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Studies on Fusarium wilt caused by *Fusarium oxysporum* in solanaceous crops

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Abstract
Fusarium wilt is a major constraint in successful cultivation of solanaceous crops. The present study was carried out in the Kashmir valley. From different regions samples of different wilted solanaceous crops were collected and were assessed for molecular and morphological variations. The sequences of internal transcribed spacers (ITS) genomic regions of isolates were studied at molecular level and they showed 99% similarity with Foc sequences by basic local alignment search tool (BLAST) analysis. *Fusarium oxysporum* was found to be a common Fusarium variant in different crops *viz*; tomato, capsicum, brinjal and chilli in Kashmir region.

Keywords: Fusarium, solanaceous, *Fusarium oxysporum*, ITS

Introduction
Fusarium wilt is regarded as one of the most devastating diseases of solanaceous crops affecting plantations in almost all solanaceous crops growing countries of the world. Solanaceous crops are one of the most common, and highly productive vegetables grown globally and cultivated widely in India. solanaceous crops belongs to the family Solanaceae, this family consists of some important genera which are cultivated as vegetable crops such as capsicum (peppers), lycopersicon (tomato), and solanum (eggplant and potato) with chromosome number 2n = 2x = 24. *Fusarium* is considered as one of the main cause of Wilt which leads to 50–80% losses in the production yield every year. Studies conducted before have concluded that *Fusarium oxysporum* has been found in crops like crocus stavis [1], fruits crops like Muskmelon [2], and tomato etc. [3]. In the present study we collected samples randomly from different regions of Kashmir *viz*, Srinagar, pulwama and anatnag. The samples were investigated through molecular and morphological identification. For morphological identification Macro conidia, Micro conidia, Chlamydospores characteristics were considered. The sequences of internal transcribed spacers (ITS) genomic regions of isolates were studied at molecular level and they showed 99% similarity with Foc sequences by basic local alignment search tool (BLAST) analysis. The study resulted in *Fusarium oxysporum* varient found in every crop plant.

Material and Method
The present study was carried out in SKUAST-K Shalimar in the year 2020. Wilt infected solanaceous crop plants were collected from different regions of Kashmir *viz*, Srinagar, Pulwama and Anatnag. The infected plant roots were washed with running water to remove dirt. Small sections 5mm^2 size were cut with a sterilized scalpel and surface sterilized in 0.1% $HgCl_2$, blotted dry and transferred aseptically onto petriplates containing potato dextrose agar medium (PDA) and incubated at 25±1 °C for 2 weeks. Morphological characteristics of the causal pathogen were studied on artificial culture medium to identify the associated pathogen. For molecular characterization PCR reaction was conducted. The sequences of internal transcribed spacers (ITS) genomic regions of isolates were studied at molecular level.

Results and Discussion
The major solanaceous vegetable crops include tomato, eggplant and peppers. These crops encompass multi health benefits and combat various multiple diseases. But the most devastating diseases which is becoming unrestrained is the wilt in terms of incidence and yield

Loss [4]. *Fusarium* wilt, caused by *Fusarium oxysporum* is considered as one of the main cause of it which leads to 50–80% losses in the production yield every year [5]. In this study, we surveyed and characterized *Fusarium oxysporum* associated with symptomatic solenaceous crops in Kashmir region using morphological and molecular analyses. Pathogenicity tests revealed varying degrees of virulence with *Fusarium oxysporum* on different solenaceous crops [4]. The plants developed initial symptoms on the second week of inoculation [6]. The initial symptoms showed light green to yellowish discoloration of leaves followed by their shriveling, drooping and finally death of whole plant at sixth week of inoculation. For morphological studies pure cultures were maintained [7] (Fig: 1). The morphological studies under microscopic conditions showed that *Fusarium oxysporum* mycelium was densely branched, cylindrical and 3.2–4.8µm in width. Micro conidia were ellipsoidal to cylindrical in shape with 0-1 septate and measuring 65.2-9.1x2.9-3.8µm in size. Macroconidia were fusiform never in chains mostly scattered, hyaline, 2-4 septate and measuring 23-54 x 3-4µm in size (Fig 2). For molecular identification the internal transcribed spacer (ITS) amplification was done using primers K-Lab-FusOxy-ITS1F2 and K-Lab-FusOxy-ITS4R2 with genera and species specific ITS primers (Table1), and were subjected to run in 1% agarose gel showed band length approx. 500bp (fig: 3). With the help of sequencing it was clearly conformed *Fusarium oxysporum* as causal agent in every crop plant [8]. The amplified sequences of the pathogens identified through sequencing were published in Gen bank and accession number obtained are given in Table number 2.

Table 1: Primers used for amplification of ITS regions

S. No.	Primer Name	Primer Sequence	Size of Amplicon	Tm °C
1	K-Lab-FusOxy-ITS1F2 Primer	5'CCTGCGGAGGATCATTA 3'	~540bp	63.7
2	K-Lab-FusOxy-ITS4R2 Primer	5'TCCTCCGCTTATTGAT3'		53.6

Table 2: Accession number of isolates in NCBI Genbank collected from different places

S. No.	Coading of Isolates	Place of Collection	Pathogen Identified	Host Plant	Accession Number
1	TBS2	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	MW850453
2	T23	Pulwama (koel)	*Fusarium oxysporum*	Brinjal	MZ868618
3	T24	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	MZ868619
4	TBS3	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	MW850454
5	T29	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	MZ868624
6	T30	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	MZ868625
7	T31	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	MZ868626
8	T35	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	MZ868630
9	T38	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	OM319466
10	T40	Anantnag (Kokarnag)	*Fusarium oxysporum*	Brinjal	OM319468
11	T42	Pulwama (koel)	*Fusarium oxysporum*	Brinjal	OM319470
12	T53	Srinagar (Shalimar)	*Fusarium oxysporum*	Brinjal	OM319481
13	TCS1	Srinagar (Shalimar)	*Fusarium oxysporum*	Chili	MW850458
14	TCS2	Srinagar (Shalimar)	*Fusarium oxysporum*	Chili	MW850459
15	TCS6	Srinagar (Shalimar)	*Fusarium oxysporum*	Chili	MW850463
16	T22	Srinagar (Shalimar)	*Fusarium oxysporum*	Chili	MZ868617
17	T39	Srinagar (Shalimar)	*Fusarium oxysporum*	Chili	OM319467
18	T41	Srinagar (Shalimar)	*Fusarium oxysporum*	Chili	OM319469
19	TTS1	Srinagar (Shalimar)	*Fusarium oxysporum*	Tomato	MW850465
20	TTS2	Srinagar (Shalimar)	*Fusarium oxysporum*	Tomato	MW850466
21	TTS3	Srinagar (Shalimar)	*Fusarium oxysporum*	Tomato	MW850467
22	T25	Srinagar (Shalimar)	*Fusarium oxysporum*	Tomato	MZ868620
23	T26	Srinagar (Shalimar)	*Fusarium oxysporum*	Tomato	MZ868621
24	T28	Srinagar (Shalimar)	*Fusarium oxysporum*	Tomato	MZ868623
25	T44	Srinagar (Shalimar)	*Fusarium oxysporum*	Tomato	OM319472
26	T56	Srinagar (Shalimar)	*Fusarium oxysporum*	Tomato	OM319484
27	T46	Srinagar (Shalimar)	*Fusarium oxysporum*	Capsicum	OM319474
28	T47	Srinagar (Shalimar)	*Fusarium oxysporum*	Capsicum	OM319475
29	T48	Srinagar (Shalimar)	*Fusarium oxysporum*	Capsicum	OM319476
30	T49	Srinagar (Shalimar)	*Fusarium oxysporum*	Capsicum	OM319477
31	T50	Srinagar (Shalimar)	*Fusarium oxysporum*	Capsicum	OM319478
32	T54	Srinagar (Shalimar)	*Fusarium oxysporum*	Capsicum	OM319482

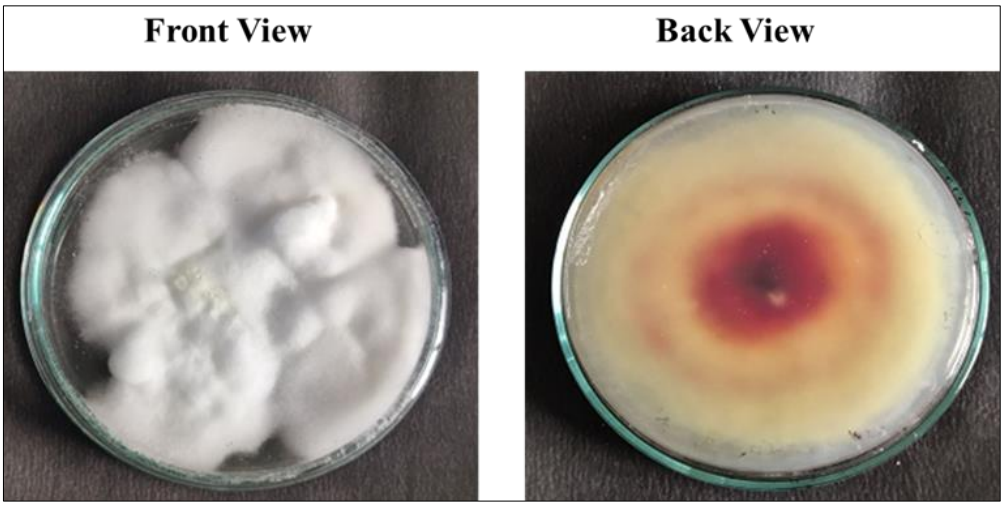

Fig 1: A Purified fungal culture of *Fusarium oxysporum* after 3 and 10 days of growth.

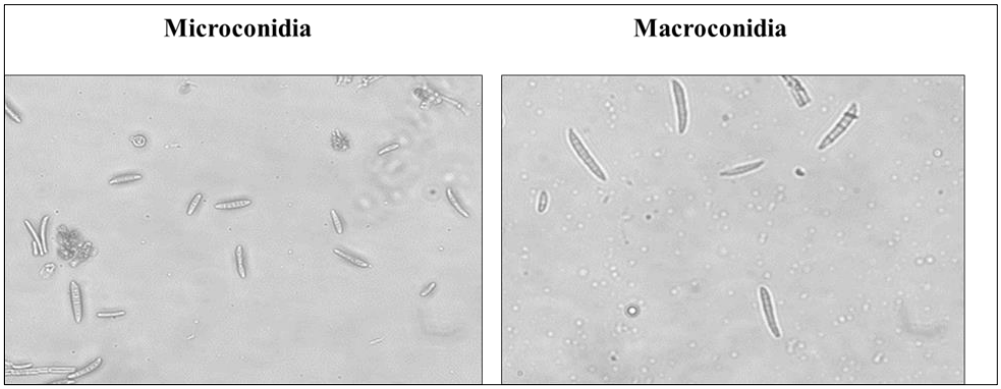

Fig 2: Morpho – cultural charactristics of *Fusarium oxysporum* causing Fusarium wilt in solanaceous crops microscopic view at 20x magnification of different spores and fruiting body of fungus

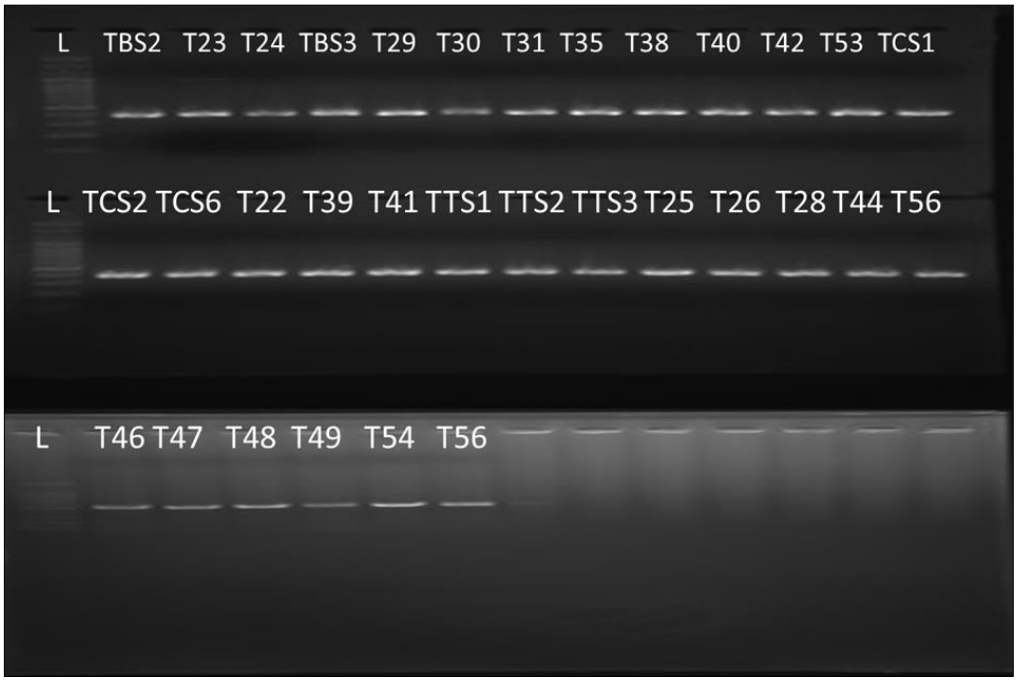

Fig 3: PCR amplification in 1% Agarose gel. L is 100bp ladder and lane TBS2 to T56 represent ITC amplified DNA samples collected from different location of Kashmir

Conclusion

We concluded that all isolates of fungi exhibited pathological infection caused by same specie *Fusarium oxysporum*. ITS sequences analysis was found to be powerful tool for authentic identification. High diversity indicate that Fusarium wilt is quickly evolving fungus in solanaceous crops and has

potential to overcome management strategies using fungicides and resistant varieties, thus management strategies need to be re-designed accordingly.

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