



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2021; 10(4): 46-50

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www.thepharmajournal.com

Received: 03-02-2021

Accepted: 16-03-2021

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Variability among the isolates of *Fusarium oxysporum* f. sp. *ciceri* in Nimar region of Madhya Pradesh

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Abstract

Chickpea (*Cicer arietinum* L.) is a major pulse crop and necessary component of in human diet. It is an annual plant of the Leguminaceae, cultivated for its nutritious seeds.. Chickpea is native of India and cultivated in tropical, subtropical and semi-arid climate. It is ranked 3rd after French bean (*Phaseolus vulgaris* L.). Chickpea is rich in minerals, vitamins (2.44%), carbohydrate (61.5%), protein (25.3-28.9% after dehulling) and fiber (3-4%). *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* is the most serious disease of chickpea. 14 isolates of *Fusarium oxysporum* f. sp. *ciceri* (FOC), collected from different blocks of Khargone and Khandwa district of Nimar region of Madhya Pradesh and cultural and morphological variability is studies among them. The radial growth of isolates ranged from 46.33 mm to 55.00 mm at seven days after inoculation on PDA medium. Cultural studies of all isolates of *Fusarium oxysporum* f. sp. *ciceri* resulted that isolates differ in the growth of mycelium, form of colony, colour of colony, elevation of colony, margin of colony, cultural pattern and pigmentation of colony on PDA medium. The size of macro-conidia was ranged varied from 6.71 × 1.78 µm to 12.30 × 2.10 µm., in micro-conidia was from 2.06 × 1.55 µm to 4.95 × 2.10 µm. The number of septa in macro-conidia was mostly 1-4 and micro-conidia are mostly no septum and some are 0-1. Conidia are hyaline. Shape of most macro-conidia is Elongated with blunt end and sickle shape while, micro-conidia are round to oval shape.

Keywords: Chickpea, *Fusarium oxysporum* f. sp. *ciceri*, Cultural, macroconidia, pigmentation

Introduction

Chickpea (*Cicer arietinum* L.) is the leading leguminous crop of South Asia and the third largest crop after French bean (*Phaseolus vulgaris* L.). Chickpea can also known as “Bengal gram”, “Egyptian pea” or “Garbanzo bean”, annual plant of the Leguminosae mainly cultivated for its nutritious seeds. It is mainly cultivated in tropical, subtropical and semi-arid climate. Chickpea has ample amount of proteins, vitamins and minerals. Chickpea seeds contains oil (4.8 to 5.5%), ash (3%), carbohydrate (61.5%), vitamins (2.44%), protein (25.3 – 28.9% after dehulling) ^[1], fiber (3-4%), phosphorus (0.3%) and calcium (0.2%) ^[2, 3]. A recent report by FAOSTAT (2020) stated that, the total production of chickpea in the world was 17.19 M tonnes and its productivity was 965 kg per hectare, for which a total area of 17.81 M hectare was available ^[4]. Globally, India occupies the first position in chickpea production with the contribution of 65 – 67%, followed by Australia and Pakistan. In India, chickpea sown in almost all states viz., Madhya Pradesh, Uttar Pradesh, Andhra Pradesh, Karnataka, Maharashtra etc., in which Madhya Pradesh is the leading state with the contribution of 42%. In India, Butler studied wilt disease for the first time in 1918, and determine that it is a soil borne disease which caused by *Fusarium oxysporum* f. sp. *ciceri*. This disease is spread in all over the globe and particularly in the South Asia, Europe and USA ^[5]. The roots of the wilting plants don't show any external rotting but when split open vertically, dark brown discoloration of internal xylem is seen ^[6]. In favorable conditions, the wilt infection damages the whole crop and cause 100% yield loss ^[7]. Present investigation was undertaken with a view to know the cultural and morphological variability of *Fusarium oxysporum* f. sp. *ciceri* from 14 locations of Nimar region of M.P.

Materials and Methods

Experimental site

The *In-vitro* experiments were conducted in Laboratory at Department of Plant Pathology, B.M. College of Agriculture, Khandwa, RVSKVV, Gwalior (M.P.).

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Isolation and Purification

A roving survey of chickpea wilt infected crop was carried out in some of the chickpea growing areas of Nimar regions of M.P. covering 14 locations *viz.*, Khargone, Bhikangaon Jhiranya, Gogawan, Bhagwanpura, Segaon, Kasrawad, Maheshwar, Barwaha, Khandwa, Khalwa, New Harsud, Pandhana and Punasa blocks (table 1). Chickpea plants exhibiting typical symptoms of wilt (*Fusarium oxysporum* f. sp. *ciceri*) disease were collected from the chickpea fields from each location and brought to the laboratory for further studies. Infected plant was surface sterilized using 0.1% mercuric chloride and washed thrice with distilled water to remove traces of HgCl₂. The diseased part was cut into pieces along with healthy tissue. These pieces were placed aseptically on sterilized Potato Dextrose Agar (PDA) medium in Petri plates. Pure culture was done by transfer of a pinch of mycelium on sterilized Potato Dextrose Agar medium in Petri plates and incubated in BOD for further studies and designated them as KNF-1, KNF-2, KNF-3, KNF-4, KNF-5, KNF-6, KNF-7, KNF-8, KNF-9, KGF-10, KGF-11, KGF-12, KGF-13 and KGF-14 respectively. The isolates from different localities were identical on the basis of morphological characters with the help of monograph on *Fusarium* by Booth (1971) [8] and cultural characters with the help of colony morphology given by Microbiology Society.

Cultural and Morphological variability

Cultural variations within the isolates were studied with regard to growth of mycelium, form of colony, color of colony, elevation of colony, margin of colony, cultural pattern and pigmentation of colony. The Morphological characters were studied with regard to length and width of macroconidia and micro-conidia and their septation, formation of chlamydospores.

Pathogenicity test

Pathogenicity test of wilt pathogen (*Fusarium oxysporum* f. sp. *ciceri*) were conducted on variety RVKG-101 in pot containing wilt sick soil. The major symptoms observed as typical wilt, drooping of leaflets, petiole and rachis. The advanced stage of disease caused drying of entire plant. The *Fusarium oxysporum* was successfully isolated from the diseased samples. The disease symptoms were similar after the inoculation of same pathogen. Initially, the upper part of the plant sheds within one or two days and then entire plant got collapsed. When the stem of the infected plant opened vertically, the color of vascular part (xylem) appears brownish or blackish in color. The pathogen was similar in morphological a study which was performed after re-isolation.

This indicates that the *Fusarium oxysporum* f. sp. *ciceri* isolated from the natural wilted plant causes wilt disease in chickpea and produced typical wilting symptoms.

Result and Discussion

Isolation of Pathogen

After 4-5 days of inoculation, partially submerged, white dense growth with smooth margin mycelial mat was developed in the plates. After 10 to 15 days of incubation, conidia were developed which were initially bright white colored and minute in size and later turned buff white in color.

Cultural Variability in isolates

The cultural characters of different isolates isolated from diseased specimen collected from different 14 locations of Khargone and Khandwa district of Nimar region of M.P. were studied. The cultural characters *viz.*, growth of mycelium, form of colony and color of colony, elevation of colony, margin of colony, cultural pattern and pigmentation of colony were recorded. The data presented in the table 2 indicated that all the isolates showed wide variation in colony.

The isolates of *Fusarium oxysporum* f. sp. *ciceri* were classified into three categories on the basis of colony form. Three isolates showed filamentous type of colony (KGF-1, KGF-2, and KGF-6), seven isolates showed circular type of colony (KGF-3, KGF-4, KGF-7, KGF-9, KNF-10, KNF-11, KNF-12) and Irregular colony type in four isolates (KGF-5, KGF-8, KNF-13, KNF-14).

Isolates are further classified on the basis of elevation of colony. Umbonate type of elevation shown by three isolates (KGF-1, KGF-5, and KNF-10), crateriform elevation seven isolates (KGF-2, KGF-7, KGF-9, KNF-11, KNF-12, KNF-13, and KNF-14), convex type of elevation includes (KGF-3) and Flat elevation includes three isolates (KGF-4, KGF-6, and KGF-8).

Isolates were also grouped into two categories based colony margin *viz.*, entire and undulate. Entire margin of colony consists of ten isolates (KGF-1, KGF-2, KGF-3, KGF-4, KGF-6, KGF-7, KGF-9, KNF-10, KNF-11, KNF-12) and Undulate margin of colony consists of four isolates (KGF-5, KGF-8, KNF-13, KNF-14).

Further, isolates were classified on the basis of cultural pattern. Cottony pattern consists eight isolates (KGF-1, KGF-5, KGF-8, KGF-9, KNF-11, KNF-12, KNF-13, and KNF-14), concentric cottony pattern consisted of three isolates (KGF-2, KGF-7, KNF-10), Fluffy cottony pattern consists only one isolates (KGF-3) and appressed cultural pattern consists two isolates (KGF-4, KGF-6).

Table 1: Survey and collection of wilted plants of chickpea from different blocks of Khargone and Khandwa district

S.N.	Region (Blocks)	Isolate Code	Varieties	No. of samples collected	Soil type	Farming type	Cropping stage	Cropping pattern
1.	Khargone	KGF-1	Vishal	8	Black and medium black	Rainfed and Irrigated	Flowering	Sole
2.	Bhikangaon	KGF-2	Vishal	6	Light black and deep black	Irrigated and Rainfed	Flowering	Sole
3.	Jhiranya	KGF-3	RVG-203	6	Light black and black	Irrigated and Rainfed	Podding	Sole
4.	Gogawan	KGF-4	Kabuli	7	Black and medium black	Rainfed and Irrigated	Flowering	Mixed
5.	Bhagwanpura	KGF-5	Kabuli	6	Light and medium black	Irrigated and Rainfed	Flowering	Mixed
6.	Segaon	KGF-6	RVG-203	6	Light black and black	Rainfed and Irrigated	Flowering	Sole
7.	Kasrawad	KGF-7	Vishal	7	Medium black and black	Irrigated and Rainfed	Podding	Sole
8.	Maheshwar	KGF-8	RVG-202	6	Medium and deep black	Irrigated and Rainfed	Flowering	Sole
9.	Barwaha	KGF-9	RVG-202	7	Medium and light black	Irrigated and Rainfed	Podding	Sole
10.	Khandwa	KNF-10	Kabuli	8	Light black and black	Rainfed and Irrigated	Flowering	Sole
11.	Khalwa	KNF-11	Uttam	5	Medium black and black	Irrigated	Flowering	Sole
12.	New Harsud	KNF-12	JG-130	6	Light black	Irrigated	Podding	Sole
13.	Pandhana	KNF-13	RVG-203	6	Deep light and light black	Rainfed and Irrigated	Flowering	Mixed

14.	Punasa	KNF-14	Kabuli	7	Light and medium black	Irrigated and Rainfed	Flowering	Sole
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KG = Khargone, KN = Khandwa, F = *Fusarium oxysporum* f. sp. *Cicero*

Table 2: Variability of Cultural characters of *Fusarium oxysporum* f. sp. *cicero* isolates from Khargone and Khandwa district

S.N.	Region (Blocks)	Isolate Code	Growth of Mycelium (in mm)*	Form of colony	Elevation of colony	Margin of colony	Cultural pattern	Colour of colony	Pigmentation of colony
1.	Khargone	KGF-1	53.66	Filamentous	Umbonate	Entire	Cottony	White	Reddish brown
2.	Bhikangaon	KGF-2	52.00	Filamentous	Crateriform	Entire	Concentric cottony	Creamy white	Light Brown
3.	Jhiranya	KGF-3	54.00	Circular	Convex	Entire	Fluffy cottony	Creamy white	Light yellow
4.	Gogawan	KGF-4	49.66	Circular	Flat	Entire	Appressed	Creamy white	Light Brown
5.	Bhagwanpura	KGF-5	47.00	Irregular	Umbonate	Undulate	Cottony	White	Brown yellow
6.	Segaon	KGF-6	50.00	Filamentous	Flat	Entire	Appressed	White	Brown yellow
7.	Kasrawad	KGF-7	53.33	Circular	Crateriform	Entire	Concentric cottony	White	Pale yellow
8.	Maheshwar	KGF-8	55.00	Irregular	Flat	Undulate	Cottony	Creamy white	Reddish brown
9.	Barwaha	KGF-9	49.66	Circular	Crateriform	Entire	Cottony	White	Brown yellow
10.	Khandwa	KNF-10	50.66	Circular	Umbonate	Entire	Concentric cottony	Creamy white	Light brown
11.	Khalwa	KNF-11	49.33	Circular	Crateriform	Entire	Cottony	White	Pale yellow
12.	New Harsud	KNF-12	46.33	Circular	Crateriform	Entire	Cottony	White	Light yellow
13.	Pandhana	KNF-13	48.00	Irregular	Crateriform	Undulate	Cottony	Creamy white	Reddish Brown
14.	Punasa	KNF-14	53.33	Irregular	Crateriform	Undulate	Cottony	White	Brown yellow
S.Em(±)			1.12	---	---	---	---	---	---
C.D. at 5%			3.24	---	---	---	---	---	---

* Means of three replications of mycelium growth

Similarly, isolates are classified on the basis of Colour of colony. White colour colony observed in eight isolates (KGF-1, KGF-5, KGF-6, KGF-7, KGF-9, KNF-11, KNF-12, KNF-14), Creamy white color colony observed in six isolates (KGF-2, KGF-3, KGF-4, KGF-8, KNF-10, KNF-13).

Next order was classified on the basis of Pigmentation. Reddish brown colony were found in three isolates (KGF-1, KGF-8, KNF-13), Light brown in three isolates (KGF-2, KGF-4, KNF-10), Light yellow colony were found in two isolates (KGF-3, KNF-12), Brown yellow colony were found in four isolates (KGF-5, KGF-6, KGF-9, KNF-14) and Pale yellow colony were found in two isolates (KGF-7, KNF-11).

The data presented in the table 2 indicated that all the isolates showed wide variation in colony diameter. The size of colony varied from 46.33 – 55.00 mm, where highest mycelial growth observed in KNF-8 (55.00 mm) and least mycelial growth is measured KNF-12 (46.33 mm).

The elevations of the colony were not found more discussed by previous workers so that is may be somewhat new generation of the categories of the isolates but colony color and the pigmentation on PDA medium were found very close to the findings of Mathivathani *et al.* (2019) [9] who discussed Previous workers revealed that isolates of *Fusarium oxysporum* collected from different districts of T.N. had fine white, pinkish, white with orange and white with yellowish tinge mycelium color. Findings are also in accordance with the previous findings as far as concerned with topography of colony, he recorded flat, raised fluffy, rings and droplets on mycelium. Venkataramanamma *et al.* (2019) [10] also studied isolates of *Fusarium oxysporum* f. sp. *cicero* from different districts of A.P. and studied the range of pigmentation and cultural characters are in accordance to the present findings.

Morphological characters

Variation in morphological characters *viz.*, size, shape and their septation of macro-conidia and micro-conidia and formation of chlamydospores of different isolates of *Fusarium oxysporum* f. sp. *cicero* have been studied using of PDA medium and depicted in table 3, figure 1 and plate1-2.

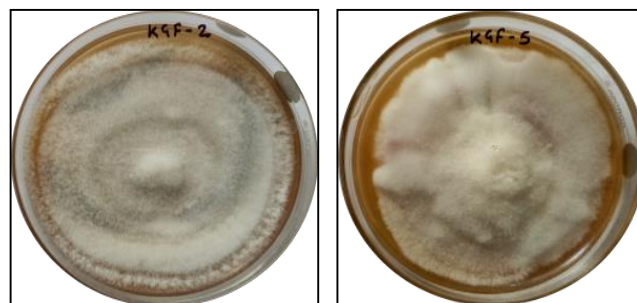


Plate 1: Cultural plate of *Fusarium oxysporum* f. sp. *Cicero*

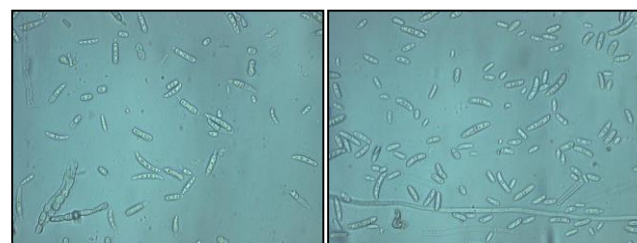


Plate 2: Microscopic view of *Fusarium oxysporum* f. sp. *cicero*

A significant conidial variation was observed amongst *Fusarium oxysporum* f. sp. *cicero* pathogen collected from different locations. Macro-conidia were noticed slightly curved, long, straight, slender, elliptical with sharp ends. All isolates are hyaline. Size of macro-conidia varied from $6.71 \times 1.78 \mu\text{m}$ to $12.30 \times 2.10 \mu\text{m}$. The maximum size of length and width of macro-conidia recorded in isolate KGF-8 ($12.30 \mu\text{m} \times 2.10 \mu\text{m}$), followed by KGF-2 ($10.47 \times 2.37 \mu\text{m}$), and KGF-5 ($10.36 \times 2.31 \mu\text{m}$) whereas minimum size of conidia was noticed in KGF-7 ($6.71 \times 1.78 \mu\text{m}$), followed by KGF-1 ($7.46 \times 1.96 \mu\text{m}$) and KGF-9 ($7.54 \times 2.03 \mu\text{m}$).

Similarly we found in our experiment that micro-conidia are ellipsoidal in nature. Microconidia size varied from $2.06 \times 1.55 \mu\text{m}$ to $4.95 \times 2.10 \mu\text{m}$. The maximum size of length and width of micro-conidia observed in isolate KGF-8 ($4.95 \times 2.10 \mu\text{m}$) followed by KGF-3 ($4.24 \times 1.80 \mu\text{m}$) and KGF-5

($4.18 \times 1.90 \mu\text{m}$), whereas smallest size of conidia was noticed in KGF-9 ($2.06 \times 1.55 \mu\text{m}$) followed by KNF-12 ($2.39 \times 1.86 \mu\text{m}$).

Apart from length and width, we also studied septation in macro-conidia and micro-conidia. In macro-conidia, number of septa varied from 1 to 4. Among 14 isolates, KGF-8 had recorded maximum number of septa varied from 2 to 4, while three isolates viz., KGF-2, KGF-6 and KNF-14 had 1 to 3 septa and ten isolates viz., KGF-1, KGF-3, KGF-4, KGF-5, KGF-7, KGF-9, KNF-10, KNF-11, KNF-12 and KNF-13 had 1 to 2 septa. Morphological study of micro-conidia showed that septa are either absent or one in number. Among 14 isolates, seven isolates viz., KGF-1, KGF-3, KGF-5, KGF-7, KGF-8, KNF-11, and KNF-14 had one septa (0-1), while remaining seven isolates viz., KGF-2, KGF-4, KGF-6, KGF-9, KNF-10, KNF-12, and KNF-13 had zero or no septa.

The results are in agreement with those reported by variability of morphological characters of different isolates with respect to size and shape of macro conidia and micro conidia and their septations, mycelium type and formation of chalymadospores are in conformity with Patra and Biswas (2016) [11] discussed that macro-conidia range from $13-15 \times 2-3 \mu\text{m}$ to $15-19 \times 3-4 \mu\text{m}$, in micro-conidia was from $3-4 \times 1-2 \mu\text{m}$ to $5-6 \times 2-3 \mu\text{m}$. The number of septa in macro-conidia was mostly 2-3 and micro-conidia had no septum and some had 0-1. Dubey *et al.* (2010) [12] reported the septation of micro and macro conidia which is in accordance to present findings. He noted the size of micro-conidia varied from $5.1-12.8 \times 2.5-5.0 \mu\text{m}$ and macro-conidia ranged from $16.5-37.9 \times 4.0-5.9 \mu\text{m}$ with 1-5 septarions which also supported the present findings.

Table 3: Size and their septation of conidia of different isolates of *Fusarium oxysporum* f. sp. *Cicero*

S. N.	Location	Isolate code	Dimension						Colour
			Macro conidia			Micro conidia			
			Size (μm) L x W	Septation	Shape	Size (μm) L x W	Septation	Shape	
1.	Khargone	KGF-1	7.46×1.96	1-2	Sickle shape	3.54×1.63	0-1	Round	Hyaline
2.	Bhikangaon	KGF-2	10.47×2.37	1-3	Sickle shape	3.35×1.96	0	Round	Hyaline
3.	Jhiranya	KGF-3	9.20×1.57	1-2	Elongated with blunt end	4.24×1.80	0-1	Round to oval	Hyaline
4.	Gogawan	KGF-4	7.69×2.05	1-2	Sickle shape	2.84×1.57	0	Round	Hyaline
5.	Bhagwanpura	KGF-5	10.36×2.31	1-2	Sickle shape	4.18×1.90	0-1	Round	Hyaline
6.	Segaon	KGF-6	8.61×2.75	1-3	Sickle shape	2.86×1.73	0	Round to oval	Hyaline
7.	Kasrawad	KGF-7	6.71×1.78	1-2	Elongated sickle shape	2.86×1.86	0-1	Round to oval	Hyaline
8.	Maheshwar	KGF-8	12.30×2.10	2-4	Elongated with blunt end	4.95×2.10	0-1	Round to oval	Hyaline
9.	Barwaha	KGF-9	7.54×2.03	1-2	Elliptical with sharp ends	2.06×1.55	0	Round	Hyaline
10.	Khandwa	KNF-10	8.49×2.18	1-2	Sickle shape	2.85×1.56	0	Round	Hyaline
11.	Khalwa	KNF-11	7.59×2.39	1-2	Elongated with blunt end	2.67×1.64	0-1	Round to oval	Hyaline
12.	New Harsud	KNF-12	8.38×2.05	1-2	Sickle shape	2.39×1.86	0	Round	Hyaline
13.	Pandhana	KNF-13	9.31×2.02	1-2	Elongated sickle shape	2.46×1.56	0	Round	Hyaline
14.	Punasa	KNF-14	8.48×2.22	1-3	Elliptical with sharp ends	3.17×1.86	0-1	Round	Hyaline

(*) means of 5 replications

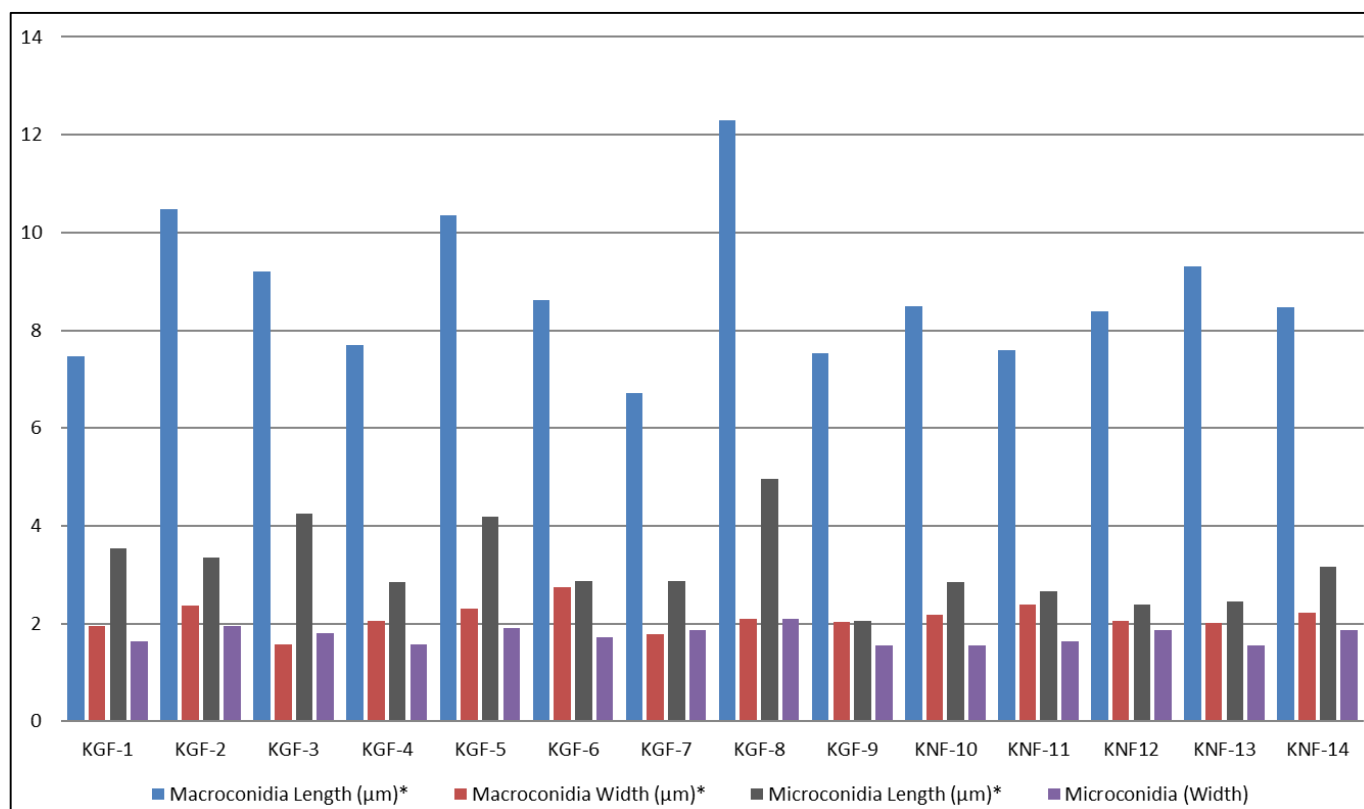


Fig 1: Characteristics of morphological variability of *Fusarium oxysporum* f. sp. *ciceri*

Conclusion

In present investigation 14 isolates of *Fusarium oxysporum* f. sp. *ciceri* were collected from different blocks of Khargone and Khandwa district of Nimar region of Madhya Pradesh and cultural and morphological variability was studied among them. The colony diameter varied from filamentous to circular to irregular colony type. The 14 isolates had four type of colony variation (umbonate, crateriform, convex and flat). The colony margin varied from entire to undulate type. Furthermore colony pattern varied from concentric cottony, fluffy cottony and appressed type colony growth pattern, having white color colony. Moreover, pigmentation among isolates varied from light brown to pale yellow in color. The colony diameter varied from 46.33 to 55.00 mm. In macroconidia, number of septa varied from 1 to 4.

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