



Original Research Article

Fusarial Wilt of *Solanum lycopersicum* L. (Tomato) at Panchgaon

Narendra Kumar* and Swati Sharma

Amity Institute of Biotechnology, Amity University Haryana, Manaser-122413,
Gurgaon, Haryana (India)

*Corresponding author

ABSTRACT

Keywords

Fusarial Wilt,
Solanum lycopersicum
L. (Tomato)

Wilted plants of tomato were collected from Panchgaon. The isolation of fungi from rhizospheric soils, rhizoplane, infected stem and infected collar region of wilted tomato were done in order to know the dominance of the pathogen. Fungal species were isolated from the rhizosphere soils by serial dilution plate technique. In the present soil types a total of 19 fungal species belonging to 13 genera and two mycelia sterile were isolated. The *Fusarium* showed maximum percent occurrence in rhizosphere soil (73.1) and minimum in rhizoplane (40.1) while *Fusarium oxysporum* showed 58.6 per cent occurrence in infected stem and 51.6 in collar region on the basis of percent occurrence. The pathogenicity of this dominant pathogen confirmed that this is responsible for tomato wilting causing similar wilting symptoms.

Introduction

Tomatoes are parasitized by a number of pathogens, including *Fusarium oxysporum* Schlecht. f. sp. *Lycopersici* (Sacc.) W.C. Snyder et H.N. Hansen, the causal agent of fusarium wilt of tomato, which is one of the most important species as tomato pathogen (Agrios, 1988; Smith *et al.*, 1988). In an indoor environment due to high temperature and humidity, *F. oxysporum* f.sp. *lycopersici* can cause significant damage. It is a soil borne pathogen in the class Hyphomycetes that causes wilt of tomato as the only host of pathogen (Rai *et al.*, 2011). Singh and Kamal (2012) reported 10–90% loss in yield of tomato in temperate region due to this disease. Healthy plants can become infected by pathogen if the soil in which they are

growing is contaminated with the fungus.

Since no systematic work on tomato wilting has been done at Panchgaon so wilted samples were brought in Laboratory in order to find out causal organism for tomato wilting. So far that dominant fungal species and their pathogenicity were studied.

Materials and Methods

Survey of Panchgaon for severity of disease

A thorough survey of tomato growing spots of Panchgaon were carried out in different months (March to June) from 2014 to 2015

to find the symptoms and severity of wilted plants of tomato. The natural diseased plants showing the typical symptoms of disease were selected from the field.

Soil preparation and collection

The rhizospheric soil samples were collected by digging out soil roots of tomato plants. Randomly selected 5 diseased plants growing in one kilometer area were uprooted carefully and the excess of soil was removed by gentle shaking and the soil adhering to roots formed composite samples. The collected samples were placed in presterilized polyethylene bags and kept at 4°C in the laboratory until processed. Five such samples were collected from each diseased spot and mixed together to prepare a single polyethylene bag.

Mycobiota analysis of rhizospheric soils, rhizoplane, infected stem and infected collar region of wilted tomato

Fungal species were isolated from the rhizosphere soils by serial dilution plate technique. A known amount (10g) of material was suspended or agitated in a known volume of sterile water blank (90ml so as to make the total volume to 100ml) to make a microbial suspension. Serial dilutions 10^{-2} , 10^{-3} , ... 10^{-7} were made by pipetting measured volumes (usually 10ml) into additional dilution blanks (having 99ml or 90ml sterile water). Finally 1ml aliquot of various dilutions were added to sterile Petri dishes (triplicate for each dilution) to which were added 15ml (approximately) of the sterile, cool, molten (45°C) media. Potato Dextrose Agar media was used for mycobiota analysis and incubated at $28 \pm 2^\circ\text{C}$ for 2–7 days.

Per cent occurrence of each fungal species associated with root sample was calculated as per formula-

Percent occurrence = $\frac{\text{Number of colonies of a particular fungus}}{\text{Total number of colonies of all the fungi}} \times 100$

Identification of fungi

Fungal identifications were confirmed on following keys and description given by Raper and Thom (1949), Gilman (1967), Raper and Fennell (1965), Booth (1971) and Ellis (1971, 1976).

When *Fusarium* species is present, the isolate was grown on Synthetic Nutrient-Poor Agar (SNA) to analyse the shape of macroconidia and microconidia. Identification was done by comparing the morphology with the atlas of *Fusarium* (Leslie and Summerell, 2006; Samson *et al.*, 2008). A single spore of *Fusarium oxysporum* was used for further test (as master isolate).

Pathogenicity trials

Twenty healthy plants of tomato were selected from field. A cut was made in the stem of 15 healthy plants with the help of a sterilized knife and inoculated with 1×2 cm block of the isolated culture of *Fusarium oxysporum* isolated from naturally infected diseased plants in the field. Following this method a cut was made in the remaining five healthy plants to serve as a control and inoculated with 1×2 cm block of only PDA and wrapped with parafilm. Plants were monitored for the development of disease symptoms and pathogens were reisolated from stem of the test plants after 25 days to confirm the pathogenicity.

Results and Discussion

The tomato plant showed yellowing of the leaves (Figure 1), often on only one side of the plant. The symptoms appeared on older plants during May to June months of year

2014-15 that was mid-growing season under warm weather conditions. One of the typical signs of the disease was leaf chlorosis. The diseased leaves wilted and dried up (Figure 1 & 2).

When the epidermis and cortical tissue (bark) on a section of the main stem, slightly above the soil line, is cut and peeled back, a distinct brown discoloration of the vascular tissue is evident. The discoloration can extend from the roots up the stem through the branches and into the petioles of the plant. A cross-section of the stem revealed necrosis of the vessels.

In the present soil types a total of 19 fungal species belonging to 13 genera and two mycelia sterile were isolated (Table 1). Among total 19 fungal species, 8 species were *Aspergillus*, 1 species each of *Fusarium*, *Colletotrichum*, *Cladosporium*, *Alternaria*, *Curvularia*, *Humicola*, *Penicillium*, *Paecilomyces*, *Rhizopus*, *Trichoderma*, *Mycelia sterilia black*, *Mycelia sterile white*. The species of *Aspergillus* isolated were *A. flavus* Link, *A. fumigatus* Fresenius *A. niger* van Tieghan, *A. ochraceus* K. Wilh. *A. sulphureus* (Fresen.) Wehmer, *A. tamari* Kita *A. versicolor* (Vuillemin) Tiraboschi. The species of *Aspergillus* showed per cent occurrence in the range of 0.2–14.0. *Alternaria solani* Keissler was in the range of 0.3–4.3, minimum in rhizosphere soil and maximum in rhizoplane on the basis of per cent occurrence. *Cladosporium cladosporioides* (Fres.) de Vries was in the range of 2.0–3.9 in different districts. The species of *Fusarium* isolated were *F. oxysporum* Schl Saccardo. The fungus *F. oxysporum* Schl are highly alarming on the basis of percent occurrence. The *Fusarium* showed maximum percent occurrence in rhizosphere soil (73.1) and minimum in rhizoplane (40.1) while *Fusarium oxysporum* showed 58.6 per cent occurrence

in infected stem and 51.6 in collar region on the basis of percent occurrence. The *Trichoderma viride* Persand Fris showed lowest percent occurrence so its low per cent occurrence is highly alarming because of being a biocontrol agent. It is visible that fungus invades plant with its sporangial germ tube or mycelium by invading the plant's roots (Figure 3 & 4). The mycelia of *Fusarium oxysporum* are delicate white and are sparse to abundant. The fungus showed three types of spores: microconidia, macroconidia and chlamydo spores. Microconidia are borne on simple phialides arising laterally and are abundant, oval-ellipsoid, straight to curved, 5-12 x 2.0-3.3 µm, and nonseptate. Macroconidia, sparse to abundant, are borne on branched conidiophores or on the surface of sporodochia and are thin walled, three- to five-septate, fusoid-subulate and pointed at both ends, have pedicellate base. Three-septate conidia measure 25-45 x 3-4 µm while five-septate conidia measure 31- 56 x 3-5 µm. Three-septate spores are more common. Chlamydo spores, both smooth and rough walled, are abundant and form terminally or on an intercalary basis (Figure 5 & 6). This can be placed as –Domain: Eukaryota; Kingdom: Fungi; Phylum: Ascomycota; Class: Ascomycetes; Subclass: Sordariomycetidae; Order: Hypocreales

It was found that during pathogenicity *Fusarium oxysporum* showed similar wilting symptoms in all 20 inoculated plants while 5 uninoculated plants remained healthy. On account of wide occurrence and their pathogenicity these were selected as test organisms.

The disease caused by this growth of tomato wilt pathogens as well as their effect on fungus is characterized by wilted plants and yellowed leaves.

Table.1 Per cent occurrence of isolated fungi from rhizospheric soils, rhizoplane, infected stem and infected collar region of wilted tomato

Fungal species	Per cent occurrence of isolated fungi			
	rhizosph ere soils	rhizoplane	infected stem	infected collar region
<i>Aspergillus aculeatus</i> Iizuka,	1.9	1.3	1.4	1.5
<i>A.flavus</i> Link,	3.1	3.3	0.2	1.4
<i>A.fumigatus</i> Fresenius	1.9	2.5	3.1	2.9
<i>A.niger</i> Van Teighmer	3.0	2.2	2.3	14.0
<i>A.ochraceous</i> K. Wilh.	3.7	4.1	1.5	1.5
<i>A.sulphureous</i> (Fresen.) Wehmer,	2.1	2.2	3.2	1.6
<i>A.tamari</i> Kita	1.4	1.5	3.5	3.6
<i>A.versicolor</i> (Vuillemin) Tiraboschi.	2.5	3.6	1.2	1.7
<i>Alternaria solani</i> Keissler	0.3	4.3	2.1	2.1
<i>Cladosporium cladosporioides</i> (Fres.) de Vries,	3.9	2.0	3.5	3.0
<i>Colletotrichum</i> sp.	10.7	14.4	16.1	14.3
<i>Curvularia lunata</i> (Wakker) Boedijn var	3.1	4.1	6.0	3.6
<i>Fusarium oxysporum</i> Schl	73.1	40.1	58.6	51.6
<i>Helminthosporium oryzae</i> Breda de Hann	0.5	6.9	5.3	4.1
<i>Humicola grises</i> Omvik	10.0	11.4	13.4	14.1
<i>Paecilomyces</i> sp	0.5	10.5	11.5	12.0
<i>Penicillium oxalicum</i> Currie & Thorn	6.8	3.9	2.5	4.1
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	11.5	13.4	12.4	11.4
Sterile mycelium black	9.5	10.4	11.4	13.4
Sterile mycelium white	0.5	1.5	0.5	1.0
<i>Trichoderma viride</i> Persand Fris	0.0	0.5	0.5	0.0

Figure.1 Yellowing of leaves of tomato



Figure.2 Wilting of entire plant



Figure.3 Root along with root hairs



Fig.4 Colonisation of *Fusarium* on root hairs kept on medium

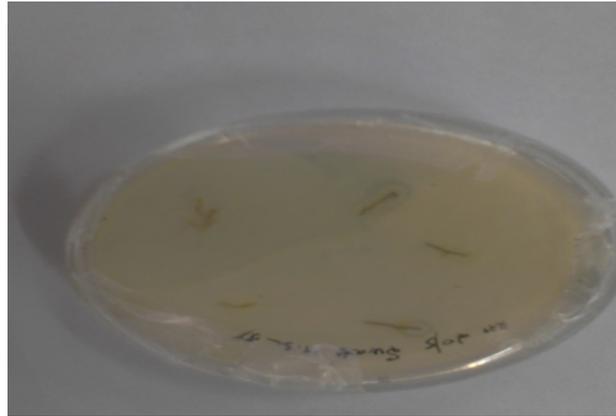


Figure.5 Culture of *Fusarium oxysporum*



Figure.6 Spores of *Fusarium oxysporum*



Kapoor (1988) highlighted that Tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is a typical example and is a serious disease wherever the crop is grown in India. Among the fungal diseases of tomato, Wilt of tomato caused by *Fusarium oxysporum* f.sp.*lycopersici* (sacc.) is one of the most destructive diseases all over the world (Beckman, 1987). This pathogen invades through wounds on roots. Infected plants become stunted, chlorotic and wilt (Jones *et al.*, 1991). *Fusarium* wilts of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) W. C. Snyder & Hansen is one of the most economically important and widespread diseases of the cultivated tomato (*Solanum lycopersicum* L.). It is one of the most important diseases which are highly destructive to tomatoes grown in greenhouse and in the field in many warm regions of the world, where it causes 10-50 % yield loss (Larkin and Fravel, 1998; Borrero *et al.*, 2004)

Ailton *et al.* (2005) recorded that *Fusarium* wilt, caused by three races of *Fusarium oxysporum* f. sp. *lycopersici*, is one of the most important diseases of tomato (*Lycopersicon esculentum*). Races 1 and 2 are distributed worldwide whereas race 3 has a more limited geographic distribution with no report thus far in Brazil. Seven *F. oxysporum* isolates were obtained from wilted tomato plants of race 1 and 2-resistant hybrids 'Carmen' and 'Alambra' in Venda Nova do Imigrante (State of Espírito Santo), Brazil. Recently Maja *et al.* (2012) found *Fusarium oxysporum* as Causal Agent of Tomato Wilt and Fruit Rot. Similarly in present investigation similar chlorosis and wilt symptoms were observed.

In conclusion, based on the findings of this investigation, it may be concluded that population of *Fusarium oxysporum* in terms of percent occurrence is increasing in soil

and this is responsible for wilting of tomato (*Lycopersicon esculentum*). This is present in rhizospheric soils, rhizoplane, infected stem and infected collar region of wilted tomato.

Acknowledgements

The authors are thankful to the Director, Amity Institute of Biotechnology Amity University Haryana for providing Laboratory facilities.

Reference

- Agrios, G.N. 1988. Plant pathology, 3rd edn. Academic Press Inc., New York. Pp. 1–803.
- Ailton Reis, Hécio Costa, Leonardo S. Boiteux, Carlos A. Lopes, 2005. First report of *Fusarium oxysporum* f. sp.*lycopersici* race 3 on tomato in Brazil. *Fitopatol. bras.*30;4.<http://dx.doi.org/10.1590/S0100-41582005000400017>
- Beckman, C.H. 1987. The nature of wilt diseases of plants. The American Phytopathological Society. St. Paul. Pp. 174.
- Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, 237 Pp
- Borrero, C., Trillas, M.I., Ordales, J., Tello, J.C., Aviles, M. 2004. Predictive factors for the suppression of *Fusarium* wilt of tomato in plant growth media. *Phytopathology*, 94: 1094–1101.
- Ellis, M.B. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. 608 Pp.
- Ellis, M.B. 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew. Surrey,

- England
- Gillman, J.C. 1967. A manual of soil fungi. Oxford and JBH publishing co. Calcutta. India
- Jones, J.B., Jones, J.P., Stall, R.E., Zitter, T.A. 1991. Compendium of tomato diseases. St. Paul, MN, USA, APS Press.
- Kapoor, I.J. 1988. Fungi involved in tomato wilt syndrome in Delhi, Maharashtra and Tamil Nadu. *Indian Phytopath.*, 41: 208–213.
- Larkin, R.P., Fravel, D.R. 1998. Efficacy of various fungal and bacterial biocontrol organisms for the control of *Fusarium* wilt of tomatoes. *Plant Dis.*, 82: 1022–1028.
- Leslie, J.F., Summerell, B.A. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Iowa.
- Maja Ignjatov, Dragana Milošević, Zorica Nikolić, Jelica Gvozdanić-Varga, Dušica Jovičić, Gordana Zdjelar, 2012. *Fusarium oxysporum* as causal agent of tomato wilt and fruit rot. *Pestic. Phytomed. (Belgrade)*, 27(1): 25–31. doi: 10.2298/PIF1201025I.
- Proceedings of International ISHS-ProMusa Symposium on Global Perspectives on Asian Challenges, Guangzhou, China, 14-18/09/2009. Acta Horticulturae 897. ISHS, Leuven, Belgium.
- Rai, G.K., Kumar, R., Singh, J., Rai, P.K., Rai, S.K. 2011. Peroxidase, polyphenol oxidase activity. protein profile and phenolic content in tomato cultivars tolerant susceptible to *Fusarium oxysporum f.sp.lycopersici*. *Pak. J. Bot.*, 43(6): 2987–2990.
- Raper, K.B., Fennell, D.I. 1965. The genus *Aspergillus*. Williams & Wilkins, Baltimore.
- Raper, K.B., Thom, C. 1949. A manual of the Penicillia. Williams Wilkins, Baltimore.
- Samson, R.A., Houbraken, J., Thrane, U., Frisvad, J.C., Andersen, B. 2008. Food and indoor fungi. CBS-KNAW Fungal Biodiversity Centre Utrecht. Netherlands
- Singh, A.K., Kamal, S. 2012. Chemical control of wilt in tomato (*Lycopersicon esculentum* L.). *Int. J. Horticulture*, 2(2): 5–6.
- Smith, I.M., Dunez, J., Phillips, D.H., Lelliott, R.A., Archer, S.A. (Eds.), 1988. European Handbook of Plant Diseases. Blackwell Scientific Publications, Oxford, UK. Pp. 1–583.