



ISSN (E): 2277-7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.23  
TPI 2023; 12(10): 540-543  
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[www.thepharmajournal.com](http://www.thepharmajournal.com)  
Received: 02-08-2023  
Accepted: 07-09-2023

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## Eco-friendly management of wilt caused by *Fusarium oxysporum* f.sp. *ciceri* in chickpea under *in-vitro* condition

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### Abstract

Chickpea (*Cicer arietinum* L.) is one of the major rabi pulse crop and is a cheap source of protein. It has also advantages in the management of soil fertility particularly in dry lands and the semiarid tropics. Despite of low productivity of chickpea is attributed to *Fusarium* wilt disease which caused by obligate bio-troph *Fusarium oxysporum* f. sp. *ciceri* is consider one of the major limiting factor. Experiment was conducted *in-vitro* efficacy of bio-agents and phytoextracts against *fusarium oxysporum* f.sp. *ciceri*. The experimental result revealed that mycelium diameter of *fusarium* fungus was recorded higher from the isolate (IFOCA-I). The maximum sporulation recorded at pH range of 6.0–6.5 while excessive growth at pH 6.0. The disease incidence range was 1.1–35.56 percent and peak disease incidence was observed during the study period. The disease incidence was significantly, whoever negatively correlated with maximum and minimum temperature and pH.

**Keywords:** Chickpea, wilt, *Fusarium oxysporum* f. sp. *ciceri*, biocontrol agents

### Introduction

Chickpea (*Cicer arietinum* L.) is one of the major pulse crops, belongs of the family leguminosae. It is also known Bengal gram. Chickpea is a cheap source of protein compared to animal protein. Chickpea also has advantages in the management of soil fertility, particularly in dry lands and the semiarid tropics (Patra and Biswas, 2017) [6]. Low yield of chickpea is attributed to several diseases and insect. Despite of different diseases, *Fusarium* wilt disease is most important disease of chickpea causes severe damage of crop. Vascular wilt caused by an important obligate biotroph *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Matuo & K. Sato, is consider one of the limiting factor for its low productivity. Although the disease is wide spread in the chickpea growing areas of the world, it is most prevalent in the Mediterranean Basin and the Indian subcontinent region. Chickpea crop is attacked by 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and phytoplasma) across the world. Among all, only a few of them have the potential to devastate the crops. Some of the serious diseases in order of their importance are wilt, dry root rot, collar rot, *Colletotrichum* blight, *Alternaria* blight respectively (Nandeesh et al., 2020) [4]. Wilt is one of the major soil or seed borne disease of chickpea. The pathogen is both seed and soil borne; facultative saprophyte and can survive in soil up to six years in the absence of a susceptible host. The spores of fungus enters the vascular system of plant via the roots. It produces enzymes that obstruct cell wall and block the plant's transport system. Discoloration of the internal tissues progresses from roots to the arial parts of the plant. That causes chlorosis of leaves and shrunken stems both above and below ground level ultimately leads to the death of plant. Bio-agent and botanicals belonging to various groups recommended for the management of *fusarium* wilt. Generally, farmers are using only the chemicals for managing the disease, but it has negative impact on the environment as well as develops resistant in the pathogen. Management of soil borne pathogens with fungicides has been attempted for long time. However, it is difficult to manage these diseases economically with fungicides alone because of their soil borne nature and wide host range. Biological control of these pathogens therefore, is an alternative possibility. Mass multiplication, efficacy of the bioagents and delivery system of bioagents have been considered the major constraints in the success of the bioagents.

## Materials and Methods

An experimental survey was conducted at various village locations in Raisen, Madhya Pradesh in chickpea was grown in larger areas. Chickpea plants root and shoot showing characteristic of fusarium wilt collected from Mendua, Chandalakhedi, Kirat Nager, Goklakundi, Chiklod, Kanora, Barbatpur, Khoha, Bhuri Tekri and Bordha villages during 2020-21 and 2021-22. Wilt infected chickpea roots and stems were kept in rough, dry envelopes and marked clearly for location, variety etc. Naturally, infected plants with disease symptoms were collected and brought to the laboratory through (Koch's postulates methods, 1883) [2] were used to confirm that the pathogen was *Fusarium oxysporum* f. sp.

(*Cicer arietinum* L.). To remove excess surface moisture, the samples were dried in the shade for 24 hours. After drying, the samples were placed in proper envelopes in a BOD incubator and kept at 6 to 8 °C for isolation. Collection of diseases plant sample should be collected for further studies. The pathogen *Fusarium oxysporum* f.sp. (*Cicer arietinum* L.) Was isolated from collected disease sample through agar plate method (PDA) after the isolation fungus should be purified through single spore isolation technique, identified, Cultural and morphological characteristics were studied according to previous results were reported by earlier works. Pathogenicity test was confirmed according to the Koch's Postulates methods as *Fusarium oxysporum* f.sp. (*Cicer arietinum* L.).

**Table 1:** Sample location of fusarium wilt of chickpea

S.No.	Village name	Altitude	Longitude
1.	Mendua	23.1220° N	77.5433° E
2.	Chandalakhedi	23°05'37.3N	77°43'39° E
3.	KiratNager	23.4058° N	77.4874° E
4.	Goklakundi	23.1173° N	77.5763° E
5.	Chiklod	23.1047° N	77.7230° E
6.	Kanora	23.1360° N	77.6079° E
7.	Barbatpur	23.1345° N	77.7195° E
8.	Khoha	23.4726° N	77.5906° E
9.	BhuriTekri	23.1331° N	77.7395° E
10.	Bordha	23.1178° N	77.7309° E

## Results and Discussion

### Disease severity of *Fusarium oxysporum* in chickpea

The data presented in table-2 experimental result revealed that the disease severity of fusarium wilt varied from 24.67 to 42.00 percent during 2020-21 and from 19.67 to 47.00 percent during 2020-21 in different agro-climate conditions. The

highest severity of the disease was recorded in the farmer's field of Kanora village during 2021-22 and Gokla Kundi during 2020-21, and the lowest in the farmer's field of Bordha during both of the years similar result found that (Dubey *et al.*, 2010) [1].

**Table 2:** Disease severity of *fusarium oxysporum* in chickpeas in various villages (Pooled data of 2021 and 2022)

Village name	Altitude	Longitude	Disease severity	
			2020-21	2021-22
Mendua	23.1220° N	77.5433° E	30.33	36.67
Chandalakhedi	23°05'37 N	77°43'39.9E	27.67	32.33
Kirat Nager	23.4058° N	77.4874° E	33.67	33.67
Gokla kundi	23.1173° N	77.5763° E	42.00	47.00
Chiklod	23.1047° N	77.7230° E	29.33	30.67
Kanora	23.1360° N	77.6079° E	41.00	37.67
Barbatpur	23.1345° N	77.7195° E	29.00	27.00
Khoha	23.4726° N	77.5906° E	35.67	35.67
Bhuri Tekri	23.1331° N	77.7395° E	38.67	46.33
Bordha	23.1178° N	77.7309° E	24.67	19.67
C.D. at 5 %			6.741	7.284
SEm (±)			2.251	2.433

### Colony diameter of *fusarium oxysporum*

The significantly largest colony diameter (91.24mm) noticed in Mendua village followed by second-best medium produced a colony diameter of 90.09 mm (Chandalakhedi), then other media. IFOCA-IV isolate from Koklakundi respectively. The smallest colony diameter (79.15mm) recorded in Gokula

Kundi village and significantly lower to each other treatments. The test fungus sporulated in all of the media that were used, but isolate IFOCA-I in PDA showed outstanding sporulation. These results are well supported by the observations made by (Shukla and Dwivedi, 2017) [7].

**Table 3:** Effect of different location on colony diameter of *Fusarium oxysporum* (Pooled data of 2021 and 2022)

S. No.	Isolates	Location	Pooled Colony Diameter (mm) (7 DAI)	Colony character	Pigmentation
1.	IFOCA-I	Mendua	91.24	White cottony mycelium	Pale yellow
2.	IFOCA-II	Chandalakhedi	90.09	White compact mycelium	Pink
3.	IFOCA-III	Kirat Nager	81.25	White compact mycelium	Pink
4.	IFOCA-IV	Gokla kundi	79.15	White fluffy mycelium	Light yellow
5.	IFOCA-IX	Bhuri Tekri	83.33	White sparse growth mycelium	Pale yellow
6.	IFOCA-V	Chiklod	86.47	White sparse growth mycelium	Pink
7.	IFOCA-VI	Kanora	85.62	White fluffy mycelium	Dark yellow
8.	IFOCA-VII	Barbatpur	87.54	White compact mycelium	Light yellow
9.	IFOCA-VIII	Khoha	89.28	White cottony mycelium	Straw yellow
10.	IFOCA-X	Bordha	86.38	White compact mycelium	Dark yellow

### Effect of pH and temperature

All of the pH levels tested resulted in growth and sporulation of the test fungus, however they peaked at pH 6.0 (85.63mm) after 168 hours of inoculation. Table 8 also indicated that pH 6.5 (79.63 mm) and pH 7.0 (76.18 mm) were beneficial. By changing the pH level from the level of 6.0, the test fungus' development was inhibited. Pathogens cannot thrive or produce spores at the most acidic or alkaline pH levels. The temperatures ranging from 10 to 40 °C. After 7 days, it was observed that there was a sizable variance in these isolates'

development at various temperatures. The highest mycelial growth was observed at 30 °C (89.36 mm), it was followed by temperatures of 25 °C (65.66 mm), 20 °C (49.33 mm), and 35 °C (21.87 mm), while no mycelial growth was observed at temperatures of 10 °C and 40 °C. Target pathogen development was most advantageous at temperatures between 25 and 30 °C. The pathogen grew most quickly and produced more spores when the temperature was 30 °C. Similar findings were observed by (Suryawanshi *et al.*, 2013)<sup>[9]</sup>.

Effect of various pH and temperature on radial growth and sporulation of *Fusarium oxysporum* (Pooled data of 2021 and 2022)

pH	Colony diameter (mm) after 168 hrs	Sporulation	Temperature (°C)	Colony diameter (mm) after 168 hrs	Sporulation
5.0	64.33	++	10	-	+
5.5	71.33	+++	15	27.63	++
6.0	85.63	++++	20	49.33	++
6.5	79.63	++++	25	65.66	+++
7.0	76.18	+++	30	89.36	++++
7.5	52.33	+++	35	21.87	+
8.0	46.00	++	40	-	+
CD at 5%	2.342	-	-	1.873	-
SEm(±)	0.970	-	-	0.642	-

### Growth habit character

On incubated seeds on blotters, the fungus formed white to deep light orange aerial mycelium and sporodochia. Mycelium was widely branched and covered the entire seed; it ranged in colour from white to light pink. Sporobochia were hardly developed, but when they did, they were entirely encased in aerial mycelium. Small, dry, fake heads generated micro conidia on short, solitary micro conidiophores. Hyaline, 1-2 celled, oval to cylindrical, straight to slightly curved, and measuring 2.5-3.5 x 5-11 m, they also had 3-5 septa, fusoid with pointed ends, and were produced on branched macro conidiophores. Globule to sub globular of thick-walled, smooth-surfaced chlamydospores was only developed intercalary in the old culture.

**Mycelial characteristics:** On incubated seed, the fungus formed white to light orange aerial mycelium and sporodochia. The mycelium is thickly branching, white to light pink in colour, and completely envelops the seed. Sporodochia are infrequently generated, but when they do exist, they are entirely encased in the aerial mycelium.

### Conidial figures

▪ **Micro-conidia:** These tiny, dry conidia are widely distributed and generated on short, unbranched monophialides (micro conidiophores). Hyaline, single-celled, 2.5-3.5x5- 11 m, oval to cylindrical, straight to

slightly curved, and hyaline micro conidia are these characteristics.

- **Macro-conidia:** These are generated on branched macro conidiophores and are few. They are 3.5-4.5x25-65 mm in size, have hyaline, fusoid pointy ends, 3-5 septa, and germinate by generating germ tubes.
- **Chlamydospores:** Chlamydospores can be generated singly or in pairs and are typically intercalary. They have smooth surfaces, thick walls, and globose to subglobose shapes. On the hyphae, a swelling that resembles chlamydospores is frequently observed.

### Conclusion

The present study, it were found that The disease severity varied from 19.67 to 47.00 percent during both the years and maximum disease infestation was observed from Kanora village during the first year and in second year from Gokla Kundi village. Mycelium diameter of *Fusarium* fungus was recorded higher from the isolate (IFOCA-I). The maximum sporulation recorded at pH range of 6.0–6.5 while excessive growth at 6.0 pH.

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