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Survey, Isolation of Soil Borne Pathogenic Fungi (Fusarium wilt) from Tomato Cultivated Areas

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A survey was conducted in Coimbatore district of Tamil Nadu to assess the incidence of Fusarium wilt disease in tomato growing areas. The maximum wilt disease incidence of 75.0% was recorded in Nachipalayam village of Coimbatore District. Pathogen was isolated and Fusarium wilt causing pathogen was identified as *F. oxysporum* f. sp. *lycopersici*. Pathogenicity of the primarily isolated fungi was proved by showing external symptoms (wilting and yellowing leaves) while transplanting the tomato seeding into pathogen inoculated pots.

Key words: Survey, Isolation, Pathogenicity, *F. oxysporum* f. sp. *lycopersici*.

Tomato (*Lycopersicon esculentum*) is one of the most popular and important commercial vegetable crops grown throughout the world. It is rich in vitamins A, B and C. In India, it occupies an area of 0.54 million ha with a production of 7.60 million tonnes [1]. Many diseases and disorders can affect tomatoes during the growing season. *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*) is a highly destructive pathogen caused wilted plants, yellowed leaves and minimal or absent crop yield. There may be a 30 to 40% yield.[2] Intially only one side of a leaf midrib, one branch, or one side of a plant will be affected. The symptoms soon spread to the remainder of the plant. To minimize losses from Fusarium wilt, it is advisable to plant resistant varieties or by the usage of biocontrol agents. This publication describes the symptoms and isolation of *FOL* pathogen for evaluating biocontrol agent for further greenhouse and field level control.

MATERIAL AND METHODS

Survey and sample collection

To assess the incidence of Fusrium wilt disease in tomato field, survey was conducted during the year 2007 in major tomato growing area in Coimbatore district of Tamil Nadu. In each village, four farmer's fields were selected and four

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plots have an average area of ten square meters each was selected randomly. Plant showing symptoms of wilt were identified and the percent disease incidence was calculated based on

$$\text{Disease Incidence (DI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

The infected plants were collected in a sterile bag from the field to the laboratory. Then it was used for the isolation of pathogens.

Isolation of plant pathogenic fungi

Direct isolation of the plant pathogen from roots or other plant parts is the most reliable way to detect many fungal pathogens. In the laboratory conditions, the infected plant material was washed several times under running tap water. Surface sterilization of root and leaf material was done by dipping in a 1% sodium hypochlorite/ 50% ethanol solution for 3 min and washed with three changes of sterile distilled water. The infected material was then dried between sheets of sterile blotting paper. Small segments was inoculated in a Potato dextrose agar medium (PDA) and incubated at room temperature (28°C) for 5 days. The culture was purified by single spore isolation and maintained in PDA slants[3].The salts were stored at 4°C until use.[4]

Pathogenicity screening test

For testing pathogenicity, the respective isolates of *FOL,* were multiplied on sand maize medium.[5] Sand and ground maize seeds were mixed in the ratio of 20:1, moistened to 50% moisture content was filled in 500ml conical flask and autoclaved for 2 hrs. The pure culture was inoculated and incubated at room temperature for 14 days. The prepared substrate was mixed with sterilized soil and infested with ten days prior to transplanting the 30-day-old seedling transplantation. Beginning a week after inoculation, external symptoms of Fusarium wilt (wilting and yellowing leaves) were assessed. Fusarium wilt was confirmed by sampling a 5-mm-long hypocotyl segment. Seedlings of 7-10 days after infection were washed free of vermiculite. Then surface sterilized small section was plated on water agar plates (2%) containing 0.1mg/ml streptomycin and incubated at 25°C for 3-4 days. The spores and the disease condition were identified.[6]

RESULTS AND DISCUSSION

A survey was conducted to assess the intensity of Fusarium wilt disease Coimbatore District of Tamil Nadu. The present study revealed that maximum disease incidence of 75.0% was recorded in Nachipalayam village and next 63.6% in Vadakkipalayam village. Wilt disease incidence in remaining villages survived was found to be minimum. (Plate 1; Table 1)

In the present study, the infected plant material collected from the Nachipalayam village was collected and plated in a PDA and incubated at RT for 5 days. The pathogenic colonies raised were subcultured in a PDA slant. The isolated pathogen was identified. The micro conidia and macro conidial structures showed that it was belonging to *F. oxysporum* f. sp. *lycopersici*.

Pathogenicity of primary isolated culture was proved by external symptom of Fusarium wilt (wilting and yellowing leaves) by transplanting tomato seedling in to *F. oxysporum* f. sp. *lycopersici* inocuclated pot. (Plate 2) The pathogenicy was proved as per standard method and the pathogen was found to be more virulent. Fusarium wilt was confirmed by sampling a 5-mm-long hypocotyl segment on water agar plates (2%) Ramamoorthy[7] reported the F. *oxysporum* f. sp. *lycopersici* in associated with tomato and same symptoms were expressed during his investigation. Browning of vascular tissue was a strong evidence of fusarium wilt. The pathogenicity study was supported by Juliano & Wagner[8]. Further invitro, invivo experiments (Green house and field application) would be necessary to assess antagonistic strain against *FOL* will now be undertaken.

Table 1. Survey for the occurrence of wilt disease incidence of tomato

S. No	Sampling filed	Disease incidence (%)
1.	Kinathukkadavu	15.5
2.	Nachipalayam	75.0
3.	Perur	11.0
4.	Vadakkipalayam	63.6
5.	Arasampalayam	53.8
6.	Arisipalayam	16.3

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