2017).

Cotton plant, also referred as the "king of fiber," is a plant that is widely cultivated all over the world for its fiber seeds, which are harvested in the fall. A total of 10 percent of Gdp of Pakistan and fifty-five percent of its foreign exchange profits come from cotton production, which represents for between two and five percent of the world's largest farmland. Cotton - based products make for 10% of Pakistan's gross domestic product (GDP) and 55% of the country's currency profits (Azam *et al.*, 2013). It is an angiosperm, a dicotyledonous plant that is a member of the Malvaceae family of plants, and it is a member of the Malvaceae family of plants. Asia and Africa are among the continents where it can be found in its natural habitat.

Cotton is a perennial plant in its natural environment, but it is currently grown as an annual crop in the United States. In addition to being harvested in the fall after the seeds were planted in the summer, cotton also has the advantage of being exceedingly cost-effective, allowing growers to cultivate two alternate harvests in the same year. The cotton species *G. hirsutum*, sometimes known as upland cotton, is the most widely cultivated of the cotton species. Noted as Egyptian cotton, this type of cotton is grown

in Egypt and accounts for more than 90 percent of global production. Egyptian cotton is known for producing cotton that is generally of exceptional quality, as well as having great strength and flexibility. In addition to cotton, *G. herbaceum* (herbaceous cotton) is found in Pakistan, India, and certain parts of Africa. It is the second most widely planted crop in the world. Herbaceous cotton (*G. herbaceum*) is another name for *G. herbaceum*. Geographical distribution of *G. barbadense* includes Egypt, Sudan, the United States, Brazil, and Peru. *G. arboreum* is a type of cotton tree that grows in the United States (native to Sri Lanka and India). Because it produces short fibres of poor quality, it is not widely planted because it is controversial (species of cotton). This study is concerned with identifying whether or not there is an enormous amount of the most world famous soil-borne fungus in cotton crop fields in the screened region located in Rajanna siricilla, District, Telangana State.

#### **Diseases of cotton:**

In additional to being prone to a wide variety of illnesses, cotton (*Gossypium* spp.) is also reported to be prone to a number of insects and pathogens, including fungi, bacteria, and viruses. As reported by Agrios (2005), diseases (fungi, bacteria, and viruses) account for 14.1 percent of total losses, insects account for 10.2 percent of total losses, and weed species account for 12.2 percent of total losses. Insects account for 10.2 percent of total losses, and weeds account for 12.2 percent of total losses. Crop damage is mostly caused by plant pathogens, which are responsible for the majority of crop losses (14.1 percent). If bacteria have a considerable effect on plant manufacturing or vegetation parts ruination (death), including such fungi (pathogens), we don't hold it against them because they only have had the capacity to slam plants whilst also gaining their life (which means food), but we do hold them responsible because they perform a given a prescription function and provide benefits to the plant population. Despite the fact that it is not the end, it is the one (fungus) that communicates with other animals in order to coexist in mutual beneficial relationships. The United States does not even have the final say, but it has the greatest impact on the well-being of the globe in a variety of ways. In a similar fashion, the fungal has contaminated the roots of the crop plant, which is called to as root based fungus, and this has both positive and negative repercussions for the crop plant, depending on the situation. A number of the most acute

diseases, as well as the fungi types that generate these, constitute a significant threat to cotton crop yields and quality.

#### **Damping-off:**

The name of this fungal infection is based upon the fact that it produces plants wilt in plants. It is believed that the fungal disease began in the roots and progressed to the stem and vascular system plugs of the plant over time. Fusarium wilt (*Fusarium oxysporum*) and Verticillium wilt (*V. oxysporum*) are two types of wilt that affect plants (*Verticillium longisporum*). These are all the plant-killing bacteria that have an effect on cotton and other crops. Fusarium wilt is one of the most devastating diseases that can affect crops. *Fusarium oxysporum f. spp. vasinfectum* is the pathogen responsible for the outbreak. In his description, Atkinson referred to it as the "first Vascular wilt illness" (Atkinson, 1892). According to Ibrahim (Ibrahim, 1966), the South Eastern United States, Egypt's Nile Valley, Tanzania, the south and east of Lake Victoria, and sections of India and China are among the locations where Fusarium wilt of cotton is most damaging (Kelman and Cook, 1977).

#### **Sclerotium wilt:**

It's a suitable soil illness that can be discovered in the tropical, subtropics, and other warmer regions. It thrives in high humidity and hot weather, thus its best avoided in these areas. It has the potential to cause a variety of plant diseases, including Sclerotium wilt, collar or stem rot, foot rot, crown rot, damping down of seedling, and blights, among others. Previous research found that *S. roffsii* infects approximately 500 monocotyledonous and dicotyledonous plant species, with the most severe effects occurring in vegetables, flowers, legumes, cereals, forage plants, and weeds. *S. roffsii* has been found to infect approximately 500 monocot and dicotyledonous plant species.

#### **Black root Rot:**

Red roots rot in different crops is caused by the fungus *R. solani*, which is an important disease in the cotton industry. When black root rot first was identified in cotton, it was discovered in the Arizona area (King and Presley, 1942). Black root rotting was first discovered in Australia on cotton (*G. hirsutum*) in North-Western South Wales, and it has since spread throughout the country (Allen, 1990). Root rot is a devastating and lethal cotton disorder that affects cotton growers in Punjab and Sindh,

particularly (Iqbal *et al.*, 2005). It is possible to contract a variety of illnesses that infect cotton roots, resulting in the destruction of cotton harvest.

### **Stem Rot:**

In addition to cotton, the pathogen *Sclerotium sclerotiorum* (Lib.) de Bary infects around 400 different plant species, including canola and rapeseed (Bolton *et al.*, 2006). *Sclerotium* can reproduce both asexually (*Myceliogenic sclerotia* germination) and sexually (*Carpogenic sclerotia* germination) depending on the environment in which it grows (Aldrich-Wolfe *et al.*, 2015). Ordóez-Valencia *et al.* (2015) describe the fungal as producing a white fluffy mycelium (white mould) on sick plants, followed by the formation of surviving components termed sclerotia, after a few days. According to the host, these are black, metalized structures with depend largely from a few millimetres (bean) to a few centimetres (sunflower) (Bolton *et al.*, 2006).

### **Aims and Objectives:**

Our aim is to isolate and identify pathogenic microbes that colonized the cotton plant's leaves, fruits, and roots, as well as to determine the proportion of pathogenic fungi that colonies the cotton plant's leaves, fruits, and roots, among other things. Despite the fact that there are a variety of ways for treating soil-borne diseases, actual infestations data would attached data the options available recommended in plant pathology guidelines (Inam-Ul-Haq *et al.* , 2015). Our research objectives were as follows:

- To harvest poor and disease-symptomatic plants from a 20-kilometer radius surrounding our college cotton fields

To extract endophytes from cotton leaf, fruit, and root samples and to determine the frequency and percentage of fungus species infection on the leaf, fruit, and root samples, respectively.

Utilizing leaves, fruits, and roots, it is possible to maintain pure cultures of fungal isolates.

Mycological media were used to identify fungal taxa and study their biology in vitro.

### **Materials and Methods**

Several fungi which are found on the leaves, fruits, and roots of the cotton plant were isolated and identified as part of the existing research project, which was done in

order to learn more about them. The experiment was carried out at the Department of Botany, Government Degree College Agraharam, in the month of October in the year 2021. Following were some of the strategies that were used throughout the experimental phase:

**Crop field survey:**

Specifically, the research was conducted in country places in the district of Rajanna Siricilla, which is located in the state of Telanagana. Areas in the neighborhood of our university, which is approximately 20 kilometers distant from our college, were the subject of the investigation. As part of the current investigation, symptomatic samples of badly infected cotton plants were collected from a range of locations, together along with specimens of the ground soil from which the cotton plants were grown. We either pressed and dried the samples for laboratory examination or placed them in polythene bags with their soil and stored them in the refrigerator until needed. Cotton samples were identified using information about the seed variety provided by the producer, as well as physical characteristics of the cotton samples. It was in the month of October in the year 2021 that the samples were collected, which was at the midst of the agricultural season.

**Mycological media preparations:**

Potato Dextrose Agar (PDA) was really the only type of histopathological media employed in the study, and it was also the only type of media that has previously been successfully grown.

**Potato dextrose agar (PDA):**

It's a good all-around gelatinous mass substrate for fungal growth because of its versatility and versatility. It is frequently used to isolate, culture, and maintain the viability of fungal species. In addition to being readily available in pre-made Petri plates and test tubes, PDA can be simply constructed using commercially available materials. As a result, we used its lab preparation, which consisted of 20 percent Dextrose, 20 percent Agar, and 200 g peeled potato extract, to create a simple and effective version that we could afford and that we could use on our PDAs if we wanted to do so (PE). It was possible to make potato extract by boiling peeled potato slices in hot water until they were mushy. Sifting the water-containing extract was accomplished with a muslin

cloth. Accumulation media was prepared to a volume of 1,000 millilitres and placed in a 02 L flask with cotton plugs and an aluminium foil lid. The solution was autoclaved for 15 minutes at 121°C and 15 pounds for 15 minutes. This composition was utilized for the production of a 1000 mL tap water solution for use in the experiment.

Dextrose.....30.0g

Agar .....20.0g

pH  $7.3 \pm 0.2$  at 25°C

### **Isolation of root fungal pathogens:**

In first instance, after the research lab was already extensively vacuumed and the hardware had been meticulously fumigated, we used the protection immunization and cleaned properly the area with an ultra - violet for thirty min and an ethyl acetate spray. In the second instance, we used the safety inoculation and thoroughly cleaned the area with an ultraviolet light for 30 minutes and an ethanol spray. In place to avert cross - infection, the chamber was normally supplied with pure oxygen, and the heat of the Bunsen burner flame was maintained at a consistent degree during the experiment. The suspected plant leaves, fruits, and roots were cut into 02 and 01 cm lengths with a Caesar knife that had been flame sterilised. It took two minutes to sterilize the root pieces in 0.2 percent NaClO (Sodium hypochlorite) solutions before they were rinsed five times serially in sterile distilled water to remove all of the chlorine and other contaminants (SDW).

The root sections were placed on sterile blotter paper in order to absorb any unwanted SDW that may have been present. The dried surface sterilised root pieces were cultured for 3+ days at 25oC in an incubator room with total darkness on medium plates that had been specified. It was necessary to monitor the possible development of fungal growth on the injected root sections on a frequent basis. Fungal entophytes were carefully transferred from the infected roots to pure media, and then transferred to SA medium filled slants for storage for a one-month period. To fully protect the roots of each questionable plant into the media, that was crucial to fulfill 05 duplicates of each experiment, with each experiment being repeated twice more than once. As part of the research's construction, entirely completely randomized configurations are employed.

### **Pathogens of the fungus genus: identification**

In addition to identifying buttons including such

- 1) Depicted genera of inherently flawed Fungi (Barnett and Hunter, 1998),
- 2) Compendium of soil fungi (Domsch *et al.*, 2007),
- 3) Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species, and
- 4) Fusarium: Laboratory guide to the identifier of the species (Barnett and Hunter, 1998), On the basis of their descriptions in the keys, Rhizoctonia species were identified. The identification of Sclerotium spp. was accomplished with the help of the keys that were provided (Aycock, 1966).

The morphology of fungal pathogens can be used to identify them.

. Leaf colonization percent:

$$\text{Colonization\%} = \frac{\text{Number of Leaf pieces colonized by the pathogen}}{\text{Total number of leaf pieces}} \times 100$$

Fruit colonization percent:

$$\text{Colonization\%} = \frac{\text{Number of fruit pieces colonized by the pathogen}}{\text{Total number of fruits pieces}} \times 100$$

Root colonization percent:

$$\text{Colonization\%} = \frac{\text{Number of root pieces colonized by the pathogen}}{\text{Total number of root pieces}} \times 100$$

Data on root colonization were converted into roots colonization index (RCI) according to a 0-5 scale of (Yaqub and Shahzad, 2011) where 0=0, 1=1-10, 2=11- 25, 3=26- 50, 4=51-75 and 5=75-100% root pieces colonized by the pathogen.

### **Statistical Analysis:**

A multivariate assessment and a way anova were performed in IBM-SPSS-19 software to assess whether or not the results were statistically significant. This programme was also used to construct the results tables and charts that were exhibited

during the presentation of the findings. On SPSS data, the homogeneity test (also known as Duncan's Multiple Range Test, or DMRT) was also performed.

## **Results and Discussion**

To achieve this goal, the researchers conducted a study in which they identified and isolated soil-borne fungus from various sites on Rajanna Siricilla plants that were grown in vitro between September and October 2021. Using the Department of Botany Government Degree College Agraharam's culture attributes, morphological characteristics, and microscopic spore and fruiting body features, the researchers were able to certify the purity of the isolated fungi. Because more modern and faster processes are more expensive, morphological characteristics should be regarded as a key tool for fungal identification, and more personnel with the appropriate competence should be recruited to the field.

### **Sampling area:**

The research has been carried out in an agricultural community in the Rajanna Siricilla district of the state of Telangana, and it covered three sample locations. The participants were recruited from a variety of backgrounds. The row technique was used to collect specimens at each spot, and samples were gathered related to the physical strength and appearance of the plant at each location. There were (25X4) hundred samples gathered from various sites during the study

### **Fungal identification**

*R. solani* and i.e. reactive were the fungus strains discovered in the roots, leaves, and fruits of the plant. *Rhizoctonia solani* is a kind of fungus. Kühn It is a brown, spreading plant with side branches that are virtually right-angled and which septate tightly between the main hyphae and side branches. Measures will help cucumeris is a member of the cucumber family. Cells that are composed of catenulate cells that have grown in an acromegaly Brown sclerotia that come in a variety of sizes and forms mm is the circumference of the circle. Isolates cultivated in pure culture on mycological medium were discovered. Hyphae are light brown or beige in colour, with lateral branches that are restricted to septa and are about right-angled in shape. Side branching are closely attached to the main hyphae. Cells that are composed of catenulate cells that



have grown in an acromegaly Sclerotia is a fungus that ranges in colour from brown to dark brown and comes in a variety of morphologies.

***Sclerotium rolfsii* Sacc.**

Conidia do not appear to have formed in this instance. Hyphae are light brown or brown in colour, branching, with septate and constricted side branches near the main hyphae, and in some isolates, clamp connections between the hyphae and the surrounding tissue. It is composed of a composed rind (upper layer) and a composed medula (inner layer). Sclerotia are brown or dark brown in colour, globose or subglobose, smooth, glossy, and compact (inner layer).

***Fusarium oxysporum* Schldl.**

People with a common with spore densities at the apical end that are simple, short, and hardly detached from of the hyphae are called hyaline conidiophores. Phalosporous conidia are hyaline, and there are two types: macro conidia are boat-shaped, with slightly tapered apical cells and hooked basal cells, and they are 4-celled, and micro conidia are ellipsoidal and 1-celled. In general, chlamydo spores are brown globular spores that are found in isolated colonies.

***Fusarium solani* (Mart.) Sacc.**

A hyaline, simple conidiophore with spore masses at the apex that is as tall as the length of macro conidia by a factor of many times the length of macro conidia, with spore masses at the apex that is as tall as the length of macro conidia by a factor of many times the length of macro conidia It is possible to differentiate between macroconidia and microconidia. Microconidia are cylindrical, 1- to 2-celled, and are usually 3- to 5-celled; macroconidia are slightly curved apical cells, two cylindrical central cells that are often slightly curved in one side, and hooked foot cells; macroconidia are slightly curved apical cells, and hooked foot cells.

***Aspergillus nidulans* (Eidam) G. Winter**

Properties of the colony: After 10 days at 25 degrees Celsius, the colony's diameter is 2.5-3.0 cm. Texture lanose; at the beginning of development, the colony surface is white, then ochre, with a light rose tint in the Centre of the colony. The reverse is a dark purple colour, similar to eggplant. On the surface, there are colourless exudates. Conidial heads are narrow and columnar in shape..

### ***Penicillium notatum* Westling**

The colonies on agar grow rapidly, reaching a diameter of 4 to 5 cm in 10 days at 25 C; some strains grow more slowly, reaching a diameter of 2.5-4.0 cm; colonies are sometimes furrowed or the conidiogenous structures are arranged in a radial pattern; colonies are mostly azonate, the margin is even or occasionally lobed, and the surface of some strains is covered by a thin.

#### **Root colonization:**

The findings of this study indicated that nine different fungus species were isolated from a sample of cotton root. *Rizopus* spp., *Aspergillus nidulans*, *Penicillium notatum*, *Rizopus solani*, *F. solani*, *F. oxysporum*, *F. chlamydosporum*, *S. rolfsii*, *S. sclerotiorum*, *Rizopus solani*, *F. solani*, *F. oxysporum*, *F. chlamydosporum*, *Rizopus* spp., The majority of the fungal isolates were from species belonging to the genera *Fusarium* and *Rhizoctonia*, which were prominent in root colonization and were shown to be associated with root colonization.

### **Discussion**

Environmental and macroscopic traits provided by were used to determine which fungus was being studied (Barnett and Hunter, 1998; Nelson, 1998; Domsch *et al.*, 2007). Upland cotton was used to support the current research, which was supported by data showing that seven genera containing 12 species of fungus, including nine *Fusarium* species, were isolated from upland cotton. The *Fusarium* species *Fusarium oxysporum*, *Fusarium solani*, and *Fusarium equiseti* were the most often encountered (Palmateer *et. al.*, 2004). According to phytopathogens, there are around 30 different types of fungus that can cause cotton plant illnesses (Kondhare *et al.*, 2014). We chose this study because it attempts to gain a better understanding of the leaf, fruit, and root soil-borne fungus diversity in cotton crops growing in the rural areas of Rajanna Siricilla District. Myco-flora dispersal is influenced by a variety of environmental factors including pH, temperature, moisture, organic carbon, and nitrogen. pH is the most important environmental factor. Because soil moisture has a direct positive influence on the population of fungus, increased soil moisture reduces tolerance and colonisation of the fungus population (Raja *et al.*, 2017).

PDA is widely recognized as the most widely used general medium in the isolation of fungi, and it provides a broad nutritious base (Agrios, 2005); it is likely that this is the explanation why colony formation was more rapid on PDA than on the other media. According to prior studies, potato dextrose was found to be the most effective substrate for fungal growth (Masudi and Bonjar, 2012). In addition, we used Potato Dextrose Agar media in our experiment, which allowed the fungus to grow to its full potential and flourish. As a result, the processes of isolation and identification were not too difficult and produced results that were satisfactory.

## **Conclusion**

Besides integrating sensitive biological mechanisms with DNA sequencing techniques, it is better to alter the accuracy of fungal agricultural disease detection in *Gossypium* spp plants. Several cotton species plant foliar fungal diseases and the mechanisms that cause environmental speciation and host shifts in them are discussed in this paper. Fungal leaf infections are responsible for significant yield losses in cotton plants that are commercially important around the world. Additionally, it is required to create technologies that will allow for the rapid identification of fungal leaf diseases in addition to evaluating their agricultural value. One strategy that could be used is the detection of infections that have evolved in response to their environment.

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