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Isolation, Purification and Pathogenicity Assessment of *Fusarium oxysporum* Schl. f.sp. *ciceris* Inciting Wilt Disease in Chickpea

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Abstract

The present study was conducted during *rabi* (November–March, 2022–23) at the Department of Plant Pathology and Central Instrumentation Cell, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India to isolate, purify and pathogenicity assessment of *Fusarium oxysporum* Schl. f.sp. *ciceris* inciting wilt disease in chickpea. The pathogen was isolated from infected chickpea and purified, with its cultural and morphological characteristics utilized for identification. The analysis revealed that pathogen grown on potato dextrose agar media exhibited traits consistent with *Fusarium oxysporum* Schl. f.sp. *ciceris*, appearing as mycelium colour, texture, pigmentation, type and speed of growth as cultural characteristics and *Fusarium oxysporum* Schl. f.sp. *ciceris* as possessing macroconidia, microconidia and chlamydospore as morphological characteristics. Furthermore, a pathogenicity test was conducted on the chickpea cultivar JG-62, cultivated in pots under controlled conditions within a net house was successfully satisfying Koch's postulates. Because of its great nutritious content, chickpeas have risen to prominence. where it plays a significant role in ensuring food security and sustainability. However, wilt disease in chickpea remains a persistent threat, leading to substantial losses in both quantity and quality. To address this issue the investigation provided valuable insights into understanding the pathogen, thereby contributing to the development of cost-effective solutions for managing wilt disease in chickpea which helps in increasing crop yield to the chickpea growing farmers of Telangana.

Keywords: Isolation, purification, pathogenicity, *Fusarium oxysporum* Schl. f.sp. *ciceris*

1. Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated, diploid (2n=2x=16), cool season legume crop ranking second in global production among food grain legumes with a total production of 15.87 mt from an area of 15.0 m ha (Anonymous, 2021). South Asia is the largest producer and consumer of chickpea that contributes about 90% of area globally. On an average from 1994–2021, production share of chickpea by Asian region includes 84.9% followed by Americas and Africa, each with 4.6% and others. It is grown primarily in semi-arid regions of South Asia, despite some locations being concentrated in the Mediterranean region. India, Turkey, Pakistan, Australia and Myanmar are major producers of chickpea (Anonymous, 2021). In India, about 104.74 lakh ha, area coverage reported under chickpea during *rabi* 2023–24 as against 110.71 lakh ha during the same period in 2022–23. Chickpea production in India for 2022–23 stands at 122.67 lakhs tons (Anonymous, 2024). According to the final estimates provided by the Telangana State Government, chickpea production for the 2023–24 yasangi season reached 2.32 lakh tons, harvested from 1.48 lakh ha, achieving a productivity rate of 1568 kg ha^{-1} (Anonymous, 2024). It is cultivated primarily for its protein-rich seeds. Besides, seeds are rich in fiber, minerals, β-carotene and unsaturated fatty acids Jukanti et al. (2012). Chickpea is known to be attacked by a variety of pathogens, viz., fungus, bacteria, viruses, mycoplasma and nematodes. More than 172 pathogens are infecting the crop worldwide Nene et al. (1996). Among these fungi are those predominantly associated with the major diseases, which are *Ascochyta* blight, *Fusarium* wilt, Dry root rot, *Botrytis* gray mold and Wet root rot. Among the biotic stresses, *Fusarium* wilt has been reported as a

devastating fungal disease. The identification of the pathogen in the Indian subcontinent dates back to Butler's discovery in 1918, followed by Padwick's elucidation of its etiology in 1941. *Fusarium oxysporum* f. sp. *ciceris* as pathogenic to chickpea, a recognition acknowledged worldwide as the causal agent of *Ciceris* spp. Haware and Nene (1980) reported four distinct physiological races of *Fusarium oxysporum* (1, 2, 3 and 4). Further exploration revealed eight races, as 0, IA, IB, 2, 3, 4, 5 and 6 each exhibiting unique characteristics and symptoms through differential pathogenic reactions (Haware and Nene, 1982a; Jimenez-Diaz et al., 1989). These races exhibit variations in pathotypes and geographic distributions, with races IA and 6 categorized as wilting pathotypes, while races 0 and 1B/C are designated as yellowing pathotypes Jimenez-Gasco et al. (2001). Similarly, in northwestern Mexico, races 0, 1B/C, 5 and 6 were identified Arvayo-Ortiz et al. (2011). The disease manifests during the seedling and flowering stages of plant growth and is characterized by various symptoms, including drooping of petioles and rachis yellowing and drying of leaves from the base upward browning of vascular bundles, improper branching, plant withering and eventual death Westerlund et al. (1974). Fungal pathogenicity genes involve spore attachment, germination, infection and colonization Swarupa et al. (2014). Wilt symptoms appearing 20–30 days after sowing are termed early wilt, while those manifesting during the flowering-podding stage are referred to as late wilt. Early wilt results in a yield decline of 77–90%, whereas late wilt causes losses of 24–65%, Partial wilt occurs when only certain plant parts Jimenez-Diaz et al. (2015). Mahajan et al. (2019) collected *Fusarium* wilt-infected plant root samples from various locations and isolated 50 *Fusarium* cultures, observed morphological and cultural variations among these isolates. The research work was done to aid understand disease development pathways, to mitigate yield losses and ensure food availability for vulnerable populations.

2. Materials and Methods

The research was undertaken during the *rabi* (November–March, 2022–23) at the Department of Plant Pathology and Central Instrumentation Cell, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India. The study focused on the Isolation, Purification and Pathogenicity assessment of *Fusarium oxysporum* Schl. f.sp. *ciceris* inciting wilt disease in chickpea.

2.1. Collection of samples

Diseased samples exhibiting typical symptoms of *Fusarium* wilt were collected in brown paper bags during the roving survey 2022–23 from different fields of chickpea growing regions and were brought to Department of Plant Pathology and Central Instrumentation Cell, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana.

2.2. Symptomatology

Fusarium wilt pathogens infiltrate the host plant through root apices or wounds, proliferating within xylem vessels to block vascular bundles. Initial symptoms typically appear on flowers, upper leaves and twigs presenting with discoloration and desiccation. Within a few days, entire plants exhibit withering and collapse. The roots look healthy but when split vertically the vascular tissues show brown to black discoloration. Wilt symptoms appearing 20-30 days after sowing are termed early wilt, while those manifesting during the flowering-podding stage are referred to as late wilt. Early wilt results in a yield decline of 77–90%, whereas late wilt causes losses of 24–65%. Partial wilt occurs when only certain plant parts are affected. Susceptible cultivars display symptoms within 25 days after sowing, with affected seedlings exhibiting drooping leaves followed by complete plant death. Generally, wilting at the early growth stage leads to greater losses than at later stages.

2.3. Isolation and purification of pathogen

The isolation of the pathogen was conducted using fresh infected plants showing typical wilt symptoms were collected during roving survey 2022-23 from different fields of chickpea growing regions were brought to Department of Plant Pathology and Central Instrumentation Cell, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India, in brown paper bags for isolation of the pathogen. Isolation of wilt fungi from affected plant samples was carried out using tissue isolation method described by (Rangaswamy and Mahadevan, 1999) under aseptic condition. The infected chickpea plant roots were washed under running tap water to remove excess soil adhered to the root zone and dried on blotter paper before isolation to avoid contamination. These roots were then cut into small pieces of size 2–3 mm with sterilized blade. These bits were then surface sterilized with 2% sodium hypochlorite solution for one minute and rinsed with sterilized water at three intervals to remove traces of sodium hypochlorite on the root. Then each bit was dried on a blotter paper and four bits of each were placed on the Potato Dextrose Agar (PDA) medium poured plates and were incubated at 28±2°C for seven days in an incubator (Aneja, 2003). The obtained pure cultures were preserved and maintained on Potato Dextrose Agar (PDA) medium throughout the investigation.

2.4. Identification of pathogen

To characterize the pathogen isolated from diseased samples, various cultural and morphological traits of the *Fusarium* was examined. The purified isolate of *Fusarium* was identified on the basis of cultural and morphological characteristics such as colony colour, mycelial growth, pigmentation and sporulation (macroconidia, microconidia and chlamydospores) by using monographs of *Fusarium* described by Booth (1971). The morphology of the conidia was observed under low power magnification (40X) of stereo binocular microscope and data was recorded.

2.4.1. Mass multiplication of *Fusarium oxysporum* f. sp. *ciceris*

Purified culture of isolate of *Fusarium oxysporum* f. sp. *ciceris*

(*FOC*) was mass multiplied on sorghum grains. The sorghum grains were soaked in water for overnight and excess water was drained out from it. Sorghum grains were filled in 500 ml conical flask and tightly closed with non-absorbent cotton and were autoclaved at 121°C 15 *psi* pressure for 30 min for 2 days. Then the flasks were allowed to cool and inoculated with 5 mm mycelial disc of *Fusarium oxysporum* f. sp. *ciceris* and incubated at 28± 2°C for 15 days in BOD incubator. The flasks were manually shaken on daily basis for a few minutes to avoid clumping in order to get early growth with uniform colonization of seeds. After 15 days, of inoculation fungal cultures are ready for further use which is fully multiplied.

2.4.2. Pathogenicity test

To confirm the pathogenicity of the isolated pathogen (following Koch's postulates) from infected plant, a pathogenicity test was conducted in a net-house environment. The earthen pots of (12×10 cm) diameter filled with three kg steam sterilized soil. The mass multiplied fungus was added to these pots @2 g kg^{-1} (10^8 cfu g^{-1}) soil and mixed thoroughly for uniform distribution. The pots were watered lightly and was incubated for 4 days. Surface sterilized seeds of chickpea cultivar JG-62 were sown in these pots (5 seeds pot^{-1}) and three replications was maintained. The pots were regularly watered to maintain sufficient moisture needed by the plants. Wilt symptoms developed was observed for 30 days after sowing and the per cent disease incidence was calculated (Jamil and Ashraf, 2020).

PDI=Number of wilted plants pot^{-1}/Total number of plants pot-1×100

3. Results and Discussion

3.1. Isolation and purification of pathogen

The presence of the pathogen was confirmed through a meticulous examination of hand sections of diseased tissue under a microscope. Samples exhibiting initial symptoms typically appear on flowers, upper leaves and twigs presenting with discoloration and desiccation. Within a few days, entire plants exhibit withering and collapse, during microscopy were identified as infected with *Fusarium* wilt (Figure 1 and 2). These identified samples were subsequently air-dried at room temperature and preserved for further analysis. The methodology employed in this study aligns with previous research. Isolation of pure fungal cultures, single spore isolation technique was followed as described by (Choi et al., 1999). Spore suspension of the isolated pathogen (*Fusarium oxysporum* f. sp. *ciceris*) was prepared by dissolving spores in sterile distilled water. Investigated cultural, morphological and pathogenic variations among races and variants of *Fusarium oxysporum* f. sp. *ciceris* from seven different locations in the central zone of India. Cultural studies revealed differences among isolates in mycelium growth pattern, pigmentation, growth rate and sporulation on potato dextrose agar media. Chaithra et al. (2019) isolated twelve *Fusarium oxysporum* f.

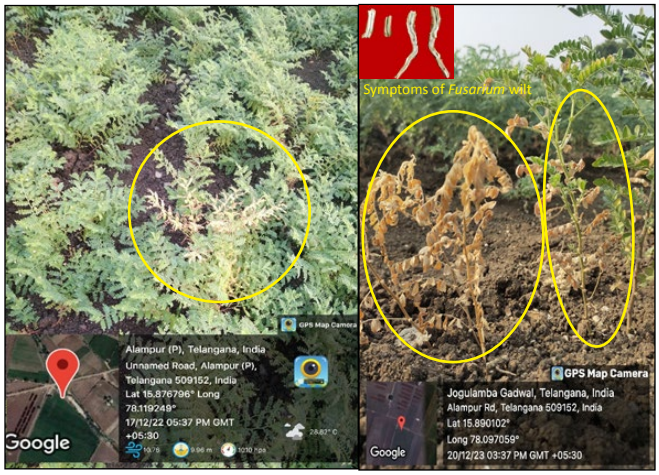

Figure 1: Young plants with dull green leaves killed by Fusarium wilt during roving survey 2022–2023

Figure 2: Young healthy plants with green leaves during roving survey 2023

sp. *ciceris* isolates obtained from chickpea-infected samples gathered from various regions of India. The variations in morphological traits such as conidia size, shape and colour as well as chlamydospore size, shape and colour across all isolates, was recorded. Venkataramanamma et al. (2021) isolated thirty-two isolates of *Fusarium oxysporum* f. sp. *ciceris* from six districts of Andhra Pradesh and three districts of Telangana. The isolates into three groups based on wilting percentage in pathogenicity tests. These isolates exhibited differences in cultural characteristics such as mycelium color, margin, texture, pigmentation, as well as morphological traits like macroconidia and microconidia length, width and chlamydospore diameter.

3.2. Identification of pathogen

The cultural and morphological examinations were conducted in accordance with the specified procedures and the obtained results were compared to the standard description of *Fusarium oxysporum* f. sp. *ciceris*.

3.2.1. Cultural characterization

Cultural characteristics of the isolated *Fusarium oxysporum* f. sp. *ciceris* were documented after incubation for seven days on potato dextrose agar medium at room temperature

(28±2°C). The analysis revealed that mycelial growth on the potato dextrose agar media exhibited traits consistent with *Fusarium oxysporum* f. sp. *ciceris*, appearing as mycelium color, texture, pigmentation, type and speed of growth (table 1 and figure 3).

Table 1: Cultural characters of the fungi on potato dextrose agar medium

Sl. No.	Characters	On Potato Dextrose Agar medium
1	Mycelium	Superficial (figure 3)
2	Colony colour	Pinkish white
3	Colony texture	Cottony
4	Pigmentation	Light orange
5	Type of growth	Radial
6	Speed of growth	Fast

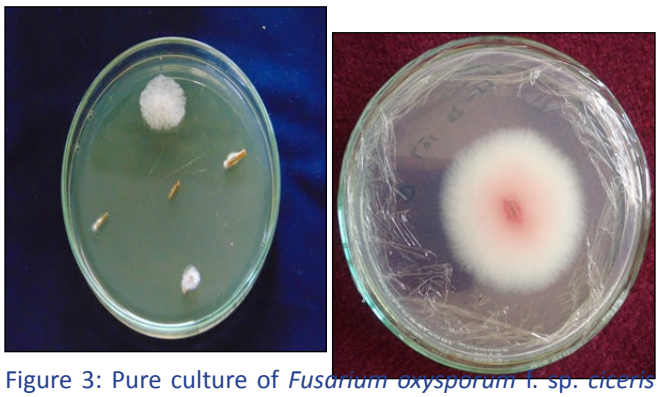

Figure 3: Pure culture of *Fusarium oxysporum* f. sp. *ciceris* isolate

3.2.2. Morphological Characterization

Additionally, the morphological assessment identified the *Fusarium oxysporum* f. sp. *ciceris* as possessing a macroconidia, microconidia and chlamydospore (Table 2 and Figure 4).

Table 2: Morphological characters of the *Fusarium oxysporum* f. sp. *ciceris*

Sl. No.	Characters	Results
1	Examination of infected tissue	Vascular tissues show brown to black discoloration
2. Conidia		
	Macroconidia	Sickle shape
	Microconidia	Oval shape
	Chlamydospores	Thick walled

3.3. Pathogenicity test and symptom development

To confirm the pathogenicity of the isolated pathogen (following Koch's postulates) from infected plant, a pathogenicity test was conducted in a net-house environment (figure 5). The earthen pots of (12×10 cm) diameter filled with three kg steam sterilized soil. The mass multiplied fungus

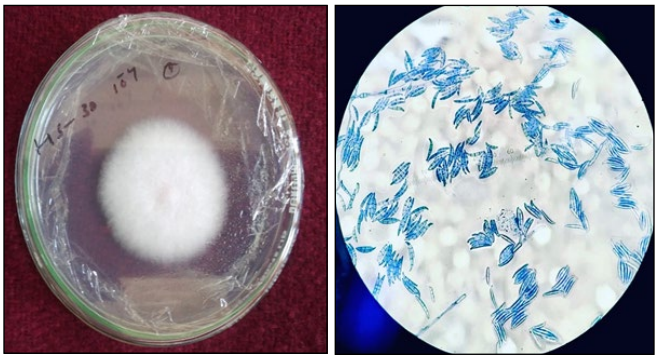

Figure 4: Macroconidia of *Fusarium* isolate

was added to these pots @2 g kg^{-1} (10^8 cfu g^{-1}) soil and mixed thoroughly for uniform distribution. The pots were watered lightly and was incubated for 4 days. Surface sterilized seeds of chickpea cultivar JG-62 were sown in these pots (5 seeds pot^{-1}) and three replications was maintained. The pots were regularly watered to maintain sufficient moisture needed by the plants. Wilt symptoms developed were observed for 30 days after sowing and the per cent disease incidence

a. *Fusarium oxysporum* f. sp. *ciceris*

b. Mass multiplied *F. oxysporum* f. sp. *ciceris* inoculum on sorghum grain

Figure 5: Continue...

c. Pathogen d. Control e. Infected

Figure 5: Pathogenicity test of *Fusarium oxysporum* f. sp. *ciceris*

was calculated. These findings are consistent with previous research, Pathogenicity test of *Fusarium oxysporum* f. sp. *ciceris* and *Fusarium redolens* isolates produced the greatest disease incidence on JG-62 and *Bivenij* cvs, whereas *Fusarium hostae*, *Fusarium equiseti* and *Fusarium acuminatum* isolates caused the least Younesi et al. (2021).

Studies on *Fusarium oxysporum* and its impact on crops like chickpeas and bananas hold profound scientific and contemporary relevance, particularly in the context of rural agricultural territories in developing countries. *Fusarium* wilt diseases caused by *Fusarium oxysporum* pose significant threats to global food security (Martinez et al., 2023). Chickpeas and bananas are staple crops for millions of people worldwide, especially in developing nations, where they contribute significantly to nutrition and livelihoods. Understanding the pathogenicity mechanisms of *Fusarium oxysporum* in these crops is crucial for developing effective disease management strategies to mitigate yield losses and ensure food availability for vulnerable populations (Olivares et al., 2021a; Campos et al., 2023). Comparative studies between *Fusarium* wilt diseases in chickpeas and bananas offer valuable insights into the variability of pathogen-host interactions across different crops. Despite being caused by the same fungal species, *Fusarium* wilt diseases may exhibit distinct symptoms, modes of transmission, and genetic variability in different host plants (Olivares et al., 2021b; Campos, 2023). By comparing these diseases in chickpeas and bananas, researchers can elucidate commonalities and differences in disease progression, which can inform the development of targeted control measures tailored to specific crops and agro-ecosystems (Vega et al., 2022; Viloria et al., 2023). Moreover, investigating the influence of agro-environmental factors on *Fusarium* wilt diseases in rural agricultural territories of developing countries is essential for sustainable crop production (Montenegro et al., 2021a; Olivares et al., 2022a). Factors such as soil health, climate conditions, irrigation practices and agronomic management can significantly affect the severity and spread of *Fusarium* wilt diseases (Montenegro et al., 2021b; Olivares et al., 2022b). Understanding how these factors interact with *Fusarium oxysporum* and host plants is crucial for implementing context-specific disease management strategies that optimize resource use, minimize environmental impacts and enhance agricultural resilience in the face of climate change and other challenges (Rodriguez-Yzquierdo et al., 2023a). Furthermore, studying *Fusarium* wilt diseases in chickpeas and bananas contributes to the broader scientific understanding of fungal plant pathogens and their evolutionary dynamics (Rodriguez-Yzquierdo et al., 2023b). *Fusarium oxysporum* is known for its genetic diversity and ability to adapt to different environmental conditions, including host plants and agricultural practices (Olivares et al., 2022c). By unraveling the genetic mechanisms underlying *Fusarium oxysporum* pathogenicity and host specificity, researchers can gain insights into fungal evolution, population dynamics, and potential avenues for disease control through breeding or biotechnological interventions (Olivares et al. 2020; Pitti et al. 2021). Studies on *Fusarium oxysporum* in chickpeas and bananas, along with the exploration of agro-environmental factors in rural agricultural territories of developing countries (Hernandez et al. 2020), are essential for addressing pressing challenges in global agriculture (Hernandez et al. 2018a; Hernandez and Olivares, 2019). By integrating scientific knowledge with practical applications, such research endeavors contribute to the development of sustainable farming practices, resilient food systems and improved livelihoods for millions of people reliant on these vital crops (Hernandez et al., 2018c; Hernandez et al., 2018b; Hernandez and Olivares, 2020).

4. Conclusion

The investigation revealed that pathogen grown on PDA media exhibited traits consistent with *FOC*, appearing as mycelium colour, texture, pigmentation, type and speed of growth as cultural characteristics and the *FOC* as possessing a macroconidia, microconidia and chlamydospore as morphological characteristics. Furthermore, a pathogenicity test was conducted on the chickpea cultivar JG-62, cultivated in pots under controlled conditions was successfully satisfying Koch's postulates.

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