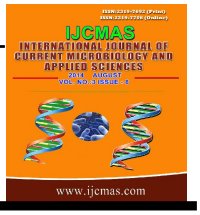
Int.J.Curr.Microbiol.App.Sci (2014) 3(8) 854-858

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 8 (2014) pp. 854-858**
http://www.ijcmas.com



Original Research Article

Study of wilt producing *Fusarium* sp. from tomato (*Lycopersicon esculentum* Mill)

Patel Harikrushana[1], Suthar Ramchandra[2] and K.R.Shah[2*]

[1]P.S.Science & H.D. Patel Arts College, Kadi, S.V.Campus, Gujarat, India
[2]Department of Biotechnology, H.D. Patel Arts College, Kadi, S.V.Campus, Gujarat, India
**Corresponding author*

A B S T R A C T

There are many possible causes of wilting of tomato plants. Successful treatment of the problem depends on accurate diagnosis and appropriate preventive measures. *Fusarium* wilt is caused by a fungus, *Fusarium*, that enters the plant through the roots and grows up through the vascular tissue. The fungus destroys cells of the vascular tissue, causing starvation in nearby branches of the plant. Disease development is favored by warm temperatures, dry weather, acidic soil and root-knot nematodes. A total of 36 *Fusarium* isolates were recovered from 108 samples collected from different geographic regions of Kadi. This study was based on both morphological and molecular levels that confirmed *Fusarium equiseti* is also pathogenic agent for tomato wilt.

Keywords

Tomato (*Lycopersicon esculentum* Mill.), Wilt, Fusarium sp

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of economically the most important vegetable crops in India, where it is grown both, indoors and outdoors on an area of about 20,000 ha in total. A number of economically important tomato diseases caused by fungi are transmitted by seed or transplants. Tomatoes are parasitized by a number of pathogens, including *Fusarim oxysporium* f. sp. *lycopersici* (Sacc.) (W.C. Snyder *et al.*,2003) the causal agent of fusarium wilt, which is one of the most important species as tomato pathogen (Jones *et al*., 1982; Agrios, 1988; Smith *et al*., 1988). An indoor environment due to high temperature and humidity, *F. oxysporum* f.sp. *lycopersici* can cause significant damage. The causal agent of *Fusarium* wilt is soil borne pathogen which can persist many years in the soil without a host. Most infections originate from the population associated with infected tomato debris. Healthy plants can become infected by *F. oxysporum* if the soil in which they are growing is infested with the pathogen (Farr *et al*., 1989). However, pathogenic fungi toof the genus *Fusarium* that is the causal agents of tomato wilt cause root and basal stem deterioration and result in the wilting of vegetable plants. Browning of the vascular tissue is strong evidence of *Fusarium* wilt (Snyder and Hans, 2003).

Materials and Methods

Wilted plants of tomato were collected from different areas of Kadi Taluka at Gujarat state of India. Isolation of fungus was done from the diseased portions of tomato plants. Infected leaves and stem portions of tomato were collected from cultivated field of North Gujarat. Infected stem and leaves sections were surface-sterilized for 3 min with a 0.01% Hgcl2 solution, rinsed twice in sterile distilled water and dried in a laminar flow cabinet. Potato Dextrose Agar (PDA) was used for fungal isolations. The plates were incubated at 25ºC in incubator for 5-7days. *Fusarium* isolates were subcultured on PDA, using a single spore technique (J. F. Leslie and B. A. Summerrell.2006). Cultural characters were observed by eye and microscopic examination. Colony morphology was observed from PDA plates. Morphological identifications of isolates were made using the criteria of Gerlach and Nirenberg.1982; J. F. Leslie and B. A. Summerrell.2006**.**

Microscopic Observation

To confirm the exact identify of pathogen morphological studies of the mycelium and conidiospores were done by measuring the length and breadth of the spore under high power magnification using ocular micrometer. Photographs were taken by attached camera with light microscope.

DNA Extraction

Fusarium isolate was grown on PDA plates for 7 days and mycelia were harvested. Total DNA was extracted from ground mycelium of isolate (~100 mg wet weight) using a Genei fungal DNA extraction Kit (Genei, India) according to the manufacturer's instructions done by Agarkar Research institute, Pune.

BLAST

The approach for identifying an isolate of *Fusarium* using the FUSARIUM-ID database it involves obtaining a pure culture of an isolate, extracting genomic DNA, amplification of the TEF gene region, and sequencing. BLAST is then used to identify the closest matches between the unknown sequence and those contained in the FUSARIUM-ID sequence database.

Results and Discussion

One hundred and eight (108) wilted plants were collected from different areas of Kadi. Total of 36 tomato fields in Kadi taluka were sampled during disease season. Each field was arbitrarily divided into five circular plots approximately 100 m in diameter and two to four samples were randomly taken from each plot. Samples were pooled in each field and two infected plant from each field were selected and used for pathogen isolation. A total of 36 *Fusarium* isolates were recovered from 108 samples collected from different geographic regions (Table-1).

Morphological Identification

Isolations from the leaves and stems of diseased plants yielded fungus is characterized by the development of abundant white aerial mycelium which turns pinkish by keeping in daylight on Potato Dextrose Agar (PDA) (figure-1 & 2). The colonies produced macro- and micro-conidia within 3-4 days at 25±2°C.

Table.1 Sample Collection from Different Geographic Regions

Sr. NO.	Village	Field	No. of sample
1	Nani Kadi	4	12
2	Sujatpura	4	12
3	Narsinhpura	3	09
4	Kasva	4	12
5	Vidaj	3	09
6	Balasar	4	12
7	Rangpurda	4	12
8	Borisana	4	12
9	Sadara	3	09
10	Daran	3	09
Total	**10**	**36**	**108**

Figure.1 Growth of *Fusarium* on PDA

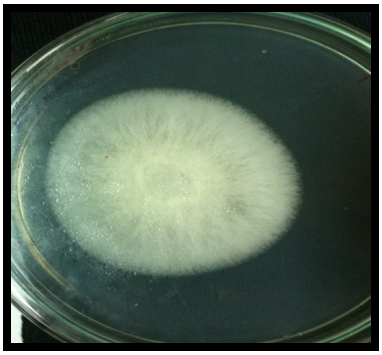


Figure.2 *Fusarium* mycelia

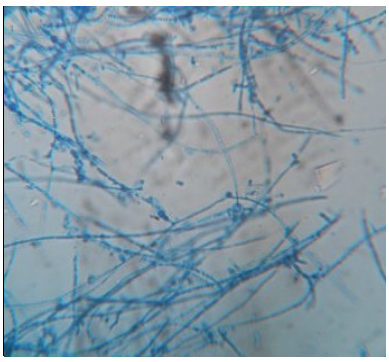


Figure.3 Observation of *Fusarium* conidia under Hemocytometer

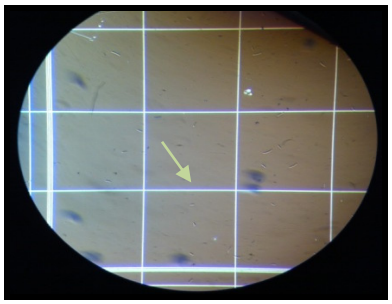
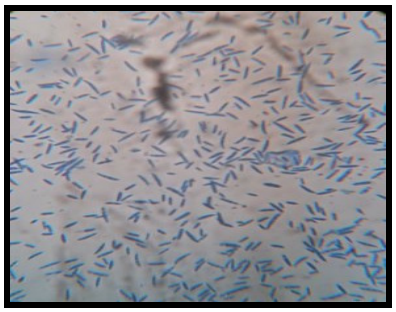


Figure.4 *Fusarium* conidia (45X)



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Acknowledgement

The authors are grateful to Mycology and Plant Pathology Group Agharkar Research Institute, Pune for identification of isolate. The authors are also grateful to Sarva vidayalaya kelavani mandal, kadi and K.S.V. University, Gandhinagar for financial support to project and research.

References

Agrios, G. N. (1988). “Plant Pathology.” 3rd. ed. Academic Press, Inc., New York, pp. 1-803**.**

Farr, D. F., Bills, G. F., Chamuris, G. P. and Rossman, A. Y. (1989). “Fungi on Plants and Plant Products in the United States.” *APS PRESS: St. Paul, USA*, pp. 1-1252**.**

Gerlach, W. and Nirenberg, H. (1982). "The genus *Fusarium*: a pictor ial at las." Mi t tei lungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany.

Jones, J. P., Jones, J. B. and Miller, W. (1982). "*Fusarium* wilt on tomato." Fla. Dept. Agric. & Consumer Serv., Div. of Plant Industry. *Plant Pathology Circular* No. 237.

Leslie, J. F. and Summerell, B. A. (2006). **"**The *Fusarium* Laboratory Manual." Blackwell Publishing, Ames, IA, USA. 388 pp.

Ramchandra , S. Suthar and Bhatt, P.N. (2011). Morphological and molecular identification of Fusarium isolated from cumin wilt. Internat. J. Plant Protec., 4(2): 359-362.

Ramchandra , S. Suthar and Bhatt, P.N. (2012).First report of fusarium equiseti causing vascular wilt of cumin in india. Plant Dis., 96: 1821-1821.1

Smith, I. M., Dunez, J., Phillips, D. H., Lelliott, R. A. and Archer, S. A. (1988). "European Handbook of Plant Diseases." *Blackwell Scientific Publications, Oxford, UK*, pp. 1-583.

Snyder, W. C. and Hans, H. N. (2003). "*Fusarim oxysporium f. sp. lycopersici* (Sacc.) and. Prepared by Mui-Yun Wong." PP728 *Soilborne Plant Pathogen Class Project*, Spring.