Pathological Survey and severity of tomato wilt disease incited by *Fusarium oxysporium f. sp. lycopersici* in different districts of Uttar Pradesh

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Abstract: Wilt disease of tomato (*Solanum lycopersicum*) is caused by *Fusarium oxysporum f. sp. lycopersici* and is a limiting factor to tomato production in India. (FOL) is the significant contributory pathogen of tomato vascular wilt. These diseases continue to present major challenges for production of this important crop world-wide. *F. oxysporum* is fungus pathogen widespread soil-borne plant pathogen. The initial symptoms of the disease appear in the lower leaves gradually, trail by wilting of the plants. A survey was conducted to investigate the incidence and severity of tomato wilt caused by *Fusarium oxysporum f. sp. lycopersici* in four major tomato growing districts of Uttar Pradesh that I had been selected for my study.

Keywords: *Fusarium oxysporum*, Vascular Wilt, Soil-born pathogen

Introduction

Tomato (*Lycopersicon esculentum L.*) is one of the most popular and important commercial vegetable crops grown throughout the world (Pritesh et al., 2011). It is second most important vegetable crop next to potato grown around the world. (Hadian et al., 2011). Easy adaptability to a wide ranges of climatic and soil conditions enable its worldwide cultivation. Many diseases and disorders can affect tomatoes during the growing season proving to be a major limiting factor for tomato production. These diseases can be classified into two groups. The first group includes those caused by non-infectious physical or chemical factors, such as adverse environmental factors, nutritional or physiological disorders and herbicide injury. Bacteria, fungi, viruses, nematodes and insects cause infectious disorders. Diseases are often a factor that limits production. Worldwide estimates of losses of fruits and vegetables have ranged as high as 50 percent (Winand et al., 1999). Soil and seed borne pathogens are important biotic constraints in sustainable crop production systems because the complexity of the soil environment makes their control with chemical difficult. Many crops throughout the world are host plants for Fusarium species causing soil-borne diseases of great economic importance. Fusarium wilts of tomato caused by Fusarium oxysporum f. sp. lycopersici (Sacc.) Synder and Hans occur worldwide and lead to high losses of tomatoes. It is primarily a soil inhabiting pathogen, once introduced, it remains in the soil for very long time and attacks tomato at all stages of its growth starting from nursery to fruiting. (Abdel-Monaim, 2012; Amini et al., 2010; Sheu et al., 2006). In the nurseries, it causes rotting of germinating seeds as pre-emergence damping off, rotting at collar region and dyeing of young seedlings as post-emergence damping off (Varma,1954). This pathogen invades through wounds on roots. Infected plants become stunted, chlorotic and wilt (Sally et al., 2006). Wilting is a result of impaired water translocation due to clogging of vessels by mycelium, spores, gels, gums and tylosis (Mui-Yun, 2003). The fungus also produces toxins such as fusaric acid and lycoparasmin which chelate with iron and cause iron deficiency in plants and consequently yellowing of leaves (Gaumann, 1951).

A survey was undertaken to assess the disease incidence variations of different variety of tomatoes infected by wilt disease caused by *Fusarium oxysporum f. sp. lycopersici*,. Percent disease incidence in major tomato growing districts of Jhansi, Prayagraj, Kanpur, Koshambi, Pratapgarh districts in Uttar Pradesh and the virulence of certain screened isolates.

Material and methods:

1. Isolation of pathogen

Isolation of Fusarium pathogen from infected tomato plant roots, collar region part of the plant was made. The infected plant material was washed with tap water to remove the surface soil and then washed again with Sterilize water. Now the sterilize pieces cut into small pieces (1-2mm) from the juncture of disease and healthy portion of roots, and collar region of the plant part with the help of sterilize blade. These small pieces were surface sterilized with 0.1% Mercuric chloride solution by dip for 10-20 seconds and then washed thrice with sterilized distilled water at the time of plating in growth chamber. These small pieces were then placed on sterilize filter paper to remove the excess moisture. The sterilized pieces were inoculated in petri plates containing sterilize potato dextrose (PDA) agar medium. 30 mg Streptocycline per liter medium was added before pouring the medium in petri plates.

2. Identification of Pathogen

The inoculated plates were labeled and incubated at $28^{O}C \pm 2^{O}C$ for 72 hours. Sub- culturing based on different cultural characteristics (color of culture, growth rate, sporulation rate and color etc.) was done by picking little pinch of mycelial mass from isolates and inoculating into new petri-dishes containing sterilized PDA with the aid of a

sterilized inoculating needle and was incubated at $28^{O}C \pm 2^{O}C$. After 7 days, a second tier sub-culturing of the hyphal ends (Hyphal tipping) of each fungal isolate was done to ensure that the cultures obtained were pure. Identification was done based on morphological and cultural characteristics exhibited by the various fungal isolates and comparing with the description of Barnett and Hunter (1998).

Symptoms of Fusarium oxysporum and disease development: Fusarium wilt caused by the soil borne fungus Fusarium oxysporum f. sp. lycopersici was formerly the most prevalent and damaging disease of field tomatoes. Symptom commonly found throughout the, Fusarium wilt is a fungal disease that attacks tomato, disease fungi Fusarium oxysporum, enter through the roots and interfere with the water conducting vessels of the plant. As the infection spreads up into the stems and leaves it restricts water flow, causing the foliage to wilt and turn yellow. Symptoms often appear later in the growing season and are first noticed on the lower (older) leaves. As the disease progresses, the younger leaves will also be affected and the plant eventually dies. In many cases, only one branch or sides of the plant show symptoms. Disease symptoms appear on the lower leaves as yellow blotches, wilting and eventually dropping off (Agrios, 2005). The pathogens are soil borne and occur throughout most tomato growing areas and infected leaves start drooping, curve downwards and turn yellow. Disease symptoms are apparent during flowering and fruiting stages, and leaflets on one side of the plants typically show more severe symptoms than leaves on the other side because of the specific vascular tissue affected by the pathogen.

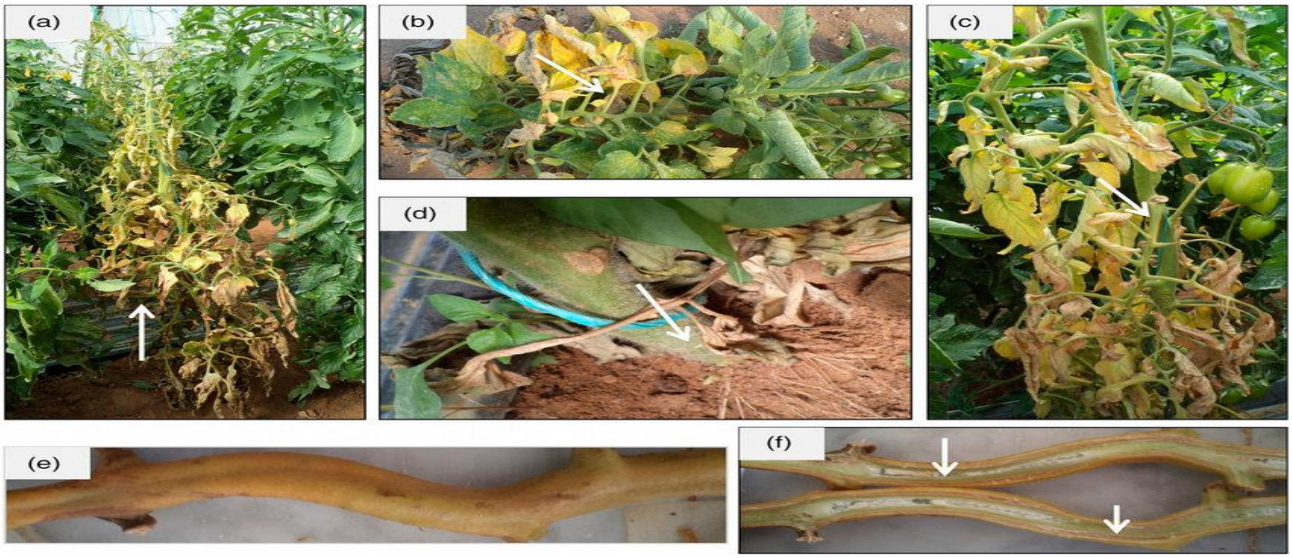


Figure 1 Fusarium wilt symptoms noted in tomato and sampled for FOL isolation. (a) Yellowing of bottom leaves. (b, c) Yellowing often on one side of the leaf, shoot or plant. (d) Free of crown and root rot signs. (e) Discoloured stems. (f) Dark brown vascular discoloration extending further up the longitudinal section of discoloured stem.

A random survey was conducted in major tomato growing areas of Jhansi, Allahabad, Kanpur and Pratapgarh districts in Uttar Pradesh and the virulence of certain screened isolates. Samples of soil and roots were collected from sufficiently wet fields from the rhizospheres of tomato. In the similar manner, totally 10 spots were selected randomly for taking infected soil and plant samples representing the whole field. Later from this, a composite sample of 200 g of soil and 5 g of root were formed. Randomly 100 plants were selected in different locations in a field and number of plants wilted was counted and the mean wilt incidence was expressed in percentage. Completely wilted plants were also collected. The per cent disease incidence was calculated by using the formula.

$$\text{Percentage of Disease incidence (PDI)} = \frac{\text{Total Number of Infected Plant}}{\text{Total Number of Plant Assesed}}$$

The grading of disease was done by following the disease scale 0-5 with few modifications, given by Winstead and Kelman (1952). The modified rating scale is given below:

The maximum rating scale (1-5) has been used for assessment of the disease severity is:

Rating Scale	Disease Percentage
1	1-5
2	5-25

3	25-50
4	50-75
5	75-100

3. Highly resistant (HR)

Plants did not show any wilt symptom Resistant (R): 1-20% plants wilted moderately resistant (MR): 21-40 % plants wilted moderately susceptible (MS): 41-60% plants wilted Susceptible (S): 61-80% plants wilted highly susceptible (HS): More than 80% plants wilted Categorization of genotypes in resistant to highly susceptible group was based on the percentage of wilt of the plants.

4. Pathogenicity test:

For pathogenicity test, conidial suspension of the pathogen was prepared (1×106 cfu ml-1) in a conical flask from cultures grown in potato dextrose broth with constant agitation (110 rpm) at 28°C for 7 days. Roots of four weeks old seedlings (three fully expanded true leaves) of susceptible cultivar Himsohna were immersed in the appropriate conidial suspension for 10 min, individually. Inoculated seedlings were transplanted into a pot containing 1 kg of sterilized soil, and grown in a greenhouse for 5 weeks. In control plants roots were dipped for 10 min in sterile water and were grown similarly. The inoculated seedlings showed typical symptoms of Fusarium wilt including yellowing, vascular necrosis and wilting after three weeks of inoculation.

STATISTICS AND DATA ANALYSIS

The common and important disease observations were surveyed and the disease incidence and severity were assessed randomly on tomato and pepper plants. Representative samples, based on visual symptoms of the disease were brought from each field at random as per methods described by the selected sites were approximately equal distance from each other along the sampling pathway.

The data have been collected from five different fields in sub zone and visited four times at an interval of three weeks in each field. All the collected data were subjected to analysis of variance at an end of the study by using GenStat Release 10.3 (2011) software application.

Results and Discussion

During the course of survey, the disease symptoms were observed in different polyhouse and protected fields - as yellowing of lower leaves, stunting growth, necrosis and wilting of the plant. The wilt symptoms moved upward with the gradual extension, discolouration of vascular tissue of root and stem and finally wilt affected plants ultimately kill or die due to multiplication of pathogen in the vascular system of plant. The culture of *Fusarium oxysporum f. sp. lycopersici* was white to pinkish in colour on PDA plate. Under microscope micro and macro types conidia were observed along with their septate hyaline mycelium. The isolated fungus was identified as *Fusarium oxysporum* on the basis of its colony, morphology and microscopic features given by Booth 1971.

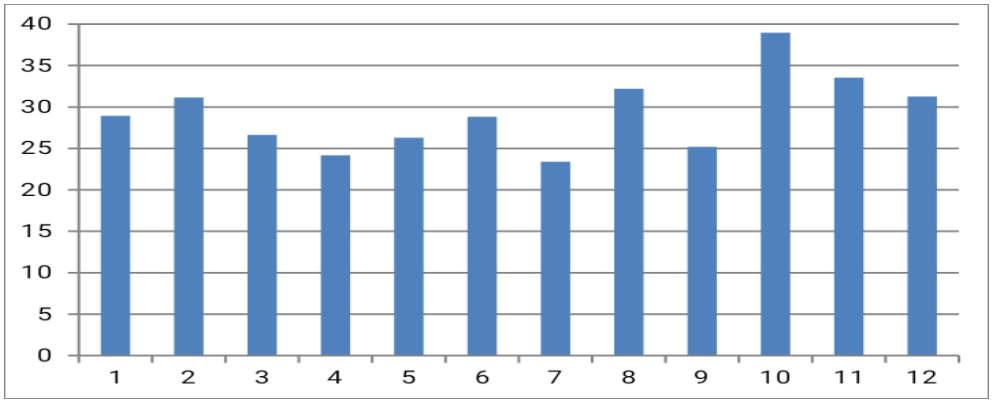
Survey and collection of wilt infected plant samples

An extensive survey was conducted in 12 selected locations of Pratapgarh, Jhansi, Kanpur and Prayagraj districts of Uttar Pradesh during 2019-20 (Table2 and Fig.1). On the basis of field symptoms infected plant samples were collected from the fields of villages adjoining to Block Headquarters of each district. For the calculation of disease incidence in each location, randomly 10 tomato plants were selected and total number of fruits and infected plant were counted.

Table 1: Incidence of Fusarium wilt of tomato grown under farmer field conditions in Uttar Pradesh, India during 2019-20

S. No.	Location/ Locality	Disease incidence per cent
A	District- Jhansi	
1	Parichha	28.93
2	Raksa	31.14
3	Bijoli	26.63
	Average	28.90
B	District- Allahabad	
1	Naini	24.17
2	Mau-ayama	26.29
3	Sorawan	28.82

	Average	26.42
C	District- Pratapgarh	
1	Uska	23.39
2	Basupur	32.20
3	Sarauli	25.19
4	Average	26.92
D	District- Kanpur	
1	Vegetable Farm, Kalyanpur	38.96
2	Mandhana	33.54
3	Choubaypur	31.26
	Average	34.58



Disease incidence of different area

Figure 2: Diseases incidence of Fusarium wilt of tomato (percent) grown under farmer field conditions in Uttar Pradesh, India during 2019-20.

The incidence of Fusarium wilts of tomato caused by Fusarium oxysporum f. sp. lycopersici (Sacc.) were recorded during the cropping season of 2019-20 in different tomato growing areas of four districts of Uttar Pradesh (Table 1).

Incidence of wilt was observed in almost all the locations surveyed and it ranged between 24.17 to 38.96 %. Overall incidence of Fusarium wilt in four districts surveyed was 34.58%. Disease incidence was maximum (38.96%) in district Kanpur out of four districts surveyed followed by district Jhansi (28.90 %). Amongst various localities surveyed highest incidence of the wilt was recorded at Vegetable Farm, Kalyanpur (38.96%) in district Kanpur followed by Mandhana (33.54%), whereas lowest incidence was recorded at Naini (24.17%) followed by Mau-ayama (26.29%) in district in district Prayagraj. Locality wise data indicated that the incidence of wilt was higher in comparatively warmer areas and also in the areas where tomato cultivation was continuously done under farmer field. The wilt incidence at locations in warmer regions in all the districts surveyed was 20% and above. Similar results have been reported by Alice, (1994).

Hence, the present study is new in this regard. However, if we see within country basis Kapoor (1988) has reported wide spread occurrence of this disease in different tomato growing states like Maharashtra, Tamilnadu, Bihar, Dehli etc. with losses between 10 to 80 per cent in different regions of the country. Continuous cropping of tomato round the year under field might be another reason for increased incidence of this disease under protected cultivation. Multiplication and spread of soil borne diseases especially *Fusarium* wilt is more when a specific crop is grown continuously (Charoenporn et al., 2010).

Conclusion

Under present investigation, wilt incidence was higher in comparatively warmer areas. *Fusarium wilt* is known to become serious in warmer areas because of the fact that this pathogen requires 25-31 ^{0}C soil temperature

for its development (Gupta and Thind, 2006). Prevalence of higher temperature is favorable for the development of wilt pathogen.

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Ethics & disclosures

Conflict of Interests

The authors have no financial or proprietary interest in the materials discussed in this article.

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