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Isolation and identification of some fungal pathogens from Solanaceous vegetable crops of Chitradurga district, Karnataka

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Abstract
The present work deals with the isolation and identification of pathogenic fungi. The fungal isolates were *Fusarium oxysporum, Fusarium solani, Fusarium incarnatum, Carynospora cassiicola, Colletotrichum gloeosporioides, Alternaria spp, Cladosporium cladosporoides, Mucor flavus, Trichoderma spp.* and *Aspergillus Niger*. These were isolated from Solanaceous vegetable crops like chilli, tomato and brinjal and were identified on the basis of colony morphology and microscopic examination on PDA medium. The morphological characteristics of these fungal elements showed various kinds of spores which was identified up to genus/species level. Out of 10 isolated fungi, all were pathogenic except *A. Niger* and *Trichoderma spp* which are saprophytes.

Keywords: isolation, identification, fungal pathogen, vegetable crops

Introduction

Vegetables are most important components of human food since they provide proteins, Vitamins, Carbohydrates and some other essential macro and micro nutrients required by the human body. Fungal diseases cause huge losses to vegetables during cultivation, transportation and storage. Phytofungal pathogens cause serious problems for the agricultural crops including vegetables. The plant is highly affected by adverse climatic conditions. The warm and cool climatic conditions provide an ideal condition for the development of many foliar, stem and soil-borne plant diseases. Fungal diseases are a major limiting factor for vegetable that cause serious yield reduction leading to severe economic losses (Pavankumar *et al*-2018). In addition, many also produce mycotoxins, which are harmful to humans and livestocks and causes a number of diseases like rusts, smuts, rots and downy mildew. Plants are infected by different kinds of microbial pathogens and the required inoculum for infection is present in the soil, water and air, in addition to plant host. Whatever may be the source of inoculum, the susceptible plant species or crop varieties may exhibit clear visible local symptoms in or on the tissues where infection is initiated. If the pathogen is able to find favourable conditions for further development, systemic symptoms are induced in tissues or organs far away from the point of pathogen entry into the plant. When the symptom of infection is not expressed externally, it is termed as latent infection. Some fungal pathogens infecting unripe fruits do not induce any visible symptom as they remain dormant. Detection of microbial pathogens refers to the process of establishing the consistent presence of a particular target organism(s) within the plant or in its environments, irrespective of the development of visible symptoms in the plant suspected to be infected by the pathogen(s) in question. Diagnosis, on the other hand relates to the identification of the nature and cause of the disease Problem under investigation (Digambar and Sahera-2016) [2]. Chitradurga district falls in central eastern parts of the state and covers a total geographical area of 8388 sq. kms. The district is divided into 6 Taluks, namely Chitradurga, Hiriyur, Hosadurga, Holalkere, challakere and Molakalmuru. It lies in the central dry agro climatic zone. The average temperature during the summer reach up to 42ºC and minimum during winter can be 12ºC. Major part of the land is utilized for the agricultural purpose which includes Rabi, kharif and other agricultural plantation. The water bodies cover an area of 384.9 sq. km which is comparatively low area with agricultural land, hence the people of this district depends on rainfall for growing the crops.

The vegetable crops are attacked by many fungal, viral, bacterial, nematodal and some other diseases leads to loss in quality and yield. Out of these diseases fungi causes more loss in field condition and post-harvest condition (Salau and Shehu-2015) [6]. Present investigation aims at identification of the fungi from the plants showing symptoms and were identified based on their morphological characters.

Materials and Methods

Study site and sample collection

Field survey was done in major vegetable growing regions of Chitradurga District from September to November 2020 to estimate the fungal diseases. A purposive and randomized sampling method is used for survey and collection of samples (Zainab and Shinkafi-2016) [8]. The fungal pathogens were able to infect various plant organs such as roots, stems, leaves, flowers and fruits. The infected part shows visible characteristic symptoms like spots, blights, wilts, rots etc. Plant parts with visible symptoms were collected from different vegetable crops in the field

condition (Thilagam *et al*-2018). Collection of infected material is performed with the pre sterilized knife, forceps, cutter and other necessary accessories. The collected materials were carried in a presterilized zip-lock cover or polyethylene bag to the laboratory for the microscopic observation and identification

Preparation of PDA Medium

200gm unpeeled potato slices were boiled in 1000ml of distilled water for 30min. Potato infusion was filtered using cheese cloth. To this, 20gms of each of Dextrose and Agar was added and sterilized using pressure cooker at 121ºC for 15 min (Digambar and Sahera-2016) [2].

The molten medium at warm temperature was poured to the pre-sterilized glass petriplates. About 20ml of medium was poured and these plates were used for the inoculation of infected plant material as well as for growing pure culture.

Isolation and identification of the fungal pathogen

The collected infected materials exhibiting clear symptoms were thoroughly washed in running tap water. The infected tissue along with adjacent small unaffected tissue were cut into small pieces (2-5mm) and they are transferred to sterile petridishes containing 1% Sodium hypochlorite (NaOCl) solution for surface sterilization of plant tissue for 1 to 2 minutes and washed repeatedly with sterile distilled water to remove disinfectants.

The sterilized pieces were aseptically transferred to petridishes containing Potato Dextrose Agar (PDA) medium and incubated at room temperatures (25±2ºc) for further growth and development of fungus. Antibiotics (ampicillin 50µg/ml) were incorporated in to the medium to prevent bacterial contamination. A portion of mycelium developing on the PDA medium was transferred to the agar slants for purification and storage for further investigation (Paulina and Sagaya-2017 and Pawar-2018) [8]. The isolated fungi were identified to the genus level and species which was possible on the basis of micro-morphological and macro-morphological characteristics using standard manual (Barnett-1998) [1].

Results and Discussion

The results are as shown in the table1. During the investigation period ten fungi were isolated from infected vegetables parts like leaves and fruits. *Carynospora cassiicola, Fusarium solani, Fusarium oxysporum, Colletotrichum gloeosporiodes, Aspergillus Niger, Fusarium incarnatum, Alternaria spp., Mucor flavus, Trichoderma spp.* and *Cladosporium cladosporoides* were observed. It was found that maximum percentage was observed for *Fusarium solani* and *F.Oxysporum*. During the study seasonal variations were also observed. Five fungal species were found in the September month. This may be due to low temperatures, percentage of humidity it was quite favourable for fungal growth and remaining fungal species were found in October and November Month. According to Paulina and Sagaya-2017 *Fusarium oxysporum* is major fungal pathogen in tomato causing wilt disease. Digamber and Sahera 2016 [2] reported that maximum percentage contribution was observed in *Fusarium moniliformi* and *F.Oxysporum* Morphological characteristics is a primary tool for fungi identification should be embraced and more personnel with the knowledge are required since modern and faster techniques are expensive. This study has provided useful information about the toxigenic fungi associated with plant parts which may affect the human health, agricultural production and also economic loss.

Table 1: Details of the identified fungi on different parts of plant at different seasons.

Sl. No	Name of Fungal Mycelium	Host	Parts	Month
1	Carynospora cassiicola	*Lycopersicon esculentum*	Leaves	September
2	Fusarium solani	*Lycopersicon esculentum*	Leaves	Sep + oct
3	Fusarium oxysporum	*Capsicum frutescens* + *Solanum melongena*	Leaves	September
4	Colletotrichum gloeosporioides	*Capsicum frutescens*	Fruits	November
5	Aspergillus niger	*Lycopersicon esculentum*	Fruits	September
6	Fusarium incarnatum	*Capsicum frutescens*	Leaves	November
7	Alternaria spp.	*Lycopersicon esculentum*	Leaves	November
8	Mucor flavus	*Capsicum frutescens*	Leaves	November
9	Trichoderma spp.	*Solanum melongena*	Fruits	Sep + October
10	Cladosporium cladosporoides	*Solanum melongena*	Fruits	October

Photoplate

Growth of pathogens on PDA medium under microscopic examination

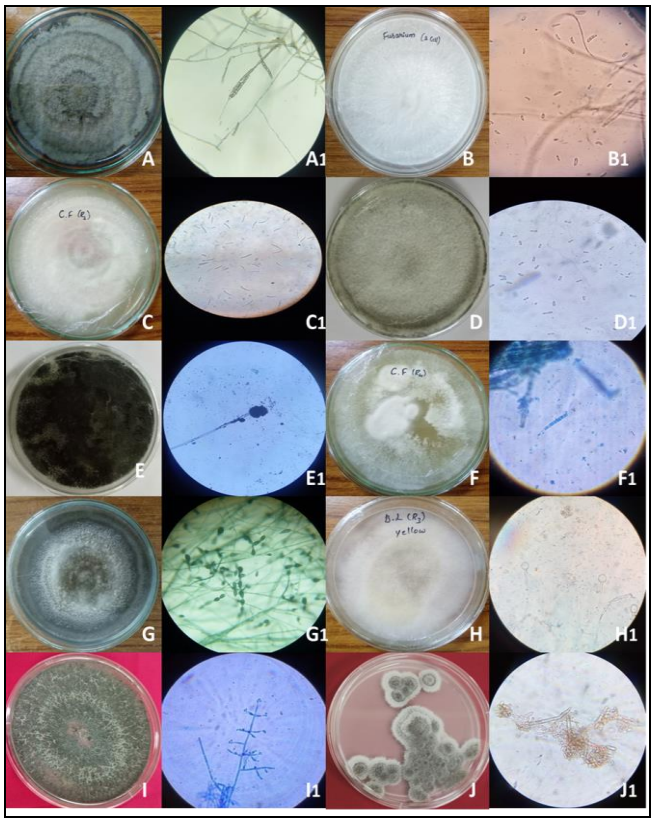

Fig 1: *Carynospora cassiicola* (A, A1), *Fusarium solani*(B, B1), *Fusarium oxysporum*(C,C1), *Colletotrichum gloeosporioides*(D, D1), *Aspergillus niger*(E, E1), *Fusarium incarnatum*(F, F1), *Alternaria spp.*(G,G1), *Mucor flavus*(H, H1), *Trichoderma spp.*(I,I1), *Cladosporium cladosporoides*(J, J1).

Conclusion

Present investigation revealed the identification of the major or prominent fungi from the infected or symptomatic plant parts. When the prevalence of the fungi on the basis of season, it was observed that *Fusarium solani* and *F. Oxysporum* was more during September and remaining

species were found to be less predominant. This may be due to the climatic conditions like temperature and humidity.

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