Isolation and Identification of *Fusarium sp.*,from Vegetable crop field

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ABSTRACT
In the present study ,investigation deals with the isolation and identification of the phytopathogens from okra field cultivated in Mylaudy of Kanyakumari District Tamil nadu.Different microbial groups on okra can have diverse effects on human health and economicloss to the farmers.

Isolation of pathogenic fungi namely *Fusarium species* was identified on the basis of morphological, cultural characteristics and staining technique.

Key words :Okra, *fusarium* sp.,Pathogenic incidence and severity

1.INTRODUCTION

It is an important vegetable suitable for cultivation in traditional agricultural systems as well as on large commercial farms. The fruits are harvested when immature and eaten or cooked in a variety of ways. It provides carbohydrates, proteins and vitamin C in large quantities and also essential and nonessential amino (Adeboye and Oputa, 1996).Moreever,its mucilage is suitable for certain medical and industrial applications (Benchasri,2012).It contain moderate levelsof vitamin A and C(Tripathi *et al*.,2011).Okra is susceptible to several diseases,both in the field and storage microorganism that cause rots do so at a high relative humidity (RH)and temperature of 25-30^{0}C(Adeniyi,1970).Okra contamination can also arise as a consequence of treating soil with organic fertilisers, such as sewage sludge.

However, culture methods leaf imprints, etc. are still thought appropriate and being widely used to characterize microbial populations from different habitat (Ritz 2007). The pathogen can spread from oozing plants by water splash and by wind-driven rain. Patel and Vala (2003) isolated *Fusarium* from wilt affected okra plant and experimentally established its pathogenic association and causal nature by confirming Koch's postulates.

The total loss of vegetable okra on this account has been estimated up to 20-30% but if the pathogens are allowed to develop, this loss may increase up to 80-90% (Glazebrook, 2005) .So the present study deals with the isolation and identification of phytopathogenin fungi from the vegetable crop field.

2. MATERIALS AND METHODS

Sterilization of media and glasswares The media were sterilized at 15lb psi steam pressure and 121 ^{0}C in an autoclave for 15 minutes. All the glassware used in laboratory studies viz. test tubes, Petri plates and flasks were immersed in chromic acid solution (Potassium dichromate 80g, sulphuric acid 400ml and water 300ml) for 24 h, then thoroughly cleaned with tap water and finally rinsed with sterile water.The glassware was sterilized in a hot air oven at 180 ̊C for an hour.

Source of plant materials

Diseased parts of okra leaves was collected from okra field then put in sterile polythene bags and brought to the laboratory

2.1. Isolation of fungal pathogen by leaf imprinting method

Infected leaf were surface sterilized in 70% alcohol and washed in three series of sterile water to remove traces of alcohol. Infected leaf was placed onto Potato Dextrose Agarpressed with the smooth end of glass rod until a clear imprint of the entire leaf was obtained on the (PDA)Potato Dextrose Agar respectively(Aneja2003 and Abdul *et al.,*2014).The plates was incubated at 25^{o}C for 7 days.Observation were made for the development of colonies.

2.2. Identification of fungal pathogen

Bacteriological tests were performed according to the determinative schemes described by Dye 1962 and by Bradbury 1986
.Identification of isolates was based on colony morphological
Characteristics and microscopic observation with reference to laboratory manual(Fawole and Oso,1988).

2.3. Sterilization of soil Field soil and farm yard manure (FYM) were mixed in the proportion of 1:1 and sterilized in autoclave at 15 lb psi for one hour for three consecutive days.

2.4. Pathogenecity test

This involved artificial inoculation of sterile ,healthy okra leaf with spore suspension *Fusarium sp*in water using a plastic spray -bottle until run -off and incubated for 7 days at 37^{o}C.Negative control plants were sprayed with sterile water. Inoculated plants were kept in a green net house in pots that was maintained at 28-30^{0}C. The pots were labelled, watered as and when required and left undisturbed in net house for germination and development of the symptoms.Re-isolation was made from artificially infected leaf and the isolates was compared with the artificially introduced inoculum .

3. RESULS AND DISCUSSION

3.1.Isolation of Associated Fungi and Pathogenicity Test

Results indicate that one fungi were isolated on Potato Dextrose Medium by leaf imprinting method from diseased okra samples collected from the cultivated field.

3.2. Identification of the phytopathogen

Observation of the cultural characteristics of the isolated organisms in the laboratory showed that they grew luxuriantly in the potato dextrose agar showed in plate:1.

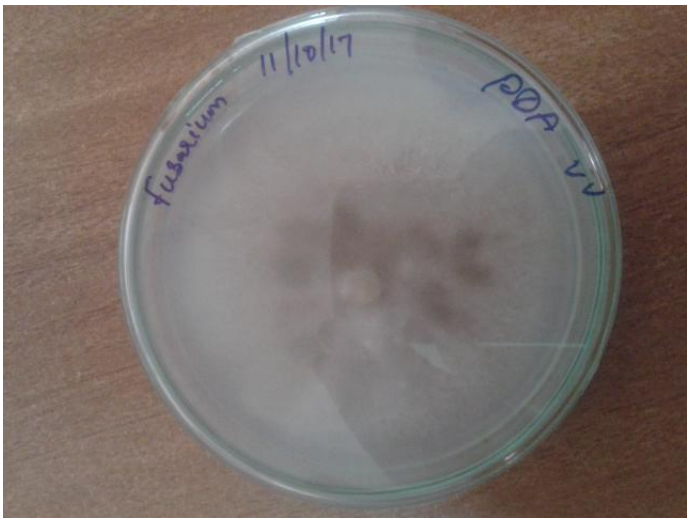

Plate 1: Cultural characteristics of *Fusarium sp*onpotato dextrose agar medium.

Identification of the pathogen causing wilt of okra was carried out by studying the cultural and morphological characters were recorded right from initiation of mycelial growth till the period of 7days. The morphological characters viz., mycelia growth and conidia formation, its size and shape were studied under low(10X) and higher (40X) power magnification from 10 days old culture of *Fusarium sp.*on PDA and were compared with those given in literature.The fungi were identified as *Fusarium sp,*..Several reports and reviews have been written on the diseases associated with okra (A. esculentus L.) both in the fields and storage

3.3.Pathogenicity test

Result of the pathogenicity test carried out revealed that the isolates namely *Fusarium sp*reproduced leaf spot in artificially inoculated okra leaf .These organisms are, therefore, pathogens of okra (A. esculentus L.) and are considered to be responsible for okra leaf spot in the pot.These organisms generally gain access into the crops by several means. While some of them utilize wounds created in different ways on the surface of the plant materials.Others may access the crops through natural openings on the surface of the leaf.

4.Discussion

According to Chattopadhyay and Basu (1957), *F. solani*, the causal agent of okra wilt produced oval shaped thick walled microconidia with rounded ends or straight,macroconidia with 1-3 septa and while chlamydospores were rounded to spherical, intercalary as well as terminal, single or in chain.In our present study oval shaped microconidia and septa macroconidia,round to spherical lchlamydospres are visualized under low and high power objective.

Bohra and Mathur (2004) isolated the virulent culture *of F.solani* on potato dextrose agar (PDA) from diseased roots of soybean.In our present study *Fusariumsp* was isolated from diseased leaf of okraon potato dextrose agar (PDA) Akinyele and Ikotun, (1989)reported that in pot study some of them create wounds in different ways on the surface of the plant material while others may access the crops through natural openings on the surface of the leaf.In our present study the pathogen create wounds on the surface of okra leaf and these organisms are, therefore, pathogens of okra (A. esculentus L.) and are considered to be responsible for okra leaf spot in the pot and thus it showed the same symptoms and it confirms Koch postulates..The cropping season (June - August) was marked with increased wetness, humid temperature with relative warmth that favoured rot development in susceptible cultivars

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