Int. Journal of Universal Science and Technology
ISSN: 2454-7263, Volume: 05 No: 01 Published : Jan., 2019
Paper ID: IJUP3101, Web: www.universalprint.org
Title Key: Fungal Pathogen Associated with Citrus …

Fungal Pathogen Associated With *Citrus Limetta* Risso.

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Abstract

Horticulture is the science and art of growing plants (fruits, vegetables, flowers and other cultivar). It also highlights the plant conservation, landscape restoration, soil management, garden design and etc. India is the second largest producer of fruits after Brazil. Fruits play an important role in human nutrition by contributing the necessary growth factors such as vitamins and essential minerals in human daily diet, which is also helpful in maintaining a good health. Rot diseases caused by fungal pathogens, provoke severe losses of agricultural and horticultural crops every year (Salman 2005; Parveen *et al*., 2016). *CITRUS LIMETTA* RISSO. belongs to the family Rutaceae.Though leaf spot and fruit rot is a common disease, severely affecting the growth and yield of the plant, it has gained, least attention of researchers. A current research includes the collection of Citrus leaf spot disease sample collected from Merkera district of Karnataka, India. The pathogen was isolated from surface sterilized small pieces of the leaves and twigs, incubated on Potato Dextrose agar (PDA) at 25°C.The study was based on the colony characteristics[morphological and cultural characteristics],Fungal pathogen ***Helimenthosporium,*** was identified as a causal agent of the disease,.Disease management studies is under process.

Key words

Helimenthosporium, Colony characteristics, Rot disease,PDA **,***CITRUS LIMETTA* RISSO.

Introduction

Citrus limetta Risso. considered a Horticultural and medicinal plant which is susceptible to only a few fungal pathogens. Epidemics of a fungal leaf spot disease with characteristic necrotic spots were evident during the onset of monsoon season every year. Proper identification and characterization of the pathogen is essential as the review of literature suggests not much work has been done on this particular disease. Preliminary diagnosis of the disease revealed the pathogen is fungus with the formation of fruiting bodies visible as black and white globose bodies in necrotized tissues of the leaves and the twigs, seen often with the presence of setae. The imperfect state as the active pathogen was detected from growth of fungi in media. The classification, nomenclature and identification of the fungi using fungal identification manual revealed the pathogen is *Helimenthosporium*. As a result of the studies conducted on leaf spot disease of Citrus limettta, *Helimenthosporium* was isolated for the first time in India. Therefore attention to the occurrence of this fungus was directed and macroscopic and microscopic features of the pathogen and host pathogen interaction was carried out in the study.

Before they can penetrate and colonize the host, they must first become attached to the host surface. Attachment takes place through the adhesive of spores, bacteria and seeds through the adhesive materials that vary significantly in composition and in the environmental factors they need to become adhesive. The germ tube is a specialized structure distinct from the fungal mycelium often growing for a very short distance before it differentiates into an appressorium. , the penetration of host tissues generally relies on formation of these specialized infection structures called appressoria (Perfect et al., 1999). Appressoria allow the fungus to penetrate the host cuticle and epidermal cell wall directly by means of narrow penetration peg that emerges the base of appressorium. Based on the host infection process of a ***Helimenthosporium*** pathogen has been studied.

Materials and Method

Isolation of the Pathogen

A total of 18 *Citrus limetta* Risso. leaf samples from Madekeri district of Karnataka were analyzed for ***Helimenthosporium*** incidence. Samples were brought to the laboratory in clean plastic bags and kept at 40 C. All the samples were subjected to mycological analysis. Fresh leaf samples showing characteristic necrotic and chlorotic spots were selected, surface sterilized using 70% ethyl alcohol and cut into small pieces of 1mmx1mm and plated on PDA medium and incubated. The plates were incubated at 25+2 □ C for 7 days. The associated microbial colonies expressed will be isolated and identified using fungal manuals and keys. Cultural isolates and their identification The ***Helimenthosporium*** isolates were cultured on to plates containing semi-synthetic PDA in order to study cultural characteristics. The colony morphology and cultural characteristics were evaluated on both sides.All the fungal isolates were maintained on PDA slants the characters such as size, shape of conidia, existence of setae, cultural characters such as colony color, growth rate and colony texture were considered for the identification of species (Barnett and Hunter 1999; Sutton 1980; Sutton 1992). Morphological and cultural characteristics Fungal isolates were grown on PDA plates at 25oC. For mycelial growth and colony characteristics on petriplates containing PDA were inoculated at the centre of each plate with 5mm diameter mycelial disc that was taken from the margin of a 7 day old culture grown on PDA. Colony diameter of each isolate was measured daily for three weeks at 25o C. Colony color of each fungal isolate was also examined about three weeks after inoculation. Three replicates of each isolate were evaluated. To determine morphological characters, conidia formed on PDA for 7 day incubation were harvested with sterile distilled water and observed under compound light microscope. One hundred conidia from each isolate were measured in length and width with 3 replicates. (Kim et al., 2008).

Colony characteristics on PDA media

Radial growth and sporulation of the isolates were compared on culture media. The media selected for cultural studies is Potato Dextrose Agar (PDA). Cultures on PDA at 4oC were used for cultural studies. 5mm diameter mycelial agar disc of each isolate were placed on the surface of the selected culture medium and incubated at 24o C for 8-12 days . Conidial morphology was examined using a compound microscope, photographed and compared.

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Pathogenicity test (In-vitro)

LEAF DETACHED BIO-ASSAY

In-vitro pathogenicity test was conducted by using healthy leaf of C. limetta using the methods described by Baiyewu and Chukwuka *et al*., To confrim the association of leaf spot disease, pathogenicity test was conducted on detached leaf. Briefly 30 healthy C. limetta leaf were taken and surface sterilized and 15 leaf were used for control and 15 were inoculated with diseased sample of *Helimenthosporium* and incubated in a moist chamber at 28±2ºC. the development of disease symptoms were observed after 15 days of incubationof post inoculation and progression of disease was recorded.

Results

Isolation of pathogen and Identification of pathogen In general, leaf lesions are dark brown to black which initially appear as minute black circular spots. A diagnostic feature of Helimenthosporium infection,it is a dematiaceous filamentous fungi.The texture is velvety to wooly.its produces hyphae conidiospores and conidia. The pathogen was isolated in pure cultures on PDA media, subcultured and maintained on PDA for further studies.

Morphological characterization of Isolated Pathogen

Colony on PDA were browinish-grey coloured, and it is a dematiaceous filamentous fungi.The texture is velvety to wooly.its produces hyphae conidiospores and conidia. Hyphae are septatae. Conidiospores are brown to dark brown in colour and ceasing to elongate when the terminal conidium is formed. Conidia are multicelluar (6 or more cell),large (9-40µm),solitary ,club shaped ,and pale to dark brown in colour. They are located along the sides of the conidio spores and their wider end is towards the conidio spores.

Colony characteristics on PDA media

The colony on PDA was compared with other six media for sporulation, acervular formation and other morphological features. All the strains of Colletotrichum tested grew quickly, usually covering the whole surface of the petriplate in 10-15 days, except CZA and showed profuse sporulation on potato carrot agar compared to other media used. This medium was better than PDA for observing the main microscopic features of the fungi. Acervular conidomata were present in all the media with the production of conidiogenous cells directly on the agar surface and/or throughout the aerial mycelium of the colony.

Potato Dextrose Agar (PDA)

Colonies on PDA grew very quickly, occupying the whole surface of the Petri dish in 5-7 days. They were browinish-grey in colour, velvete to wooly, Hyphae are septatae. Conidiospores are brown to dark brown in colour and ceasing to elongate when the terminal conidium is formed. Conidia are multicelluar (6 or more cell),large (9-40µm),solitary ,club shaped ,and pale to dark brown in colour. They are located along the sides of the conidio spores and their wider end is towards the conidio spores.

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Fig-1: A- Field photo. B- C.limetta black spot back view. C,D- C.limetta black spot front view

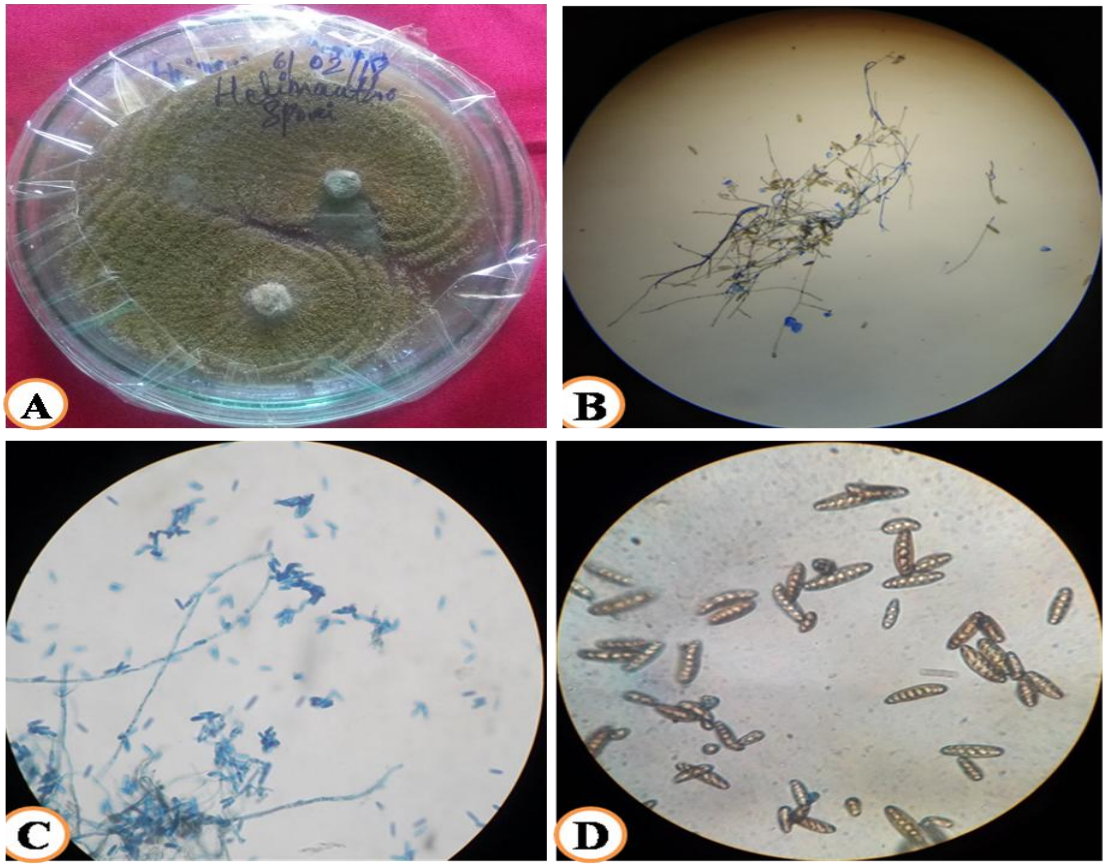

FIGURE 02:A- Isolation of *Helimenthosporium* species on PDA media.
B,C-spores of Helimenthosporium along with mycelia under 10X
D-spores of *Helimenthosporium* under 40Xmagnification.

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DISCUSSION

The main aim of this objective was to identify the causative agent responsible for leaf spot disease of *Citrus limetta* Risso.which appear be a destructive epidemic in many regions of Karnataka and Kerala. In a morpho-taxonomic comparison, isolates from necrotic and chlorotic spots of the leaf and twig samples of leaf spot affected plants were uniform in appearance and identified as Helimenthosporium. This species is one of the most economically important groups of fungi, because of its economic importance as a plant.

Fungi are one of the most important heterotrophic organisms have the ability to cause disease in both animals and plants. Many fungi are also useful in many ways and play an important role in industry, medicine, and agriculture. They also causes severe disease in fruit crops and causes more than 60% of economic loss in India.The present study conducted to determine the incidence severity and identification of the fungal pathogen associated with the leaf spot of *C.limetta.* A field survey conducted in infected C. limetta fields revealed that, the leaf spot disease was spreading fast in recently planted C.limetta plantation. Hence this plot was surveyed further for disease incidence .

- The disease samples subjected to mycological examination by agar plating method. The fungal pathogen was isolated and subsequently sub cultured on PDA medium to obtained pure culture after 7 days of incubation at room temperature(28±2ºC). fungal pathogen was isolated and identified as Heliminthosporium.
- Helimenthosporium, **is** the fungal pathogen causes leaf spot disease in C.limetta. The morpho-cultural characters of Alternaria is the fungus produce brown coloured fungal colonies with ovoid, ellipsoidal often smooth walled conidia with short conical or cylindrical beak.

The fungus heliminthosporium produce velvety to wooly texture. The conidiospores are brown to dark in colour. Conidia aremulticellural, solitary, club shaped and pale to dark brown in colour.

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