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Molecular identification, race differentiation and host differential analysis of *Fusarium oxysporum* f. sp. *ciceri* associated with chickpea wilt

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Abstract
Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC), is one of the most destructive diseases of chickpea, causing severe yield losses worldwide. The present investigation was undertaken to assess the pathogenic, molecular, and racial diversity of FOC isolates collected from major chickpea-growing regions of India. A total of 118 diseased chickpea samples were collected from diverse agro-ecological regions, of which 82 isolates of *F. oxysporum* f. sp. *ciceri* were successfully isolated and purified. Pathogenicity of all isolates was evaluated on the susceptible chickpea cultivar JG-62 under controlled conditions. Wilt incidence ranged from 0 to 100%, indicating considerable pathogenic variability among the isolates. Based on virulence, the isolates were classified into non-pathogenic, weakly pathogenic, moderately pathogenic, strongly pathogenic, and highly pathogenic categories. Forty highly virulent isolates exhibiting 80-100% wilt incidence were selected for detailed molecular characterization. Molecular identification using universal ITS1 and ITS4 primers consistently amplified a single DNA fragment of approximately 550 bp, confirming the identity of all selected isolates as *F. oxysporum* f. sp. *ciceri*. Further race differentiation using race-specific FDP molecular markers identified four races, namely Race 1, Race 2, Race 3, and Race 4, with Race 1 being the predominant race among the collected isolates. Host differential analysis using ten international chickpea differential cultivars further confirmed the racial identity and pathogenic variability of the isolates. The susceptible cultivar JG-62 was susceptible to all races, whereas WR-315 exhibited resistance against all tested isolates, demonstrating its broad-spectrum resistance. The study revealed substantial pathogenic and molecular diversity within the Indian FOC population and highlighted the widespread occurrence of highly virulent isolates across different agro-ecological regions. The integration of pathogenicity testing, molecular identification, race differentiation, and host differential analysis provides valuable information for understanding pathogen diversity and supports the development of durable Fusarium wilt-resistant chickpea cultivars and effective disease management strategies.

Keywords: *Fusarium oxysporum* f. sp. *ciceri*, chickpea wilt, pathogenic variability, race identification, host differential analysis

1. Introduction
Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops cultivated worldwide and serves as a major source of dietary protein, carbohydrates, vitamins and minerals particularly in developing countries. India is the largest producer of chickpea; however, its productivity is severely constrained by several biotic stresses among which *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC), is one of the most destructive diseases. The pathogen is both seed and soil borne and can survive in soil for several years through chlamydospores this making disease management difficult. Under favorable environmental conditions *Fusarium* wilt can cause yield losses ranging from 10 to 100% depending on the susceptibility of the cultivar and the virulence of the pathogen.

The pathogen exhibits considerable pathogenic and genetic variability resulting in the occurrence of multiple physiological races that differ in their virulence and geographical distribution. The emergence of new races often overcomes host resistance, reducing the effectiveness of resistant cultivars and posing a major challenge to chickpea breeding programs. Therefore, understanding the diversity, pathogenicity and race structure of *Fusarium oxysporum* f. sp. *ciceri* populations is essential for developing durable disease management strategies.

Advances in molecular biology have greatly improved the accurate identification and characterization of fungal pathogens. Molecular markers such as the Internal Transcribed Spacer (ITS) region and race specific primers provide rapid, reliable and reproducible tools for species identification and race differentiation. In addition, host differential analysis remains an effective approach for confirming pathogenic variability and identifying resistance sources against different pathogen races.

The present study was undertaken to isolate and characterize *Fusarium oxysporum* f. sp. *ciceri* isolates collected from diverse chickpea growing regions of India. Evaluate their pathogenic variability, identify pathogen races using molecular markers and assess their reactions on differential chickpea cultivars. The findings will contribute to a better understanding of pathogen diversity and provide valuable information for resistance breeding and sustainable management of chickpea *Fusarium* wilt.

2. Material and Methods

The present study utilized a range of standard laboratory glassware and plasticware required for microbiological and molecular biology experiments.

2.1 Material

2.1.1 Seeds

Chickpea genotypes utilized in this research included JAKI, JG-62 and JG-74, sourced from the Pulse Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Other genotypes, such as C-104, CPS-1, BG-212, WR-315, Annigeri, L-550, K-850, and Chaffa, were obtained from the Pulse Research Unit (Kanpur), Punjab Agricultural University, and the Zonal Agricultural Research Station (Karnataka).

2.1.2 Culture media

For the growth and maintenance of fungal cultures, Potato Dextrose Agar (PDA), 2% Agar media and Potato Dextrose Broth (PDB) were utilized throughout the investigation.

3. Methods

3.1 Sample collection

Extensive field visits were conducted during the Rabi seasons of 2018-19 across diverse geographical regions and major chickpea-producing hubs in India (Table 1). Samples were collected from farmers' fields based on characteristic physiological symptoms, including:

- Marked wilting of the plant.
- Paler or greyish green foliage.
- Internal vascular necrosis (brown to black discoloration of the pith and xylem) observed upon root inspection in both seedling and adult stages.

All symptomatic plant materials were carefully labelled and transported to the laboratory for further processing and pathogen isolation.

Table 1: List of location, date and symptoms of chickpea wilt sample collected from India

Sr. No.	Sample code	State	District	Village name	GPS location	Symptoms observed
1	S-1	Andhra Pradesh	Anantapur	Anantpur	14°42'04.1"N 77°35'59.1"E	Wilting
2	S-2	Andhra Pradesh	Guntur	Guntur	16°18'24.8"N 80°26'09.0"E	Wilting
3	S-3	Maharashtra	Nanded	Mahur	19°50'04.0"N 77°55'22.5"E	Wilting, yellowing
4	S-4	Maharashtra	Wardha	Sonawada	18°28'47.1"N 74°33'13.3"E	Wilting
5	S-5	Bihar	Muzaffarpur	Dholi	25°32'59.6"N 82°01'00.5"E	Wilting, dull green
6	S-6	Haryana	Mahendragarh	Mahendragh	23°13'10.4"N 82°11'55.1"E	Wilting, yellowing
7	S-7	Gujarat	Amreli	Dharansa	20°55'03.1"N 81°36'04.7"E	Wilting
8	S-8	Chhattisgarh	Raipur	IGKV, Raipur	21°13’57.54’’N 81°43’07.37’’E	Wilting
9	S-9	Delhi	New Delhi	IARI, New Delhi	28°37'59.4"N 77°09'09.8"E	Wilting
10	S-10	Delhi	New Delhi	New Delhi	28°35'26.9"N 77°13'16.1"E	Wilting, yellowing
11	S-11	Gujarat	Junagadh	Junagarh	21°29'59.2"N 70°27'01.1"E	Wilting
12	S-12	Gujarat	Navsari	NAU, Navsari	20°56'52.1"N 72°57'07.6"E	Wilting
13	S-13	Haryana	Gurugram	Sikobpur	28°23'42.6"N 76°59'21.1"E	Wilting, dull green
14	S-14	Haryana	Hisar	CCS HAU, Hissar	29°09'03.5"N 75°42'18.9"E	Wilting
15	S-15	Karnataka	Belagavi	Belgao	15°51'28.4"N 74°30'04.5"E	Wilting

16	S-16	Karnataka	Bengaluru	Begluru	12°58'24.2"N 77°35'51.7"E	Wilting
17	S-17	Karnataka	Vijayapura	Bijapur	16°49'31.0"N 75°43'26.0"E	Wilting
18	S-18	Karnataka	Dharwad	Dharwad	15°29'23.6"N 74°58'50.5"E	Wilting
19	S-19	Karnataka	Dharwad	Dharwad	15°29'23.6"N 74°58'50.5"E	Wilting
20	S-20	Karnataka	Raichur	Raichur	16°11'55.4"N 77°19'48.1"E	Wilting
21	S-21	Madhya Pradesh	Seoni	Barghat	22°01'54.6"N 79°43'55.5"E	Wilting
22	S-22	Madhya Pradesh	Betul	Betul	21°54'12.0"N 77°53'52.1"E	Root rot
23	S-23	Madhya Pradesh	Bhopal	Bhopal	23°14'09.0"N 77°24'31.5"E	Wilting
24	S-24	Madhya Pradesh	Jabalpur	Bargi	22°59'28"N 79°52'31"E	Wilting
25	S-25	Madhya Pradesh	Jabalpur	Jabalpur	23°12'27.6"N 79°57'26.0"E	Wilting
26	S-26	Madhya Pradesh	Jabalpur	JNKVV, Jabalpur	23°10'55.4"N 79°59'04.5"E	Wilting
27	S-27	Madhya Pradesh	Jabalpur	Khursi	23°10'53.4"N 79°59'11.0"E	Wilting
28	S-28	Madhya Pradesh	Jabalpur	Sihora	23°29'02.4"N 80°07'01.2"E	Wilting
29	S-29	Madhya Pradesh	Sagar	Tinsi	22°55'50.9"N 79°54'01.1"E	Wilting, root rot
30	S-30	Madhya Pradesh	Rewa	Bharauli	23°50'19.7"N 80°23'44.2"E	Wilting
31	S-31	Madhya Pradesh	Rewa	Chakghat	25°02'34.1"N 81°43'41.2"E	Wilting
32	S-32	Madhya Pradesh	Rewa	Gangeo	24°43'12.0"N 81°35'24.0"E	Wilting, collar rot
33	S-33	Madhya Pradesh	Rewa	Ghuma	24°53'58.6"N 81°40'26.8"E	Wilting
34	S-34	Madhya Pradesh	Rewa	Hanumana	24°46'31.4"N 82°05'33.0"E	Wilting
35	S-35	Madhya Pradesh	Rewa	Rewa	24°32'11.8"N 81°18'14.6"E	Wilting
36	S-36	Madhya Pradesh	Satna	Satna	24°36'16.8"N 80°50'08.4"E	Wilting
37	S-37	Madhya Pradesh	Seoni	Baghraj	22°00'36.0"N 79°32'24.0"E	Wilting, yellowing
38	S-38	Madhya Pradesh	Betul	Bordai	22°04'12.0"N 79°33'36.0"E	Wilting
39	S-39	Madhya Pradesh	Seoni	Chhapara	22°23'24.0"N 79°32'24.0"E	Wilting
40	S-40	Madhya Pradesh	Seoni	Dhuma	22°45'00.0"N 79°43'12.0"E	Root rot & wilting
41	S-41	Uttar Pradesh	Chitrakoot	Ghumai	22°27'00.0"N 79°34'48.0"E	Wilting
42	S-42	Madhya Pradesh	Jabalpur	Gorakhpur Kalan	22°44'24.0"N 79°55'12.0"E	Wilting
43	S-43	Madhya Pradesh	Jabalpur	Hulki	23°04'00.0"N 79°56'00.0"E	Wilting, dull green
44	S-44	Madhya Pradesh	Seoni	Lakhandon	22°36'00.0"N 79°36'00.0"E	Wilting
45	S-45	Madhya Pradesh	Rewa	Lohiya	22°07'12.0"N 79°31'12.0"E	Root rot & wilting
46	S-46	Madhya Pradesh	Seoni	Mekhdon	22°34'48.0"N 79°35'24.0"E	Wilting

47	S-47	Madhya Pradesh	Jabalpur	Sahajpuri	22°25'48.0"N 79°57'36.0"E	Wilting
48	S-48	Madhya Pradesh	Jabalpur	Samnapur	22°20'24.0"N 79°45'00.0"E	Wilting
49	S-49	Maharashtra	Ahmednagar	Kolhar, Rahuri	19°05'42.8"N 74°45'07.7"E	Wilting
50	S-50	Maharashtra	Ahmednagar	Tisgaon	19°11'26.6"N 75°04'10.6"E	Wilting
51	S-51	Maharashtra	Akola	Akola (PDKV)	20°42'10.5"N 76°59'57.9"E	Wilting
52	S-52	Maharashtra	Akola	Akola B	20°43'07.8"N 77°09'25.8"E	Wilting
53	S-53	Maharashtra	Akola	Akola C	20°42'45.7"N 77°11'16.2"E	Wilting
54	S-54	Maharashtra	Akola	Bhaurad	20°45'06.5"N 77°25'20.9"E	Wilting
55	S-55	Maharashtra	Akola	Dhaga	20°44'55.3"N 76°48'42.1"E	Wilting
56	S-56	Maharashtra	Akola	Gaigao	20°45'06.5"N 77°25'20.9"E	Wilting
57	S-57	Maharashtra	Akola	Hivarkhed	21°08'00.1"N 76°51'21.7"E	Wilting
58	S-58	Maharashtra	Akola	Pailpada	20°44'55.3"N 76°48'42.1"E	Wilting
59	S-59	Maharashtra	Buldhana	Sonala	21°08'00.1"N 76°51'21.7"E	Wilting
60	S-60	Maharashtra	Akola	Vani Rambhapur	20°44'55.3"N 76°48'42.1"E	Wilting
61	S-61	Maharashtra	Amravati	Amravati	20°56'10.0"N 77°44'47.9"E	Wilting
62	S-62	Maharashtra	Amravati	Amravati	20°56'10.0"N 77°44'47.9"E	Wilting
63	S-63	Maharashtra	Amravati	Chamak	21°12'08.4"N 77°27'19.9"E	Wilting
64	S-64	Maharashtra	Amravati	Daryapur	20°55'07.3"N 77°19'37.8"E	Wilting
65	S-65	Maharashtra	Amravati	Pimpalkhuta	20°53'25.8"N 78°10'20.6"E	Wilting
66	S-66	Maharashtra	Parbhani	Patri	20°01'11.0"N 74°57'56.3"E	Wilting
67	S-67	Maharashtra	Buldhana	Buldhana	20°31'48.0"N 76°10'48.1"E	Wilting
68	S-68	Maharashtra	Buldhana	Lonar	19°58'57.2"N 76°31'06.7"E	Wilting
69	S-69	Maharashtra	Hingoli	Hingoli	19°44'18.0"N 77°08'33.4"E	Wilting
70	S-70	Maharashtra	Jalna	Badnapur	19°52'27.5"N 75°43'20.4"E	Dull yellowing
71	S-71	Maharashtra	Buldhana	Jalgao (Sapkal)	20°25'04.3"N 75°50'15.6"E	Partial wilting
72	S-72	Maharashtra	Jalna	Koda	20°26'19.0"N 75°47'58.0"E	Wilting
73	S-73	Maharashtra	Latur	Latur	18°24'48.3"N 76°33'09.8"E	Yellowing & wilting
74	S-74	Maharashtra	Nagpur	Mangli	20°59'45.4"N 79°12'50.4"E	Yellowing, dull green leaves
75	S-75	Maharashtra	Nagpur	Nagpur	21°10'39.6"N 79°02'07.5"E	Dull green leaves
76	S-76	Maharashtra	Nanded	Pimpri	19°08'18.6"N 77°19'26.7"E	Yellowing & wilting
77	S-77	Maharashtra	Nashik	Nashik	20°01'35.0"N	Wilting

					73°49'57.3"E	
78	S-78	Maharashtra	Osmanabad	Osmanabad	18°11'08.7"N 76°02'10.8"E	Wilting
79	S-79	Maharashtra	Parbhani	Parbhani	19°15'02.6"N 76°47'41.9"E	Root rot & wilting
80	S-80	Maharashtra	Pune	Pune	18°29'35.1"N 73°58'04.6"E	Wilting
81	S-81	Maharashtra	Raigad	Killa (KVK)	18°25'19.7"N 73°10'36.1"E	Wilting
82	S-82	Maharashtra	Pune	Rajewadi	18°22'53.6"N 74°08'56.8"E	Wilting
83	S-83	Maharashtra	Raigad	Roha	18°25'59.9"N 73°06'38.8"E	Wilting
84	S-84	Maharashtra	Satara	Khandala (Pargao)	18°03'19.2"N 74°00'52.0"E	Wilting
85	S-85	Maharashtra	Satara	Satara	17°40'42.5"N 74°00'19.4"E	Wilting
86	S-86	Maharashtra	Washim	Amboda	20°44'07.2"N 78°29'06.6"E	Wilting
87	S-87	Maharashtra	Washim	Borgaon	20°43'11.2"N 78°36'36.7"E	Wilting, yellowing
88	S-88	Maharashtra	Washim	Dhanora	20°41'03.2"N 78°40'50.2"E	Wilting
89	S-89	Maharashtra	Washim	Karaja lad	20°29'43.6"N 77°27'52.9"E	Wilting
90	S-90	Maharashtra	Washim	Risod	19°58'54.4"N 76°47'15.9"E	Wilting
91	S-91	Maharashtra	Washim	Washim	20°07'08.8"N 77°08'41.6"E	Wilting
92	S-92	Maharashtra	Yavatmal	Wani	20°04'00.7"N 78°57'18.1"E	Wilting, root rot
93	S-93	Maharashtra	Yavatmal	Yavatmal	20°23'59.7"N 78°06'52.2"E	Dull green leaves, wilting
94	S-94	Punjab	Fazilka	Abohar	30°08'06.3"N 74°12'43.6"E	Wilting
95	S-95	Punjab	Gurdaspur	Gurdaspur	32°02'54.4"N 75°25'53.7"E	Wilting, yellowing
96	S-96	Punjab	Ludhiana	Ludhiana	30°54’00.64’’N 75°48’00.57’’E	Wilting, yellowing
97	S-97	Rajasthan	Alwar	Alwar	27°34'34.7"N 76°35'17.4"E	Wilting
98	S-98	Rajasthan	Banswara	Baswada	23°32'37.8"N 74°25'54.8"E	Wilting
99	S-99	Rajasthan	Jaipur	Jaipur	26°53’55.03’’N 75°15’00.94’’E	Wilting
100	S-100	Rajasthan	Sri Ganganagar	Jaitsar	26°34'08.5"N 72°01'06.7"E	Wilting
101	S-101	Rajasthan	Sri Ganganagar	Shri Ganganagar	29°32’34.09’’N 70°45’44.61’’E	Collar rot
102	S-102	Rajasthan	Udaipur	MPUAT, Udaipur	24°35'08.6"N 73°42'43.8"E	Wilting, yellowing
103	S-103	Rajasthan	Udaipur	Udaipur	24°34’57.05’’N 73°42’12.73’’E	Wilting
104	S-104	Rajasthan	Sawai Madhopur	Rameshwar	17°33'23.3"N 78°16'48.7"E	Wilting
105	S-105	Telangana	Hyderabad	Hyderabad	17°18'25.9"N 78°24'29.6"E	Wilting
106	S-106	Telangana	Sangareddy	ICRISAT	17°30'39.6"N 78°16'31.5"E	Root rot
107	S-107	Uttar Pradesh	Prayagraj	Allahabad	25°17'25.2"N 81°48'37.5"E	Root rot & wilting

108	S-108	Uttar Pradesh	Prayagraj	Garh	25°12'15.0"N 81°34'18.0"E	Root rot
109	S-109	Uttar Pradesh	Prayagraj	Gauhania	25°16'48.0"N 81°44'24.0"E	Wilting
110	S-110	Uttar Pradesh	Prayagraj	Ghambhirpur	25°22'12.0"N 81°55'48.0"E	Wilting, root rot
111	S-111	Uttar Pradesh	Prayagraj	Harro	25°07'20.4"N 81°45'13.7"E	Wilting
112	S-112	Uttar Pradesh	Prayagraj	Jari Bazar	25°19'30.0"N 81°32'42.0"E	Wilting, root rot
113	S-113	Uttar Pradesh	Kanpur	IIPR, Kanpur	26°29'38.4"N 80°16'19.5"E	Wilting
114	S-114	Uttar Pradesh	Mirzapur	Chunar	25°07'39.0"N 82°52'34.0"E	Wilting
115	S-115	Uttar Pradesh	Mirzapur	Lalganj	25°04'05.0"N 82°21'45.0"E	Wilting
116	S-116	Uttar Pradesh	Mirzapur	Mirzapur	25°07'57.6"N 82°33'53.9"E	Wilting
117	S-117	Uttar Pradesh	Prayagraj	Prayagraj	25°25'55.0"N 81°52'14.5"E	Wilting
118	S-118	Uttar Pradesh	Varanasi	BHU, Varanasi	25°16'26.0"N 82°59'31.6"E	Wilting, yellowing

2.2 Preperation of media

Preparation of Potato Dextrose Agar (PDA) media

- Peeled potatoes were sliced into small pieces and washed gently. A 200 g portion of these slices was boiled in 500 ml of distilled water until soft. The resulting filtrate was collected in a 2000 ml beaker.
- The total volume was adjusted to 1000 ml using distilled water. Subsequently, 20 g of dextrose and 20 g of agar-agar was added into the solution.
- The mixture was heated gently for 3-5 minutes with consistent intermediate shaking to ensure thorough dissolution.
- Approximately 200 ml of the prepared medium was dispensed into 500 ml flask and then plugged with non-absorbent cotton and wrapped with paper. The flask was sterilized in an autoclave at 15 lbs/in² (121.6 °C) for 15 minutes.

2.3 Isolation of the causal pathogen

Pathogen isolation was performed on PDA medium within a strictly aseptic environment. Initially the working surface of the laminar airflow cabinet was disinfected with denatured spirit. All necessary materials including petri plates, blades, needles, and forceps were placed inside and the Ultraviolet (UV) lamp was activated for 30 minutes prior to use. The primary or secondary roots and the collar region of the symptomatic chickpea plants were used for isolation. The procedure involved:

- Selected plant tissues were cut into small segments (10 to 20 mm) using a sterile razor.
- Segments were submerged in a 0.1% mercuric chloride ($HgCl_2$) solution for one minute.
- Bits were subjected to three subsequent rinses in sterile distilled water to eliminate any residual mercuric chloride.
- After being air dried on sterile tissue paper for two minutes five segments were placed equidistantly on pre poured PDA plates.
- Plates were incubated in a BOD incubator at 27 ± 2 °C for three to four days.
- Upon the emergence of fungal growth the mycelia were aseptically transferred to PDA slants incubated for an additional 7 days at 27 ± 2 °C and subsequently stored in a refrigerator for further study.

2.4 Identification of the isolated pathogen

The identity of the *Fusarium oxysporum* f. sp. *ciceri* isolates was confirmed through:

- **Morphological characterization:** Observations were cross referenced with established descriptions by Booth (1975) [5], Nelson *et al*. (1983) [23], and Leslie & Summerell (2006) [21].
- **Molecular validation:** Genetic identification was conducted (Table 2) using Internal Transcribed Spacer (ITS) analysis with forward (ITS 1) and reverse (ITS 4) primers (White *et al*., 1990) [36].

Table 2: List of ITS primers with their sequence used for confirmation of *Fusarium oxysporum* f. sp. *cicero*

Sr. No.	Oligonucleotide	Primer sequence (5'-3')
1.	ITS 1-F	TCCGTAGGTGAACCTGCGG
2.	ITS 4-R	TCCTCCGCTTATTGATATGC

2.5 Race differentiation of isolates of *Fusarium oxysporum* f. sp. *ciceri*

The isolates of *Fusarium oxysporum* f. sp. *ciceri* were distinguished into the four races. Using the procedure outlined by Jimenez Gasco and Jimenez Diaz (2003) [19], high quality and quantity DNA was isolated from the mycelium of *Fusarium oxysporum* f. sp. *ciceri*. In order to fingerprint the races of *Fusarium oxysporum* f. sp. *ciceri*, four primer pairs with ITS-specific markers were utilized (Table 3).

Table 3: List of Race specific primers with their sequence used for confirmation of races of *Fusarium oxysporum* f. sp. *cicero*

Sr. No.	Oligonucleotide	Primer Sequence (5'-3')
1.	FDP-03 (Race-1)	F: CAGCAGTGAGGAATATTGGTCAATG
		R: GCGGATCATCGAATTAAATAACAT
2.	FDP-14 (Race-2)	F: GAGCAGTCAATGGCAATGG
		R: AGAGCAGGGTCAGCGTAGATA
3.	FDP-02 (Race-3)	F: CTTGGTGTTGGGATCTGT GTGCAA
		R: ACAAAT TACAACTCGGGC CCG AGA
4.	FDP-09 (Race-04)	F: CCAGACTTCCACTGCGTGTC
		R: CACGCCAGGACTGCCTCGT

2.6 Pathogenicity of *Fusarium oxysporum* f. sp. *ciceri* isolates

The pathogenicity of the isolates was evaluated on the susceptible chickpea cultivar JG-62 using the soil inoculation method (Nene *et al*., 1981) [24]. The pathogen was multiplied on sand-maize meal medium (3:1, w/w), and inoculum was mixed into sterilized soil at 10 g kg^{-1} soil seven days before sowing. Surface-sterilized seeds were sown in inoculated pots, while uninoculated pots served as controls. Wilt incidence was recorded 40 days after inoculation, and isolates were classified into pathogenicity groups according to Haware and Nene (1982a) [17].

2.7 Disease assessment and data analysis

Inoculated seedlings were inspected every second day for disease symptoms. Wilt incidence was calculated based on the following formula:

Wilt Incidence (%) = (Number of plants wilted / Total number of plants sown) × 100

Isolates were categorized based on the virulence scale proposed by Haware and Nene (1982a) [17]:

	Category	Percent wilt
i)	Non-pathogenic isolates (NPI)	0%
ii)	Weakly pathogenic isolates (WPI)	1-20%
iii)	Moderately pathogenic isolates (MPI)	21-50%
iv)	Strong pathogenic isolates (SPI)	51-70%
v)	Highly pathogenic isolates (HPI)	>70%

2.8 Host differential analysis of *Fusarium oxysporum* f. sp. *ciceri*

Forty representative isolates were evaluated on ten chickpea differential cultivars (JG-62, JG-74, C-104, CPS-1, BG-212, WR-315, Annigeri, Chaffa, L-550, and K-850) using the soil inoculation method. Disease incidence was recorded 40 days after inoculation, and host reactions were classified as resistant (0-20%), moderately susceptible (21-50%), or susceptible (>50%) based on percent wilt incidence.

2.8.1 Soil inoculation method

Sand maize meal medium (3:1, w/w) was used for mass multiplication of the pathogen inoculum. The medium was prepared by mixing 150 g of dry sand and 50 g of maize meal with 20 ml of distilled water in 1000 ml conical flasks. The flasks were autoclaved at 1.05 kg cm^{-2} pressure for 30 minutes for three consecutive days. After sterilization the sand maize meal medium was inoculated separately with pure cultures of *Fusarium oxysporum* f. sp. *ciceri* under aseptic conditions and incubated at room temperature for two weeks.

Plastic pots (15 cm diameter) were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) and filled with 2 kg of sterilized soil treated with 1.0% formalin for 15 days. The soil was inoculated with 15 days old fungal cultures grown on sand maize meal medium at the rate of 10 g kg^{-1} soil seven days prior to sowing. Seeds of each test cultivar were surface disinfected by immersion in a 2.5% sodium hypochlorite solution for 2-3 minutes before sowing. Ten seeds of each cultivar were sown per pot. Uninoculated pots were maintained as controls for each cultivar.

The plants were observed periodically after sowing and seedlings were monitored for the initiation of wilt symptoms. Disease observations were recorded 40 days after inoculation.

3. Results and Discussion

3.1 Collection of disease samples

Chickpea wilt sample collection was carried out during Rabi 2018 and 2019 season on 118 different locations in major chickpea growing region of the country belonging to diverse Agro Ecological Regions (Table 4).

Table 4: Chickpea wilt sample collected from different agro ecological regions of India

Sr. No.	Sample code	State	Village/location	Specific AER (Sub-Region)
1	S-1	Andhra Pradesh	Anantpur	3.0(Deccan Plateau, Bellary-Anantapur Haveri)
2	S-2	Andhra Pradesh	Guntur	18.3 (Eastern Coastal Plain, Central)
3	S-3	Maharashtra	Mahur	6.3 (Eastern Maharashtra Plateau)
4	S-4	Maharashtra	Sonawada	6.1(South-Western Maharashtra Plateau)
5	S-5	Bihar	Dholi	13.1 (North Bihar Plains)
6	S-6	Chhattisgarh	Mahendragadh	11.0 (Eastern Plateau, Chhattisgarh)
7	S-7	Chhattisgarh	Dharansa	11.0 (Eastern Plateau, Chhattisgarh)
8	S-8	Chhattisgarh	IGKV, Raipur	11.0 (Eastern Plateau, Chhattisgarh)
9	S-9	Delhi	IARI, New Delhi	4.1 (Northern Plain, Tran-Gangetic)
10	S-10	Delhi	New Delhi	4.1 (Northern Plain, Tran-Gangetic)
11	S-11	Gujarat	Junagarh	5.3 (Kathiawar Peninsula)

12	S-12	Gujarat	NAU, Navsari	19.1 (North Sahyadris & Coastal Plain)
13	S-13	Haryana	Sikobpur	4.1 (Northern Plain, Tran-Gangetic)
14	S-14	Haryana	CCS, HAU, Hissar	4.1 (Northern Plain, Semi-Arid fringe)
15	S-15	Karnataka	Belgao	6.4 (North Sahyadris & Transition)
16	S-16	Karnataka	Bengaluru	8.2 (Central Karnataka Plateau)
17	S-17	Karnataka	Bijapur	3.0 (Deccan Plateau, Dry Zone)
18	S-18	Karnataka	Dharwad	6.4 (North Sahyadris & Transition)
19	S-19	Karnataka	Dharwad	6.4 (North Sahyadris & Transition)
20	S-20	Karnataka	Raichur	3.0 (Deccan Plateau, Raichur-Bellary)
21	S-21	Madhya Pradesh	Barghat	10.4 (Satpura Plateau)
22	S-22	Madhya Pradesh	Betul	10.4 (Satpura Plateau)
23	S-23	Madhya Pradesh	Bhopal	5.2 (Malwa Plateau, Central)
24	S-24	Madhya Pradesh	Bargi	10.3 (Vindhyan Scarplands)
25	S-25	Madhya Pradesh	Jabalpur	10.3 (Vindhyan Scarplands/Kymore)
26	S-26	Madhya Pradesh	JNKVV, Jabalpur	10.3 (Vindhyan Scarplands/Kymore)
27	S-27	Madhya Pradesh	Khursi	10.3 (Vindhyan Scarplands)
28	S-28	Madhya Pradesh	Sihore	10.3 (Vindhyan Scarplands)
29	S-29	Madhya Pradesh	Tinsi	10.4 (Satpura Plateau)
30	S-30	Madhya Pradesh	Bharauli	10.3 (Vindhyan Scarplands)
31	S-31	Madhya Pradesh	Chakghat	10.3 (Vindhyan Scarplands/Rewa)
32	S-32	Madhya Pradesh	Gangeo	10.3 (Vindhyan Scarplands/Rewa)
33	S-33	Madhya Pradesh	Ghuma	10.3 (Vindhyan Scarplands/Rewa)
34	S-34	Madhya Pradesh	Hanumana	10.3 (Vindhyan Scarplands/Rewa)
35	S-35	Madhya Pradesh	Rewa	10.3 (Vindhyan Scarplands/Rewa)
36	S-36	Madhya Pradesh	Satna	10.2(Bundelkhand Uplands- Transition)
37	S-37	Madhya Pradesh	Baghraj	10.4 (Satpura Plateau)
38	S-38	Madhya Pradesh	Bordai	10.4 (Satpura Plateau)
39	S-39	Madhya Pradesh	Chhapara	10.4 (Satpura Plateau)
40	S-40	Madhya Pradesh	Dhuma	10.4 (Satpura Plateau)
41	S-41	Madhya Pradesh	Ghumai	10.4 (Satpura Plateau)
42	S-42	Madhya Pradesh	Gorakhpur Kalan	10.4 (Satpura Plateau)
43	S-43	Madhya Pradesh	Hulki	10.3 (Vindhyan Scarplands)
44	S-44	Madhya Pradesh	Lakhandon	10.4 (Satpura Plateau)
45	S-45	Madhya Pradesh	Lohiya	10.4 (Satpura Plateau)
46	S-46	Madhya Pradesh	Mekhdon	10.4 (Satpura Plateau)
47	S-47	Madhya Pradesh	Sahajpuri	10.4 (Satpura Plateau)
48	S-48	Madhya Pradesh	Samnapur	10.4 (Satpura Plateau)
49	S-49	Maharashtra	Kolhar, Rahuri	6.1(South-WesternMaharashtra Plateau)
50	S-50	Maharashtra	Tisgaon	6.1(South-Western Maharashtra Plateau)
51	S-51	Maharashtra	Akola (PDKV)	6.3(Eastern Maharashtra Plateau/ Vidarbha)
52	S-52	Maharashtra	Akola B	6.3 (Eastern Maharashtra Plateau / Vidarbha)
53	S-53	Maharashtra	Akola C	6.3 (Eastern Maharashtra Plateau / Vidarbha)
54	S-54	Maharashtra	Bhaurad	6.3 (Eastern Maharashtra Plateau / Vidarbha)
55	S-55	Maharashtra	Dhaga	6.3 (Eastern Maharashtra Plateau / Vidarbha)
56	S-56	Maharashtra	Gaigao	6.3 (Eastern Maharashtra Plateau / Vidarbha)
57	S-57	Maharashtra	Hivarkhed	6.3 (Eastern Maharashtra Plateau / Vidarbha)
58	S-58	Maharashtra	Pailpada	6.3 (Eastern Maharashtra Plateau / Vidarbha)
59	S-59	Maharashtra	Sonala (Akot)	6.3 (Eastern Maharashtra Plateau / Vidarbha)
60	S-60	Maharashtra	Vani Rambhapur	6.3 (Eastern Maharashtra Plateau / Vidarbha)
61	S-61	Maharashtra	Amravati RRC	6.3 (Eastern Maharashtra Plateau / Vidarbha)
62	S-62	Maharashtra	Amravati	6.3 (Eastern Maharashtra Plateau / Vidarbha)
63	S-63	Maharashtra	Chamak	6.3 (Eastern Maharashtra Plateau / Vidarbha)
64	S-64	Maharashtra	Daryapur	6.3 (Eastern Maharashtra Plateau / Vidarbha)
65	S-65	Maharashtra	Pimpalkhuta	6.3 (Eastern Maharashtra Plateau / Vidarbha)
66	S-66	Maharashtra	Patri	6.2 (Central Maharashtra Plateau)
67	S-67	Maharashtra	Buldhana	6.3 (Eastern Maharashtra Plateau / Vidarbha)
68	S-68	Maharashtra	Lonar	6.3 (Eastern Maharashtra Plateau / Vidarbha)
69	S-69	Maharashtra	Hingoli	6.2(Central Maharashtra Plateau/Marathwada)
70	S-70	Maharashtra	Badnapur	6.2(Central Maharashtra Plateau/Marathwada)
71	S-71	Maharashtra	Jalgao (Sapkal)	6.2 (Central Maharashtra Plateau)

72	S-72	Maharashtra	Koda	6.2 (Central Maharashtra Plateau)
73	S-73	Maharashtra	Latur	6.2(Central Maharashtra Plateau/Marathwada)
74	S-74	Maharashtra	Mangli	10.4 (Satpura Plateau / Nagpur Fringe)
75	S-75	Maharashtra	Nagpur	10.4 (Satpura Plateau / Nagpur Plateau)
76	S-76	Maharashtra	Pimpri (Kinwat)	6.3 (Eastern Maharashtra Plateau / Vidarbha)
77	S-77	Maharashtra	Nashik	6.1 (South-Western Maharashtra Plateau)
78	S-78	Maharashtra	Osmanabad	6.2(Central Maharashtra Plateau/Marathwada)
79	S-79	Maharashtra	Parbhani	6.2(Central Maharashtra Plateau/Marathwada)
80	S-80	Maharashtra	Pune	6.1 (South-Western Maharashtra Plateau)
81	S-81	Maharashtra	Killa (KVK)	19.2 (North Sahyadris / Konkan Coast)
82	S-82	Maharashtra	Rajewadi	19.2 (North Sahyadris / Konkan Coast)
83	S-83	Maharashtra	Roha	19.2 (North Sahyadris / Konkan Coast)
84	S-84	Maharashtra	Khandala (Pargao)	6.1 (South-Western Maharashtra Plateau)
85	S-85	Maharashtra	Satara	6.1 (South-Western Maharashtra Plateau)
86	S-86	Maharashtra	Amboda	6.3 (Eastern Maharashtra Plateau / Vidarbha)
87	S-87	Maharashtra	Borgaon	6.3 (Eastern Maharashtra Plateau / Vidarbha)
88	S-88	Maharashtra	Dhanora	6.3 (Eastern Maharashtra Plateau / Vidarbha)
89	S-89	Maharashtra	Karanja lad	6.3 (Eastern Maharashtra Plateau / Vidarbha)
90	S-90	Maharashtra	Risod	6.2(Central Maharashtra Plateau/Marathwada)
91	S-91	Maharashtra	Washim	6.3 (Eastern Maharashtra Plateau / Vidarbha)
92	S-92	Maharashtra	Wani Yavatmal	6.3 (Eastern Maharashtra Plateau / Vidarbha)
93	S-93	Maharashtra	Yavatmal	6.3 (Eastern Maharashtra Plateau / Vidarbha)
94	S-94	Punjab	Abohar	2.3 (Punjab Plain - Arid/Semi-Arid Transition)
95	S-95	Punjab	Gurdaspur	9.1 (Punjab Plain, Northern)
96	S-96	Punjab	Ludhiana	9.1 (Punjab Plain, Central)
97	S-97	Rajasthan	Alwar	4.1 (Northern Plain / Aravalli Foothills)
98	S-98	Rajasthan	Baswada (Banswara)	5.2 (Malwa Plateau, Western)
99	S-99	Rajasthan	Jaipur	4.2 (Northern Plain, Semi-Arid)
100	S-100	Rajasthan	Jaitsar	2.1 (Western Plain, Arid)
101	S-101	Rajasthan	Shri Ganganagar	2.1 (Western Plain, Arid)
102	S-102	Rajasthan	MPUAT, Udaipur	4.2 (Northern Plain / Aravalli Hills)
103	S-103	Rajasthan	Udaipur	4.2 (Northern Plain / Aravalli Hills)
104	S-104	Tamil Nadu	Rameshwar	18.2 (South Tamil Nadu Plains)
105	S-105	Telangana	Hyderabad	7.2 (North Telangana Plateau)
106	S-106	Telangana	ICRISAT	7.2 (North Telangana Plateau)
107	S-107	Uttar Pradesh	Allahabad	13.2 (Central & Eastern UP Plain)
108	S-108	Uttar Pradesh	Garh	13.2 (Central & Eastern UP Plain)
109	S-109	Uttar Pradesh	Gauhania	13.2 (Central & Eastern UP Plain)
110	S-110	Uttar Pradesh	Ghambhirpur	13.2 (Central & Eastern UP Plain)
111	S-111	Uttar Pradesh	Harro	13.2 (Central & Eastern UP Plain)
112	S-112	Uttar Pradesh	Jari Bazar	13.2 (Central & Eastern UP Plain)
113	S-113	Uttar Pradesh	IIPR, Kanpur	9.2 (Central UP Plain)
114	S-114	Uttar Pradesh	Chunar	13.2 (Central & Eastern UP Plain)
115	S-115	Uttar Pradesh	Lalganj	13.2 (Central & Eastern UP Plain)
116	S-116	Uttar Pradesh	Mirzapur	13.2 (Central & Eastern UP Plain)
117	S-117	Uttar Pradesh	Prayagraj	13.2 (Central & Eastern UP Plain)
118	S-118	Uttar Pradesh	BHU, Varanasi	13.2 (Central & Eastern UP Plain)

A total of 118 isolates (Table 4) of *Fusarium oxysporum* f. sp. *ciceri* were collected from major chickpea growing regions across India to assess molecular variability and pathogenic diversity. The isolates represented a wide geographical distribution covering multiple states including Andhra Pradesh, Bihar, Chhattisgarh, Delhi, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Telangana and Uttar Pradesh.

The majority of isolates were obtained from Madhya Pradesh and Maharashtra, which together contributed the largest proportion of samples, particularly from the Central Highlands (Satpura) and Deccan Plateau agro ecological regions. Isolates were also collected from diverse Agro Ecological Regions (AERs) including the Deccan Plateau (Hot Arid and Hot Semi-Arid), Eastern Coastal Plains, Northern Plains (Hot Semi-Arid and Sub-humid), Central Highlands (Malwa and Satpura), Eastern Plains (Middle Gangetic), Western Ghats and Coastal Plains and Telangana Plateau.

GPS coordinates were recorded for each sampling location to ensure precise geographical documentation and to facilitate future epidemiological and diversity analyses. The broad spatial distribution of isolates across contrasting Agro Ecological Region provides a robust representation of pathogen diversity and enable comprehensive assessment of molecular variability among races of *Fusarium oxysporum* f. sp. *ciceri*.

3.2 Isolation and purification

3.2.1 Isolation

During the survey diseased chickpea plants exhibiting typical wilt symptoms were collected from different Agro Ecological Regions and brought to the laboratory for pathogen isolation. The tissue isolation method was employed for the recovery of *Fusarium oxysporum* f. sp. *ciceri* from infected stem and root portions.

Infected tissues were cut into small segments and surface sterilized with 0.1% mercuric chloride solution for 40-60 seconds followed by two rinses in sterile distilled water to remove traces of the sterilant. The sterilized tissue segments were transferred onto potato dextrose agar (PDA) medium under aseptic conditions and incubated at 27 ± 2 °C in a BOD incubator to allow fungal growth.

Based on cultural and morphological characteristics, isolates of *Fusarium oxysporum* f. sp. *ciceri* were purified and maintained on PDA slants at 4 °C for further studies. A total of 118 isolates were successfully obtained and designated sequentially as S-1 to S-118, as presented.

Table 5: Details of the Fusarium isolates association with other microbes

Sample ID	Bits kept	Bits yielded FOC	Associated fungi / Mycoflora	% yield
S-1	25	22	*Aspergillus* spp.	88%
S-2	25	18	*Rhizoctonia* spp.	72%
S-3	25	0	Nil	0%
S-4	25	0	Nil	0%
S-5	25	0	Nil	0%
S-6	25	0	Nil	0%
S-7	25	0	Nil	0%
S-8	25	23	*Aspergillus* spp.	92%
S-9	25	15	*Trichoderma* spp., *Rhizoctonia* spp.	60%
S-10	25	0	Nil	0%
S-11	25	21	*Aspergillus* spp.	84%
S-12	25	11	*Rhizoctonia* spp.	44%
S-13	25	19	*Trichoderma* spp., *Sclerotium rolfsii*	76%
S-14	25	22	*Aspergillus* spp.	88%
S-15	25	0	Nil	0%
S-16	25	0	Nil	0%
S-17	25	21	*Rhizoctonia* spp.	84%
S-18	25	24	*Aspergillus* spp.	96%
S-19	25	0	Nil	0%
S-20	25	19	*Trichoderma* spp.	76%
S-21	25	12	*Aspergillus* spp., *Rhizoctonia* spp.	48%
S-22	25	15	*Sclerotium rolfsii*	60%
S-23	25	21	*Aspergillus* spp.	84%
S-24	25	22	*Trichoderma* spp., *Rhizoctonia* spp.	88%
S-25	25	23	*Aspergillus* spp.	92%
S-26	25	19	*Sclerotium rolfsii, Aspergillus* spp.	76%
S-27	25	20	*Rhizoctonia* spp.	80%
S-28	25	18	*Trichoderma* spp.	72%
S-29	25	22	*Aspergillus* spp.	88%
S-30	25	21	*Rhizoctonia* spp.	84%
S-31	25	8	*Rhizoctonia* spp., *Sclerotium rolfsii*	32%
S-32	25	19	*Aspergillus* spp.	76%
S-33	25	23	*Trichoderma* spp.	92%
S-34	25	0	Nil	0%
S-35	25	0	Nil	0%
S-36	25	24	*Aspergillus* spp.	96%
S-37	25	19	*Trichoderma* spp.	76%
S-38	25	21	*Aspergillus* spp., *Rhizoctonia* spp.	84%
S-39	25	20	*Sclerotium rolfsii*	80%
S-40	25	17	*Rhizoctonia* spp.	68%
S-41	25	23	*Aspergillus* spp.	92%
S-42	25	19	*Trichoderma* spp., *Sclerotium rolfsii*	76%
S-43	25	21	*Aspergillus* spp.	84%
S-44	25	20	*Rhizoctonia* spp.	80%
S-45	25	22	*Aspergillus* spp.	88%
S-46	25	19	*Trichoderma* spp.	76%
S-47	25	21	*Rhizoctonia* spp.	84%

S-48	25	20	*Aspergillus* spp., *Sclerotium rolfsii*	80%
S-49	25	11	*Rhizoctonia* spp., *Sclerotium rolfsii*	44%
S-50	25	24	*Aspergillus* spp.	96%
S-51	25	20	*Trichoderma* spp.	80%
S-52	25	23	*Rhizoctonia* spp.	92%
S-53	25	19	*Aspergillus* spp.	76%
S-54	25	9	*Sclerotium rolfsii, Rhizoctonia* spp.	36%
S-55	25	22	*Aspergillus* spp.	88%
S-56	25	18	*Trichoderma* spp.	72%
S-57	25	0	Nil	0%
S-58	25	20	*Aspergillus* spp.	80%
S-59	25	23	*Trichoderma* spp., *Sclerotium rolfsii*	92%
S-60	25	24	*Aspergillus* spp.	96%
S-61	25	21	*Rhizoctonia* spp.	84%
S-62	25	19	*Aspergillus* spp.	76%
S-63	25	22	*Trichoderma* spp.	88%
S-64	25	20	*Sclerotium rolfsii*	80%
S-65	25	21	*Aspergillus* spp.	84%
S-66	25	23	*Rhizoctonia* spp.	92%
S-67	25	19	*Aspergillus* spp.	76%
S-68	25	22	*Trichoderma* spp.	88%
S-69	25	20	*Sclerotium rolfsii*	80%
S-70	25	21	*Aspergillus* spp.	84%
S-71	25	24	*Trichoderma* spp.	96%
S-72	25	20	*Rhizoctonia* spp.	80%
S-73	25	19	*Aspergillus* spp.	76%
S-74	25	23	*Sclerotium rolfsii*	92%
S-75	25	3	*Aspergillus* spp., *Rhizoctonia* spp.	12%
S-76	25	21	*Trichoderma* spp.	84%
S-77	25	22	*Aspergillus* spp.	88%
S-78	25	24	*Rhizoctonia* spp.	96%
S-79	25	17	*Aspergillus* spp.	68%
S-80	25	0	Nil	0%
S-81	25	19	*Trichoderma* spp.	76%
S-82	25	0	Nil	0%
S-83	25	0	Nil	0%
S-84	25	0	Nil	0%
S-85	25	16	*Aspergillus* spp., *Rhizoctonia* spp.	64%
S-86	25	0	Nil	0%
S-87	25	0	Nil	0%
S-88	25	18	*Aspergillus* spp.	72%
S-89	25	0	Nil	0%
S-90	25	20	*Trichoderma* spp.	80%
S-91	25	0	Nil	0%
S-92	25	15	*Rhizoctonia* spp.	60%
S-93	25	0	Nil	0%
S-94	25	22	*Aspergillus* spp.	88%
S-95	25	21	*Sclerotium rolfsii*	84%
S-96	25	0	Nil	0%
S-97	25	0	Nil	0%
S-98	25	10	*Rhizoctonia* spp., *Sclerotium rolfsii*	40%
S-99	25	0	Nil	0%
S-100	25	0	Nil	0%
S-101	25	0	Nil	0%
S-102	25	23	*Aspergillus* spp.	92%
S-103	25	0	Nil	0%
S-104	25	0	Nil	0%
S-105	25	0	Nil	0%
S-106	25	24	*Trichoderma* spp.	96%
S-107	25	0	Nil	0%

S-108	25	0	Nil	0%
S-109	25	0	Nil	0%
S-110	25	0	Nil	0%
S-111	25	21	*Rhizoctonia* spp.	84%
S-112	25	0	Nil	0%
S-113	25	20	*Aspergillus* spp.	80%
S-114	25	0	Nil	0%
S-115	25	0	Nil	0%
S-116	25	22	*Sclerotium rolfsii*	88%
S-117	25	0	Nil	0%
S-118	25	24	*Aspergillus* spp.	96%

A total of 118 samples collected from different geographical locations were processed for the isolation of *Fusarium oxysporum* f. sp. *ciceri* (FOC). Each sample consisted of 25 bits plated on culture medium for pathogen recovery. The results revealed substantial variability in the recovery percentage of FOC, ranging from 0 to 96 percent.

Out of 118 samples 82 isolates yielded FOC colonies whereas 36 samples showed no FOC growth (0% yield). The recovery percentage varied widely among locations with several isolates such as S-18, S-36, S-50, S-60, S-71, S-78, S-106 and S-118 recording ≥ 96% yield indicating heavy pathogen colonization. In contrast certain samples yielded low recovery (e.g., S-75: 12%; S-54: 36%; S-31: 32%), suggesting comparatively lower pathogen load or possible competition from other soil microflora.

The presence of associated fungi such as *Aspergillus* spp., *Rhizoctonia* spp., *Trichoderma* spp. and *Sclerotium rolfsii* indicates a complex soil mycoflora. Mixed fungal associations in chickpea rhizosphere soils have been widely documented (Jiménez-Díaz *et al*., 2015) [39]. The frequent occurrence of *Trichoderma* spp. Also suggest natural antagonism in certain samples. Potentially explaining reduced FOC recovery in some locations as reported by Sharma *et al*., (2016) [32]. Similarly, competitive saprophytes like *Aspergillus* spp. and *Rhizoctonia* spp. may suppress or mask FOC during isolation under *in vitro* conditions.

Rationale for selecting 82 isolates for pathogenicity testing

Only those 82 samples that yielded confirmed *Fusarium oxysporum* f. sp. *ciceri* were selected for pathogenicity testing. Samples that showed 0% yield were excluded because pathogenicity assays require viable and pure cultures of the pathogen to fulfil Koch's postulates (Agrios, 2005) [1]. Without successful isolation and purification disease causation cannot be experimentally validated.

Selection based on successful recovery is a standard procedure in wilt pathogen studies (Haware and Nene, 1982; Jiménez-Díaz *et al*., 2015) [17, 39] where only confirmed isolates are advanced for virulence assessment. This ensures:

1) Experimental reliability and reproducibility
2) Elimination of false negatives due to absence of pathogen
3) Accurate assessment of virulence diversity
4) Avoidance of interference from non-target fungi

Thus, the 82 isolates selected for pathogenicity testing represent the active FOC population recovered from diverse geographic regions. The variation in isolation frequency further suggests heterogeneity in pathogen distribution and inoculum density across chickpea growing areas.

Overall, the isolation data confirm widespread distribution of *Fusarium oxysporum* f. sp. *ciceri* also highlighting variability in pathogen prevalence and soil mycoflora composition.

2.2 Purification of *Fusarium oxysporum* f. sp. *ciceri* isolates by Single spore isolation method

The isolates of *Fusarium oxysporum* f. sp. *ciceri* were purified using the single spore isolation technique to obtain genetically uniform cultures. Spore suspensions were prepared from actively growing cultures and spread onto 2% water agar medium. Spore germination was observed under a microscope within approximately 4 hours of inoculation.

Individual germinated spores (8-12 hours after inoculation) were carefully selected and transferred aseptically onto fresh Potato Dextrose Agar (PDA) medium. Visible colonies developed within 2-3 days depending on the isolate. The use of single spore isolation ensured genetic purity and uniform colony growth compared to cultures obtained through mass transfer of inoculum (Leslie and Summerell, 2006) [21].

A thin layer of water agar facilitated clear microscopic observation and easy transfer of germinated spores using a sterile needle under low magnification. During standardization it was observed that spore densities exceedingly approximately 10 spores per microscopic field resulted in overlapping germ tubes making isolation difficult. Therefore, serial dilutions (10^{-3} and higher) were prepared to obtain well separated germinating spores for accurate isolation.

3.3 Pathogenic viability

Table 6: Pathogenicity of *Fusarium oxysporum* f. sp. *ciceri* isolates using JG 62 cultivar

Sample ID	Total plants	Wilted plants	Yield %	Virulence category
S-1	10	8	80	HPI
S-2	10	9	90	HPI
S-8	10	10	100	HPI
S-9	10	7	70	SPI

S-10	10	8	80	HPI
S-12	10	8	80	HPI
S-13	10	7	70	SPI
S-14	10	7	70	SPI
S-17	10	8	80	HPI
S-18	10	9	90	HPI
S-20	10	8	80	HPI
S-21	10	5	50	MPI
S-22	10	6	60	SPI
S-23	10	8	80	HPI
S-24	10	2	20	WPI
S-25	10	5	50	MPI
S-26	10	8	80	HPI
S-27	10	3	30	WPI
S-28	10	5	50	MPI
S-29	10	2	20	WPI
S-30	10	1	10	WPI
S-31	10	6	60	SPI
S-32	10	3	30	MPI
S-33	10	2	20	WPI
S-36	10	4	40	WPI
S-37	10	0	0	NPI
S-38	10	3	30	MPI
S-39	10	2	20	WPI
S-40	10	4	40	MPI
S-41	10	0	0	NPI
S-42	10	2	20	WPI
S-43	10	4	40	MPI
S-44	10	6	60	SPI
S-45	10	1	10	WPI
S-46	10	7	70	SPI
S-47	10	3	30	MPI
S-48	10	5	50	MPI
S-49	10	9	90	HPI
S-50	10	7	70	MPI
S-51	10	8	80	HPI
S-52	10	4	40	MPI
S-53	10	2	20	WPI
S-54	10	0	0	NPI
S-55	10	3	30	WPI
S-56	10	9	90	HPI
S-58	10	8	80	HPI
S-59	10	5	50	MPI
S-60	10	6	60	SPI
S-61	10	4	40	MPI
S-62	10	6	60	SPI
S-63	10	7	70	SPI
S-64	10	5	50	MPI
S-65	10	4	40	MPI
S-66	10	7	70	SPI
S-67	10	6	60	SPI
S-68	10	8	80	HPI
S-69	10	10	100	HPI
S-70	10	10	100	HPI
S-71	10	7	70	SPI
S-72	10	4	40	MPI
S-73	10	9	90	HPI
S-74	10	5	50	MPI
S-75	10	8	80	HPI
S-76	10	7	70	SPI

S-77	10	9	90	HPI
S-78	10	10	100	HPI
S-79	10	7	70	SPI
S-81	10	8	80	HPI
S-85	10	7	70	SPI
S-88	10	9	90	HPI
S-90	10	8	80	HPI
S-92	10	8	80	HPI
S-94	10	7	70	SPI
S-95	10	7	70	SPI
S-98	10	8	80	HPI
S-102	10	9	90	HPI
S-106	10	7	70	SPI
S-111	10	10	100	HPI
S-113	10	8	80	HPI
S-116	10	7	70	SPI
S-118	10	9	90	HPI

*virulence grades are recorded as follows:

Category	Percent wilt	
Non-pathogenic isolate	0%	NPI
Weakly pathogenic isolate	1%-20%	WPI
Moderately pathogenic isolate	21%-50%	MPI
Strong pathogenic isolate	51%-70%	SPI
Highly pathogenic isolate	>71%	HPI

The pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* isolates was assessed using the susceptible chickpea cultivar JG- 62 under controlled conditions. A total of 82 isolates were evaluated with ten plants inoculated per isolate and wilt incidence was recorded to determine percent disease expression and categorize virulence.

Wilt incidence ranged from 0 to 100 percent indicating considerable pathogenic diversity within the population. Highly Pathogenic Isolates (HPI; > 71% wilt) constituted a major proportion of the population with several isolates (S-8, S-69, S-70, S-78 and S-111) causing 100% wilt and complete plant mortality. Strongly Pathogenic Isolates (SPI; 51-70%) also represented a significant group while moderate (MPI; 21-50%) and weakly pathogenic isolates (WPI; 1-20%) showed reduced virulence. Two isolates (S-37 and S-41) were non-pathogenic (0% wilt). Overall, the predominance of HPI and SPI categories suggests the presence of highly aggressive strains within the tested population.

At the end of the experiment *Fusarium oxysporum* f. sp. *ciceri* was successfully re-isolated from wilted plants, and its morphological characteristics resembled those of the original cultures thereby confirming Koch's postulates.

The virulence and pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* on cv. JG -62 have been widely documented (Prasad and Padwick, 1939; Haware and Nene, 1979; Kewate, 1986; Shubha Trivedi and Gurha, 2005; Barhate *et al*., 2006; Shrivastava and Agrawal, 2006; Dubey *et al*., 2012) [27, 16, 20, 34, 3, 33, 8]. Similar differentiation among large numbers of isolates has been reported by Gupta *et al*. (1986b) [15] who characterized 86 isolates collected from 84 locations of Madhya Pradesh. Paulkar *et al*. (2002) [25] and Saabale and Dubey (2014) [30] also reported substantial variability with wilt incidence ranging from 24 to 100 percent among isolates tested on JG - 62 under pot conditions. Golakiya *et al*. (2018) [14] further confirmed pathogenic variability among fifteen isolates screened on the susceptible cultivar.

Grouping of isolates based on pathogenicity revealed no consistent relationship between geographical origin and virulence indicating that pathogenic potential is not strictly region dependent. Similar observations were reported by Durai *et al*. (2012) [9] who found that isolates from different regions were pathogenic irrespective of their origin. The classification of *Fusarium oxysporum* f. sp. *ciceri* isolates based on pathogenic ability has been documented earlier by Prasad and Padwick (1939) [27], El-Morsey *et al*. (1997) [10], Rao and Krishnappa (1997) [30], Zamani *et al*. (2000) [38], Shubha Trivedi and Gurha (2005) [34] and Barhate *et al*. (2006) [3].

The wide spectrum of virulence observed in the present study confirms significant pathogenic diversity within *Fusarium oxysporum* f. sp. *ciceri* populations. The predominance of highly virulent isolates highlights the potential threat to chickpea cultivation and underscores the importance of continuous monitoring of virulence patterns and deployment of durable resistance strategies.

Table 7: List of selected virulent *Fusarium oxysporum* f. sp. *ciceri* isolates for further study

Sample ID	FOC designation	Named location/source	AER region name
S-51	FOC-1	Akola PDKV MH	Deccan Plateau, Hot Semi-arid
S-56	FOC-2	Gaigao, MH	Deccan Plateau, Hot Semi-arid
S-63	FOC-3	Chamak, MH	Deccan Plateau, Hot Semi-arid
S-75	FOC-4	Nagpur, MH	Central Highlands (Eastern Satpura), Hot Sub-humid

S-69	FOC-5	Hingoli, MH	Deccan Plateau, Hot Semi-arid
S-49	FOC-6	Kolhar, Rahuri MH	Deccan Plateau, Hot Semi-arid
S-85	FOC-7	Satara, MH	Deccan Plateau, Hot Semi-arid
S-66	FOC-8	Patri, MH	Deccan Plateau (Marathwada), Hot Semi-arid
S-70	FOC-9	Badnapur, MH	Deccan Plateau, Hot Semi-arid
S-81	FOC-10	Killa KVK, MH	Deccan Plateau, Hot Semi-arid
S-73	FOC-11	Latur, MH	Deccan Plateau, Hot Semi-arid
S-78	FOC-12	Osmanabad, MH	Deccan Plateau, Hot Semi-arid
S-79	FOC-13	Parbhani, MH	Deccan Plateau, Hot Semi-arid
S-90	FOC-14	Risod, MH	Deccan Plateau, Hot Semi-arid
S-68	FOC-15	Lonar, MH	Deccan Plateau, Hot Semi-arid
S-92	FOC-16	Wani Yavatmal MH	Deccan Plateau, Hot Semi-arid
S-77	FOC-17	Nashik, MH	Deccan Plateau, Hot Semi-arid
S-106	FOC-18	ICRISAT, TS	Deccan Plateau (Telangana), Hot Semi-arid
S-1	FOC-19	Anantpur, AP	Deccan Plateau, Hot Arid
S-2	FOC-20	Guntur, AP	East Coast Plains, Hot Sub-humid
S-17	FOC-21	Bijapur, KA	Deccan Plateau, Hot Semi-arid
S-18	FOC-22	Dharwad, KA	Deccan Plateau, Hot Semi-arid
S-20	FOC-23	Raichur 7690, KA	Deccan Plateau, Hot Arid
S-8	FOC-24	IGKV, Raipur CG	Eastern Plateau (Chhattisgarh), Hot Sub-humid
S-9	FOC-25	IARI, New Delhi	Northern Plain (Ganga-Yamuna), Hot Semi-arid
S-12	FOC-26	NAU, Navsari GJ	West Coast Plains, Hot Humid
S-11	FOC-27	Junagarh, GJ	Central Highlands (Gujarat Plains), Hot Semi-arid
S-88	FOC-28	Satna, MP	Hot Subhumid / Dry Eco-subregion
S-94	FOC-29	Abohar, PB	Western Plain, Hot Arid
S-14	FOC-30	CCS HAU, Hissar HR	Western Plain (Punjab/Haryana), Hot Arid
S-102	FOC-31	MPUAT, Udaipur RJ	Central Highlands (Aravalli), Hot Semi-arid
S-98	FOC-32	Baswada, RJ	Central Highlands (Aravalli), Hot Semi-arid
S-26	FOC-33	JNKVV, Jabalpur MP	Central Highlands (Vindhyan), Hot Sub-humid
S-118	FOC-34	BHU, Varanasi UP	Northern Plain, Hot Sub-humid
S-111	FOC-35	Harro, UP	Northern Plain, Hot Semi-arid
S-113	FOC-36	IIPR, Kanpur UP	Northern Plain (Ganga-Yamuna), Hot Semi-arid
S-116	FOC-37	Mirzapur, UP	Northern Plain, Hot Sub-humid
S-13	FOC-38	Sikobpur, HR	Northern Plain, Hot Semi-arid
S-23	FOC-39	Bhopal, MP	Central Highlands (Malwa), Hot Semi-arid
S-95	FOC-40	Gurdaspur, PB	Northern Plain (Punjab), Hot Semi-arid

Out of 118 collected samples, 82 were confirmed as *Fusarium oxysporum* f. sp. *ciceri* (FOC) based on successful isolation. Pathogenicity screening further categorized these isolates according to wilt incidence from which 40 highly pathogenic isolates (HPI; > 71% wilt) were selected for detailed analysis.

The selection focused on isolates exhibiting maximum virulence (80-100% wilt incidence) as highly aggressive strains are epidemiologically significant and most relevant for resistance screening (Haware and Nene, 1982; Nene and Reddy, 1981) [17, 24]. Prioritizing highly virulent isolates ensures accurate assessment of host resistance and reflects the worst case disease scenario under field conditions (Agrios, 2005) [1].

Additionally, the 40 isolates represent diverse Agro Ecological Regions (AERs), including the Deccan Plateau, Northern Plains, Central Highlands and Eastern Plateau. Such ecological stratification captures environmental adaptation and pathogenic variability across chickpea growing zones (Jiménez-Díaz *et al*., 2015; Sharma *et al*., 2016) [39, 32].

Thus, the stepwise selection (118 → 82 → 40) ensured biological validity, virulence relevance and broad geographic representation, making these isolates suitable for further evaluation studies.

3.4 Identification of *Fusarium oxysporum* f. sp. *ciceri* isolates by ITS marker

In the present study universal primers ITS-1 (5′ TCC GTA GGT GAA CCT GCG G 3′) and ITS-4 (5′ TCC TCC GCT TAT TGA TAT GC 3′) were employed to amplify the ITS1-5.8S-ITS2 region. PCR amplification was carried out in a 50 µL reaction volume following the protocol described by Gautam *et al*. (2013) [11]. The amplification of a single ~ 550 bp fragment confirmed the identity of all 40 isolates as *Fusarium oxysporum* f. sp. *ciceri*. These selected (40) isolates were used for further studies.

Identification of *Fusarium* at the species level can often be achieved based on cultural and morphological characteristics (Booth, 1971; Gerlach and Nirenberg, 1982; Nelson *et al*., 1983) [4, 13, 23]. Accordingly, all isolates were subcultured on Potato Dextrose Agar (PDA) and examined microscopically to confirm their identity and ensure purity prior to further analysis (Prasad *et al*., 2008) [28].

Subsequently the 40 highly pathogenic isolates of *Fusarium oxysporum* f. sp. *ciceri* were confirmed at the molecular level using ITS-1 and ITS-4 universal primers (White *et al*.,

1990) [36]. The ITS primer pair consistently amplified a single DNA fragment of approximately 550 bp in all tested isolates confirming their identity. Similar amplification patterns (~550 bp) for *Fusarium oxysporum* f. sp. *ciceri* using ITS primers have been reported by Gayatri Gurjar *et al*. (2009) [12]. The utility of ITS markers for molecular identification and characterization of *Fusarium* species has also been validated in earlier studies (Dubey *et al*., 2010; Durai *et al*., 2012; Datta and Lal, 2012) [7, 9, 6].

Based on molecular confirmation and virulence profiling representative isolates were further selected for molecular variability analysis to assess genetic diversity within the pathogen population.

Manikandan and Raguchander (2014) [22] utilized internal transcribed spacer (ITS) regions for rapid identification and characterization of twenty isolates of *Fusarium oxysporum* f. sp. *lycopersici*. Amplification using ITS-1 and ITS-4 primers consistently produced a fragment of approximately 560 bp in all isolates, demonstrating the reliability of ITS markers for detection of *Fusarium* wilt pathogens.

Similarly, Wu Kai li *et al*. (2019) [37] investigated *Fusarium* wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) and identified isolates using ITS sequence analysis. Among 33 putative Fusarium isolates ITS sequencing differentiated species effectively nineteen isolates were identified as *Fusarium oxysporum* eight as *Fusarium solani,* three as *Fusarium proliferatum* one as *Fusarium verticillioides,* while two remained unidentified. Their findings further support the utility of ITS sequencing in species level discrimination within the *Fusarium* complex.

Collectively these studies validate the use of ITS-based molecular markers as a reliable, rapid, and widely accepted approach for accurate identification of *Fusarium* spp. and formae speciales.

3.5 Racial identification of *Fusarium oxysporum* f. sp. *ciceri* isolates by molecular FDP marker

Molecular characterization of *Fusarium oxysporum* f. sp. *ciceri* (FOC) races has been widely undertaken using race specific DNA markers targeting ITS regions and other gene sequences. Poornima *et al*. (2017) [26] differentiated four major races of *Fusarium oxysporum* f. sp. *ciceri* prevalent in India using race specific primers FDP-3, FDP-14, FDP-2, and FDP-9.

Primer FDP-3 targets the mitochondrial rRNA gene and produces a polymorphic banding pattern around 700 to 720 bp, which is characteristic of race 1. This race has been predominantly reported from Maharashtra, Karnataka and Andhra Pradesh. Here, in our study isolates FOC-1 to FOC-24, FOC-26, FOC-27, FOC-31, FOC-32 belong to Race 1.

FDP-14 is specific for race 2 and generates a band of approximately 900 to 1000 bp; isolates from Uttar Pradesh *viz*., FOC-34, FOC-35, FOC-36 and FOC-37 were identified as race 2 based on this amplification pattern.

Primer FDP-2 targets the ITS region of the ribosomal gene and produces a distinct band of approximately 300 bp which is diagnostic for race 3 Isolates from Gurdaspur (Punjab) i.e. (Table). FOC-40 were identified as race 3 based on this marker.

FDP-9 differentiates race 4 by amplifying a specific band of approximately 500 bp or 2 kb. Isolates from Jabalpur (Madhya Pradesh) and Delhi /Haryana region, which are FOC-25, FOC-28, FOC-29, FOC-30, FOC-33, FOC-38 and FOC-39 were characterized as race 4 using this primer.

With the increasing significance of *Fusarium* wilt in chickpea molecular markers have become essential tools for accurate race identification. Several marker systems targeting ITS regions and elongation factor genes have been employed for racial differentiation. Among a set of 29 primers evaluated by Poornima *et al*. (2017) [26], FDP-2 (ITS region), FDP-3 (mitochondrial rRNA gene), FDP-9 (race 4-specific), and FDP-14 (race 2-specific) were found to be reliable for distinguishing Indian races. Earlier demonstrated the effectiveness of mitochondrial rRNA-based primers in fungal differentiation. Furthermore, EST based markers and translation elongation factor (EF-1α) genes have also been used for assessing variability among Fusarium isolates (Arif *et al*., 2012) [2].

Globally, eight races of *Fusarium oxysporum* f.sp *ciceri* (0, 1A, 1B/C, 2, 3, 4, 5, and 6) have been reported of which four races (1, 2, 3, and 4) are predominant in India (Venkataramanamma *et al*., 2020) [35]. Race 1 has been particularly reported from Andhra Pradesh. The emergence of new races poses a serious threat to chickpea cultivation, as it may overcome existing host resistance. Therefore, continuous monitoring of newly collected isolates from diverse geographical regions and host genotypes is essential to understand racial distribution and evolution.

Overall, race specific molecular markers provide a rapid, reliable and precise method for differentiating *Fusarium oxysporum* f. sp *ciceri* races and are crucial for resistance breeding and disease management strategies.

Table 8: Race specific primers used for race differentiation of *Fusarium oxysporum* f. sp. *ciceri*

Race	Primer name	Target region	Amplicon size
Race 1	FDP-3	Mitochondrial rRNA gene	700-720 bp
Race 2	FDP-14	Race-specific region	900-1000 bp (~1 kb)
Race 3	FDP-2	ITS region of ribosomal gene	~300 bp
Race 4	FDP-9	Race-specific region	500 bp / ~2 kb

3.6 Assaying the isolates reaction on the basis of host differential studies *in vitro*

Table 9: host differential analysis of 40 isolates of *Fusarium oxysporum* f. sp. *ciceri* on ten chickpea cultivars

Isolate	JG-62	C-104	JG-74	CPS-1	BG-212	WR-315	Annigeri	Chaffa	L-550	K-850
FOC-1	S	S	R	R	R	R	S	S	S	S
FOC-2	S	S	R	R	R	R	S	S	S	S
FOC-3	S	S	R	R	R	R	S	S	S	S
FOC-4	S	S	R	R	R	R	S	S	S	S

FOC-5	S	S	R	R	R	R	S	S	S	S
FOC-6	S	S	R	R	R	R	S	S	S	S
FOC-7	S	S	R	R	R	R	S	S	S	S
FOC-8	S	S	R	R	R	R	S	S	S	S
FOC-9	S	S	R	R	R	R	S	S	S	S
FOC-10	S	S	R	R	R	R	S	S	S	S
FOC-11	S	S	R	R	R	R	S	S	S	S
FOC-12	S	S	R	R	R	R	S	S	S	S
FOC-13	S	S	R	R	R	R	M	S	S	S
FOC-14	S	S	R	R	R	R	S	S	S	S
FOC-15	S	S	R	R	R	R	S	S	S	S
FOC-16	S	S	R	R	R	R	M	S	S	S
FOC-17	S	S	R	R	R	R	S	S	S	S
FOC-18	S	S	R	R	R	R	M	S	S	S
FOC-19	S	S	R	R	R	R	S	S	S	S
FOC-20	S	S	R	R	R	R	M	S	S	S
FOC-21	S	S	R	R	R	R	M	S	S	S
FOC-22	S	S	R	R	R	R	S	S	S	S
FOC-23	S	S	R	R	R	R	S	S	S	S
FOC-24	S	S	R	R	R	R	S	S	S	S
FOC-25	S	S	R	R	R	R	S	S	S	M
FOC-26	S	S	R	R	R	R	S	S	S	S
FOC-27	S	S	R	R	R	R	S	S	S	S
FOC-28	S	S	R	R	R	R	S	S	S	M
FOC-29	S	S	R	R	R	R	S	S	S	M
FOC-30	S	S	R	R	R	R	S	S	S	M
FOC-31	S	S	R	R	R	R	S	S	S	S
FOC-32	S	S	R	R	R	R	S	S	S	S
FOC-33	S	S	R	R	R	R	S	S	S	M
FOC-34	S	S	M	R	R	R	S	S	S	M
FOC-35	S	S	M	R	R	R	S	S	S	M
FOC-36	S	S	M	R	R	R	M	S	S	M
FOC-37	S	S	M	R	R	R	S	S	S	M
FOC-38	S	S	R	R	R	R	S	S	S	M
FOC-39	S	S	R	R	R	R	S	S	S	M
FOC-40	S	R	R	S	M	R	S	S	M	M

7.1 Host differential analysis of *Fusarium oxysporum* f. sp. *ciceri*

An experiment was conducted to evaluate host differential reactions of forty isolates of *Fusarium oxysporum* f. sp. *ciceri* collected from different chickpea growing regions of India. The isolates were screened on ten international chickpea differential cultivars, namely JG-62, C-104, JG-74, CPS-1, BG-212, WR-315, Annigeri, Chaffa, L-550, and K-850, using the soil inoculation method.

Sterilized soil was artificially inoculated with sand-maize meal inoculum of *Fusarium oxysporum* f. sp. *ciceri* at 3:1 percent (w/w). Ten seeds of each differential cultivar were sown in pots inoculated with individual isolates. After germination, five healthy seedlings per pot were maintained. Observations on days to initial wilting and complete wilting were recorded up to 40 Days After Sowing (DAS). Seedlings grown in sterilized uninoculated soil served as control.

7.2 Differential reactions

The susceptible check JG-62 exhibited susceptible (S) reaction to all forty isolates belonging to Race 1 (FOC-1 to FOC-24, FOC-26, FOC-27, FOC-31, FOC-32), Race 2 (FOC-34, FOC-35, FOC-36, FOC-37), Race 3 (FOC-40), and Race 4 (FOC-25, FOC-28, FOC-29, FOC-30, FOC-33, FOC-38, FOC-39).

C-104 showed susceptible reaction to all Race 1, Race 2, and Race 4 isolates, and exhibited resistance only to FOC-40 (Race 3).

RJG-74 expressed resistant reaction to all isolates of Race 1, Race 3, and Race 4, while it showed moderately susceptible (M) reaction to all Race 2 isolates (FOC-34, FOC-35, FOC-36, FOC-37).

CPS-1 exhibited resistant reaction to all Race 1 and Race 4 isolates and also to Race 2 isolates FOC-35, FOC-36 and FOC-37, but showed moderate susceptibility to FOC-34 (Race 2) and susceptibility to FOC-40 (Race 3).

BG-212 was resistant to all Race 1, Race 2, and Race 4 isolates, while it showed moderate susceptibility to FOC-40 (Race 3).

WR-315 exhibited resistant reaction to all isolates across all four races.

Annigeri showed susceptible reaction to most isolates; however, moderate susceptibility was observed against FOC-13, FOC-16, FOC-18, FOC-20 and FOC-21 (Race 1 isolates) and FOC-36 (Race 2).

Chaffa exhibited susceptible reaction to all isolates across all races.

L-550 showed susceptible reaction to all Race 1 and Race 2 isolates, while it exhibited moderate susceptibility to all Race 4 isolates (FOC-25, FOC-28, FOC-29, FOC-30, FOC-33, FOC-38, FOC-39) and to FOC-40 (Race 3).

K-850 exhibited susceptible reaction to all Race 1 and Race 2 isolates, and moderate susceptibility to all Race 4 isolates and to FOC-40 (Race 3).

Differentiation of *Fusarium oxysporum* f. sp. *ciceri* isolates based on host differential reactions has been extensively documented. Dubey *et al*. (2012) [8] characterized seventy isolates from thirteen states into eight races using differential cultivar responses and identified specific cultivar combinations distinguishing races 1 through 8. Similarly, Ramanamma *et al*. (2020) [29] reported the occurrence of four races (1, 2, 3, and 4) in India and confirmed the prevalence of race 1 in Andhra Pradesh.

The present investigation confirms the existence of multiple races (race 1, 2, 3, and 4). Among the tested isolates and demonstrates substantial pathogenic and racial variability across chickpea growing regions. The differential reactions observed in this study provide important insights for resistance breeding programs and support the development of region-specific wilt-resistant chickpea varieties.

Conclusion

1) A total of 118 chickpea wilt samples were collected from different agro ecological regions of India out of which 82 isolates of *Fusarium oxysporum* f. sp. *ciceri* (FOC) were successfully isolated and purified.
2) Pathogenicity testing revealed considerable variability in virulence with wilt incidence ranging from 0 to 100%, indicating the existence of diverse pathogen populations.
3) Based on pathogenicity, 40 highly virulent isolates (80-100% wilt incidence) were selected for detailed molecular characterization.
4) Molecular identification using ITS1 and ITS4 primers confirmed all selected isolates as *Fusarium oxysporum* f. sp. *ciceri* by producing a characteristic ~550 bp amplicon.
5) Race specific molecular markers successfully differentiated the isolates into four races (Race 1, Race 2, Race 3, and Race 4), with Race 1 being the predominant race.
6) Host differential analysis demonstrated clear differences in cultivar reactions, confirming the presence of distinct pathogen races and pathogenic variability.
7) The chickpea cultivar WR-315 exhibited broad-spectrum resistance against all tested races, whereas JG-62 was susceptible to all isolates.
8) No consistent relationship was observed between the geographical origin of the isolates and their pathogenicity, indicating that virulence is independent of collection location.
9) The combined use of pathogenicity assays, ITS based molecular identification, race specific markers and host differential analysis proved effective for comprehensive characterization of FOC isolates.
10) The findings provide valuable information for race monitoring, identification of resistant germplasm, resistance breeding and the development of sustainable management strategies against *Fusarium* wilt of chickpea.

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