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Prevalence and Diversity of *Fusarium oxysporum* f. sp. *lycopersici* at Bhilwara Region of Rajasthan

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ABSTRACT: Tomato ranks third among the vegetable crops (*Lycopersicon esculentum*) with an annual productivity of 115.5 million tons but is constrained by wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici.* Hence a survey was conducted for fourteen locations at Bhilwara region. Results reveal that Bardod (L_1- 36.17) had the highest disease incidence in comparison to other location followed by Mangrop (L_6- 32.45). Further, variability of isolates were tested with respect to virulence, cultural and morphological characteristics. It was found that pathogenicity of isolate belonging to Bigod (84.67) showed best pathogenic virulence in comparison to Atoon (6.65) isolate having lowest pathogenecity. Morphological variability was studied by observing mycelium pattern, colour of mycelium and pigmentation on medium for isolates of *Fusarium oxysporum* f. sp. *lycopersici* collected from different locations. It shows that mycelium patterns were aerial fluffy, aerial cottony, slightly aerial to submerged and submerged on potato dextrose agar media. The mycelium colour variations were from dirty white to pure white and light pink to violet. Even the pigmentation on PDA was also distinguished from dirty white to white, pink, light violet to violet, light yellow.

The macro-conidia ranged from 16.8 – 37.9 × 4.06- 5.92 µm in size with septations ranging from 3-6 and micro-conidia ranged from 6.22 – 12.84 × 3.12 – 5.18 µm in size mostly non-septate or single septations.

Keywords: Tomato, *Fusarium oxysporum* f. sp. *lycopersici,* eco-friendly, bio-agents, botanicals.

INTRODUCTION

One of the most essential vegetable crops is the tomato (*Lycopersicon esculentum*), which is planted on 4.5 million hectares and yields 115.5 million metric tonnes globally. Indian agriculture produced 333.25 metric tonnes from an area of 27.56 million hectares, ranking it third globally in terms of yield and area. Numerous chronic conditions limit tomato production, causing in crop losses of 100%. In greenhouse and outdoor environments, *Fusarium oxysporum* f. sp. *lycopersici* (FOL) wilt is the most detrimental of all diseases. The average worldwide agricultural output lost due to disease was about 12.8%, whereas the tomato alone suffered a decline of 21.8 percent (Anonymous, TSFAO 2020). In particular, the management of these pathogenic fungus is still challenging due to the wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*, resulting in a significant financial loss. On a number of different host plants, *Fusarium* sp. causes a wide spectrum of illnesses. More than 150 hosts have been harmed by wilt disease, which is caused by the *Fusarium oxysporum* species and its several distinct previous species (Bertoldo *et al.,* 2015). The tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hansen (FOL), one that causes vascular wilt, was indeed the reason of the greatest yield reduction (Asha *et al.,* 2011). Many studies have revealed that there are cultural, morphological, and pathogenic variations in *Fusarium oxysporum* f. sp. *lycopersici* strains isolates from wilted tomato plants and soil samples collected from different tomato fields (Nirmaladevi and Srinivas 2012). *Fusarium oxysporum* f. sp. *lycopersici* shows cultural and morphological variations on potato dextrose agar, a semi-solid medium, and produces the best mycelium development. The isolates vary in terms of colony growth, mycelium bulk, macroconidia, and microconidia production (Sonkar *et al.*, 2013).

MATERIALS AND METHODS

The study entitled "Prevalence and diversity of *Fusarium oxysporum* f. sp. *lycopersici* at Bhilwara region of Rajasthan" was carried out during *Rabi* seasons in 2020-2022 where fourteen locations cultivating tomato having high prevalence of wilt

caused by *Fusarium oxysporum* f.sp. *lycopersici* were visited to record the incidence, plants showing peculiar wilt symptoms were collected as samples.The samples were brought to the Research laboratory, 28 isolates were isolated and identified. Isolates with similar character resemblance were discarded and further studies were carried out with 14selected isolates of *Fusarium oxysporum* f. sp. *lycopersici*. The samples were further tested for virulence at School of Agriculture Science and Technology, Sangam University, Bhilwara. *In vitro* studies were taken up to study the pathogenic, cultural and morphological variations.

To test the pathogenic variability, an experiment was conducted in field by inoculating the soil with different isolates to prepare wilt sick plot with fungal culture. Inoculum (5g. / Kg of soil) of *Fusarium oxysporum* f. sp. *lycopersici,* mass multiplied on sorghum seeds in 500 ml conical flasks, polypropylene bags and incubated at 28±2°C for 15 days was mixed in plot regularly to build the conidia inoculum potential, having C.F.U. of 2-3 × 10^7. Thereafter, healthy seedlings of tomato variety Pusa Ruby were disinfected (surface sterilization) with 2.5% Sodium hypo-chlorite solution and sown at the depth of 2-3 cm.

Studies for morphological variability was carried out on 11 isolates based on pathogenicity, by observing mycelium pattern, colour of mycelium and pigmentation on medium which showed the significant variations among isolates of *Fusarium oxysporum* f. sp. *lycopersici* collected from different locations.

To study the cultural variations, the isolates were further subjected to radial growth (mm.) obseravtions on potato dextrose agar and the colony growth was recorded at regular intervals of 48, 72, 96 and 120 hours after inoculation.

RESULTS AND DISCUSSION

Survey data reveals that Bardod [L_1- 36.17] had the highest disease incidence in comparison to other location followed by Mangrop [L_6- 32.45], Gadarmala (Pur) [L_{13}- 29.04], Sawaipur [L_{12}- 27.67], Ojayada (Hameergarh) [L_4- 25.17], Arani (Shahpura) [L_7- 23.43], Mandalgarh [L_3- 21.41], Kotari [L_2- 20.87], Bigod [L_5- 20.23], Bherukhera (Banera) [L_{11}- 19.28] and Patliyas [L_9- 18.97]. The lowest disease incidence percent was recorded at Atoon [L_{14}- 12.50] followed by Malola [L_{10}- 16.26] and Sardarpura [L_8- 18.10] of Bhilwara District.

From the data recorded for pathogenic variability, it reveals that highest disease incidence was recorded for 5 isolates. The highest wilt disease incidence percent in this category was recorded in FOL-5, Bigod (84.67) followed byFOL-3, Mandalgarh (80.55), FOL-8, Sardarpura (78.58), FOL-11, Bherukhera (Banera) (75.89) and FOL-1, Bardod (50.12). Since the isolates were highly pathogenic on the tomato plants they were categorizedto highly pathogenic category. Three isolates having pathogenic incidence between 21 – 50 percent were categorized as moderately pathogenic isolates.The highest wilt disease incidence percent in this category was recorded in FOL-6, Mangrop (48.95) followed by FOL-9, Patliyas (46.54) and FOL-10, Malola. Remaining six isolates having incidence below 30 percent were placed in weekly pathogenic category which includes FOL-2, Kotari (30.83) followed by FOL-4, Ojayada (Hameergarh) (29.55), FOL-7, Arani (Shahpura) (20.5), FOL-13, Gadarmala (Pur) (18.89), FOL-12, Sangam university campus (9.5) and FOL-14, Atoon (6.65)

Cultural variations for the isolates was studied with the help of radial growth (mm.) obseravtions on potato dextrose agar and the colony growth was recorded at regular intervals of 48, 72, 96 and 120 hours after inoculation and were categorized as Fast growing, moderate growing and slow growing isoloates. From the data, it shows that three isolates had the fastest radial growth was observed for FOL-3: Mandalgarh (90) followed FOL-8: Sardarpura (85.33) and FOL-11: Bherukhera (Banera) (84.67). Moderate radial growth was exhibited by FOL-5: Bigod (78.67), FOL-9: Patliyas(73.5) and FOL-10: Malola (70.33) whereas slowest radial growth was recorded for FOL-1: Bardod (67.83), FOL-4: Ojayada (65.17), FOL-6: Mangrop (63.5), FOL-7: Arani (Shahpura) (61.83) and FOL-2: Kotari (61.5).

Morphological variability was studied by observing mycelium pattern, colour of mycelium and pigmentation on medium for isolates of *Fusarium oxysporum* f. sp. *lycopersici* collected from different locations. It shows that mycelium patterns were aerial fluffy, aerial cottony, slightly aerial to submerged and submerged on potato dextrose agar media. The mycelium colour variations were from dirty white to pure white and light pink to violet. Even the pigmentation on PDA was also distinguished from dirty white to white, pink, light violet to violet, light yellow.

The macro-conidia ranged from 16.8 – 37.9 × 4.06-5.92 µm in size with septations ranging from 3-6 and micro-conidia ranged from 6.22 – 12.84 × 3.12 – 5.18 µm in size mostly non-septate or single septations.

Therefore, as per the data recorded it clearly shows that *Fusarium oxysporum* f. sp. *lycopersici* isolates have variations in terms of pathogenicity, cultural and morphological characteristics collected from different locations of Bhilwara which is a similar finding to Sonkar *et al.* (2013) where he studied the cultural and morphological characteristics of the tomato wilt (*Fusarium oxysporum* f. sp. *lycopersici*). The results showed that potato dextrose agar, a semi-solid medium, produced the greatest mycelium development. The isolates varied in terms of colony growth, mycelium bulk, macroconidia, and microconidia production. Regarding cultural and morphological traits, these variances represented each isolate. Similarly, Chopada *et al.* (2014) conducted an experiment utilizing 10 isolates of *Fusariumo xysporum* f. sp. *lycopersici* acquired from various tomato-growing regions in south Gujarat to examine the diversity. Studies revealed that they had morphological and cultural variations such as mycelial color, growth, dry mycelial weight, sporulation, conidial size, and chlamydospore production. Also, Khan *et al.* (2016) reported the prevalence of the tomato disease fusarium wilt in a few

districts in Uttar Pradesh. Three blocks and three tomato-growing villages were chosen for the survey from each district that was chosen. The Masauli block in the Barabanki district had the greatest incidence (80.34%), which was followed by the Arniya block in the Bulandshahr district with 74.5%. The Bamaur Block of Jhansi districts showed the lowest illness incidence at 10.67%. In all blocks of the chosen districts, the disease incidence ranged from 10.67 to 80.34%. Sivakumar *et al.* (2018) investigated for variation of twenty isolates of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) were in terms of cultural, morphological, and pathogenic characteristics. The sizes of the Chlamydospores, macro conidia, and micro conidia varied significantly among all of the isolates. The pathogenicity of the isolates of FOL, specifically 2, 4, 6, 9, 11, 12, 18, and 19, were extremely virulent when tested against susceptible types PKM1 and CO1, whereas remaining isolates were moderately virulent.

Table 1: Survey of Tomato Wilt Incidence in Bhilwara and adjacent districts.

Sr. No.	Location	Incidence (%)
L_1	Bardod	36.17^{a}
L_2	Kotari	20.87^{de}
L_3	Mandalgarh	21.41^{cd}
L_4	Ojayada (Hameergarh)	25.17^{bcd}
L_5	Bigod	20.23^{de}
L_6	Mangrop	32.45^{ab}
L_7	Arani (Shahpura)	23.43^{bcd}
L_8	Sardarpura	18.10^{def}
L_9	Patliyas	18.97^{def}
L_{10}	Malola	16.26^{ef}
L_{11}	Bherukhera (Banera)	19.28^{de}
L_{12}	Sawaipur	27.67^{abc}
L_{13}	Gadarmala (Pur)	29.04^{ab}
L_{14}	Atoon	12.50^{f}
C. D. (0.05)		**3.845**

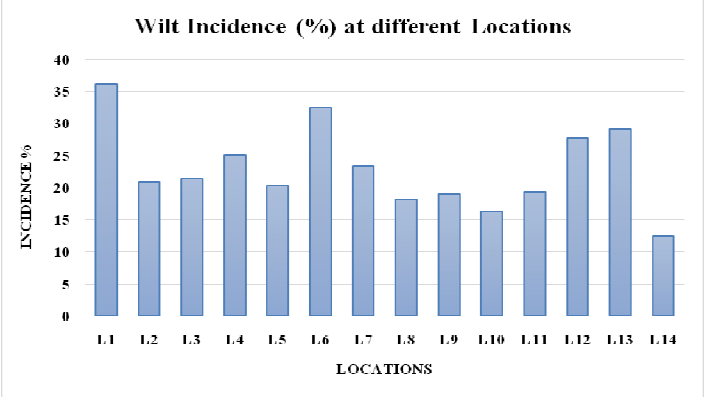

Fig. 1. Survey of Tomato Wilt Incidence in Bhilwara and adjacent districts.

Table 2: Categorization of isolates on the basis of pathogenic variability caused by *Fusarium oxysporum* f. sp. *lycopersici*.

Highly Pathogenic Isolate (Wilt incidence < 50%)			
Sr. No.	**Isolate Identity**	**Place of Collection**	**Wilt Incidence percent**
1.	FOL-3	Mandalgarh	80.55
2.	FOL-5	Bigod	84.67
3.	FOL-8	Sardarpura	78.58
4.	FOL-11	Bherukhera (Banera)	75.89
Total Highly Pathogenic Isolates			**4**
Moderately Pathogenic (Wilt Incidence 31- 50%)			
5.	FOL-1	Bardod	50.12
6.	FOL-6	Mangrop	48.95
7.	FOL-9	Patliyas	46.54
8.	FOL-10	Malola	38.25
Total Moderately Pathogenic Isolates			**4**
Weakly Pathogenic (Wilt Incidence >20%)			
9.	FOL-2	Kotari	30.83
10.	FOL-4	Ojayada (Hameergarh)	29.55
11.	FOL-7	Arani (Shahpura)	20.5
12.	FOL-12	SangamUniversity Campus	9.5
13.	FOL-13	Gadarmala (Pur)	18.89
14.	FOL-14	Atoon	6.65
Total Weakly Pathogenic Isolates			**6**

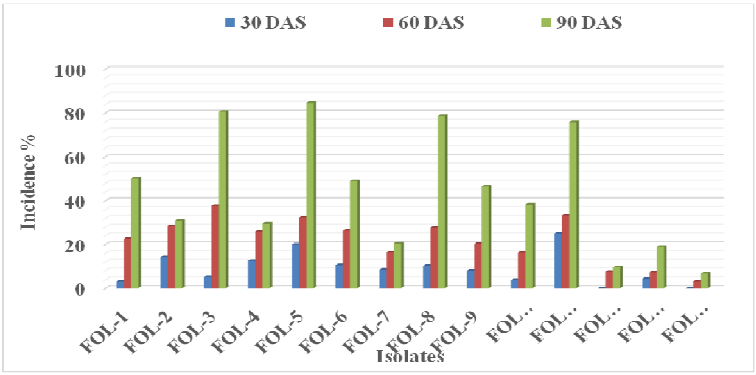

Fig. 2. Pathogenic variability of *Fusarium oxysporum* f. sp. *lycopersici* at 30, 60 and 90 DAT:

Table 3: Categorization of different isolates of *Fusarium oxysporum* f. sp. *lycopersici* on the basis of radial growth (mm.) variations collected from Bhilwara and adjoining locations.

Fast Growing Isolates		Moderate Growing Isolates		Slow Growing Isolates	
FOL – 3	90	**FOL – 5**	78.67	**FOL – 1**	67.83
FOL – 8	85.33	**FOL – 9**	73.5	**FOL – 2**	61.5
FOL – 11	84.67	**FOL – 10**	70.33	**FOL – 4**	65.17
Total No. of Isolates	**3**	**Total No. of Isolates**	**3**	**FOL – 6**	63.5
				FOL – 7	61.83
				Total No. of Isolates	**5**

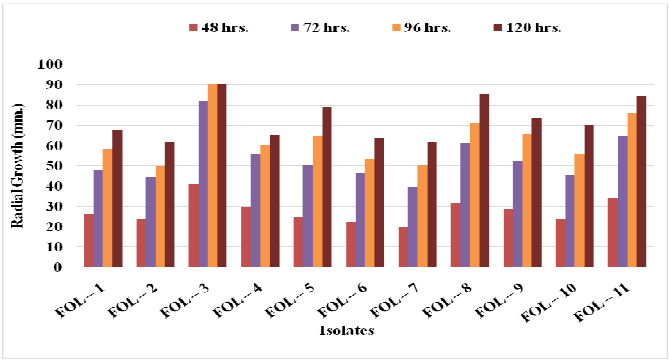

Fig. 3. Radial growth (mm.) of different isolates of *Fusarium oxysporum* f. sp. *lycopersici* collected from Bhilwara and adjoining locations.

Table 4: Cultural variability based on mycelial pattern, colour and pigmentation produced by *Fusarium oxysporum* f. sp. *lycopersici* collected from different locations.

Sr. No.	Isolate	Mycelium Color	Mycelium Pattern	Pigmentation
1.	FOL -1	Dirty white	Aerial, fluffy	Dirty white
2.	FOL -2	White	Aerial, fluffy	White
3.	FOL -3	Light Pink	Slightly aerial to submerged	Pink
4.	FOL -4	White	Submerged	Dirty white
5.	FOL -5	Pure white	Aerial, fluffy	White
6.	FOL -6	Light Pink	Aerial, cottony	Violet
7.	FOL -7	Violet	Submerged	Violet
8.	FOL -8	Violet	Slightly aerial to submerged	Violet
9.	FOL -9	Pure white	Aerial, fluffy	Light yellow
10.	FOL -10	Light Pink	Aerial, fluffy	Pink
11.	FOL -11	Pure white	Aerial, cottony	Light violet

Table 5: Morphological variability (conidial size and septations) of *Fusarium oxysporum* f. sp. *lycopersici* collected from different locations.

Isolate No.	Macro-conidia	Septations	Micro-conidia	Septations
FOL 1	26.4-33.6 × 5.52	2-6	7.20 - 12.84 × 4.32	0-1
FOL 2	19.2-27.6 × 4.80	2-3	8.16 - 11.76 × 4.08	0-1
FOL 3	24.0-28.8 × 4.06	2-3	7.20 - 12.24 × 4.08	0
FOL 4	31.2-37.9 × 5.76	3-5	11.76 - 12.48 × 5.18	0-1
FOL 5	19.2-27.6 × 5.04	3-4	9.60 - 10.8 × 4.80	0-1
FOL 6	15.6-24.0 × 5.28	2-4	7.68 - 12.0 × 4.20	0-1
FOL 7	31.2-38.2 × 4.32	3-6	11.52 - 12.48 × 3.96	0-1
FOL 8	19.2-11.5 × 3.36	3-4	8.40- 10.08 × 4.32	0-1
FOL 9	24.0-37.9 × 5.28	3-4	9.60 - 11.76 × 3.96	0-1
FOL 10	27.6-31.2 × 5.92	2-4	11.52 - 11.88 × 3.12	0
FOL 11	16.8-26.4 × 4.80	3-5	6.22 - 7.68 × 4.32	0-1

CONCLUSIONS

The isolates collected from various locations of Bhilwara region have huge variations in terms of pathogenic incidence as Bardod [L_1-36.17] has the highest incidence whereas it was lowest in the Sardarpura [L_8-18.10] area. The pathogenicity of isolate belonging to Bigod (84.67) showed best pathogenic virulence in comparison to Atoon (6.65) isolate having lowest pathogenecity.

Cultural and morphological studies shows that isolates from different region had varied sizes of mycelial colour, pigmentations and growth.

It can be concluded that isolates of different locations have distinguished diversity and characters.

Overall, the future scope for variability studies of fungal pathogens is vast and multidisciplinary, involving genomics, phenomics, ecology, antifungal resistance, and host-pathogen interactions. Such research can provide crucial insights into the mechanisms of fungal pathogenesis, drug resistance, and host susceptibility, leading to improved strategies for managing fungal infections and protecting human health.

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Conflict of Interest. None.

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