WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 7, 1383-1397. Research Article ISSN 2277– 7105

MORPHOLOGICAL, PATHOGENIC AND MOLECULAR CHARACTERIZATION OF *FUSARIUM OXYSPORUM* CAUSING DRY ROOT ROT OF CHICKPEA FROM SANGLI DISTRICT OF MAHARASHTRA STATE

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Article Received on
21 April 2022,

Revised on 11 May 2022,
Accepted on 01 June 2022

DOI: 10.20959/wjpr20227-24447

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ABSTRACT

Fusarium dry root rot of chickpea is caused by *Fusarium spp*. Ten isolates of *Fusarium* were isolated from chickpea growing areas of Sangli district to study the cultural and morphological characters of *Fusarium* sp. From the survey, it was found that the highest PDI was recorded in Kavalapur 1 with 52.63%. The mycelial diameter of ten isolates was ranged from 6.2 to 9.0 cm at seven days of incubation on PDA. The highest colony diameter of 9.0cm was observed in FO 2 and FO 7 followed by FO5, FO8, FO9, FO4, FO6, FO10 and FO3. Minimum colony diameter was observed in FO1 with 6.1cm. Different media studies have shown that *Fusarium oxysporum* could grow well in PDA and PSA. All ten isolates have produced micro, macroconidia and chlamydospores. The size (µm) of microconidia ranged from (9.2- 11.8) x (3.2-4.6) and macroconidia is (15.7-24.4) x (3.8 – 5.0) and they are hyaline in color. There are 2-4 septations in macroconidia. Macroconidia are sickle shaped with pointed tips and microconidia are elongated oval in shape. Chlamydospores were seen on intercalary, terminal portion of mycelium and oval in shape. PCR amplification with universal primers of ITS1 and ITS4 yielded approximately at 560 bp. Sequencing of ITS regions followed by BLAST search confirmed the associated fungus as *Fusarium oxysporum*.

INTRODUCTION

Pulses are an important part of the daily diet for most indians as they contain 2 to 3 times more protein than cereals. Chickpea is the most important pulse food crop among major rabi pulses of India and belongs to family Leguminosae. Chickpea is not only important human

food but also used in traditional farming systems. According to (Sreegayathri *et al.*, 2018) in the dry land it fixes atmospheric nitrogen in the soil and increases soil fertility. It has very great nutritional value. Raw chickpea seeds contains per 100g: 357 calories, 140-440 mg Ca, 2-4.8 g ash, 190-382 mg P, 4.5-15.69 percent moisture 0.8 to 6.4 percent fat, 9mg Fe, 1.3-2.9 mg niacin, 0.12-0.33 mg riboflavin and 0- 225 µg β carotene. According to Chiranjeevi*et al*., 2002 chickpea is very richest source of fibre, fat and proteins. Chickpea is used as medicine for cholera, diarrhea, snakebite, warts and blood purification. Chickpea is most hypocholesteremic legume among all food legumes. Seeds are antibilious. Chickpea seeds are eaten fresh, roasted and boiled. Seed flour can be used to make soup, tasty food stuffs, bread and served as side dish.

In general, *Fusarium* dry root rot is caused by *Fusarium oxysporum* and served as a major reason for great loss on some economically important cucurbit crops like watermelon, cucumber. In chickpea, this *Fusarium* dry root rot was found to be caused by the pathogen *Fusarium oxysporum* (Sreegayathri *et al.*, 2018). The major symptoms of *Fusarium* dry root rot are damping off, seedling disease and dry root roting at any developmental stage of the crop. On matured plants the symptoms appeared as dull grey green leaves followed by yellowing of foliage, dry root roting during day hours and eventually leads to death. The characterisation of pathogen is important to decide on the plant protection measure and hence in this study, *Fusarium spp*. which causes *Fusarium* dry root rot in chickpea were isolated from different regions of Sangli, and their cultural and morphological characters were studied and the molecular characterisation was done. This will be helpful for the further studies related to *Fusarium* dry root rot of chickpea.

MATERIALS AND METHODS

A random field survey was conducted in the chickpea cultivating areas of Sangli district, percent disease incidence was calculated (PDI = No. of infected plants x100 / Total no. of plant assessed) and symptoms were collected to isolate the pathogen. The typical symptom of vascular discoloration was observed. The discolored portions were cut into small pieces and surface sterilized with 70% ethanol and then washed well with sterile water for three times. All these steps have done under the aseptic condition. For isolation of pathogen, Potato Dextrose Agar (PDA) medium was used. The PDA medium was added with Cefotaxime antibiotic to prevent the bacterial contamination. The infected bits were placed in sterile petri dishes containing PDA medium and incubated at laboratory condition (28±2°C). After five

days of incubation, pure culture of *Fusarium* was obtained by single spore isolation technique and maintained in PDA slant at 5°C for further study.

Pathogenicity

The pathogenicity of *Fusarium* was proved by the soil inoculation method. The virulent isolate FO2 was multiplied in the sand maize meal containing sand and ground maize at the ratio of 9:1. The mixture was slightly moistened with water and sterilized at 121oC at 15 psi for 2 hours. The fungus was inoculated into autoclaved medium and incubated for 15 days at room temperature for multiplication. Chickpea seeds (variety: Co 1) were raised in propagation tray. Potting soil (red soil: sand: cow dung manure @ 1: 1: 1 ratio) was sterilized in autoclave for 2 consecutive days. The inoculum on sand maize medium was incorporated into sterilized soil at the rate of 50 g kg-1of the soil. Twenty-five days old seedlings were transplanted into the pots and maintained under glasshouse conditions. Three replications were maintained and monitored regularly. Control plant was maintained without inoculating the pathogen. From the infected plants, pathogen was reisolated and Koch's postulates were proved.

Cultural and morphological characterization of pathogen

Ten isolates of *Fusarium* were grown on PDA for studying their cultural characters. Ten-millimeter mycelial disc of the fungus from actively growing culture plates were taken and placed on PDA media contained in (90mm) sterile petri dishes. They were incubated at room temperature for 7 days and observations were recorded on parameters like colony diameter, colony character, pigmentation and colony margin. The colony diameter (cm) was measured at 48, 72, 96, 144 and 168 hours after incubation. The experiment was laid out in completely randomized design with three replications. The colony diameter was measured from all the four sides. Spores of all the ten *Fusarium* isolates were collected. Spores were observed under high power objectives (40 X) and their images were captured. The average size of the spores was measured and shape of the spores were also recorded. For micro and macroconidia, length (µm) and breadth (µm) of twenty-five (25) spores for all the isolates were measured from fifteen days old culture.

Molecular characterization

DNA were extracted for all the isolates of *Fusarium* and PCR amplification was performed with universal primers ITS 1 (5' – CTTGGTCATTTAGAGGAAGTAA - 3') and ITS 4 (5' – TCCTCCGTTATTGA TATGC – 3'). PCR reactions were performed in 10µl final volume

containing 5µl of Taq 2x master mix (Ampliqon) which contains 1.5 Mm MgCl2, 2µl double distilled water, 1µl of each primer, and 1µl of DNA. Optimal PCR efficacy was obtained with an initial denaturation of 95˚C for 3 min followed by 35 amplification cycles (denaturation of 94˚C for 40sec, annealing with 58˚C for 40 sec, extension with 72˚C for 40 sec and a final extension at 72˚C for 5 min.) PCR products were visualized on 1.2% agarose gel along with 1 kb DNA ladder. The sequencing was done for two virulent isolates. The obtained sequences were then compared with sequences available in Gen Bank by using BLAST server from the NCBI website (http://www.ncbi.nlmsdjk.nih.govt/BLAST).

Growth rate of *Fusarium sp* isolates on different media

For two virulent isolates, differences in colony growth were observed by growing it in six different media. The different media includes Potato Dextrose Agar media (PDA) (extract of 250g of potato, dextrose: 20g, agar: 20g, 1000ml of distilled water), Potato Sucrose Agar media (PSA) (extract of 250 g of potato, sucrose: 20g, agar:20g, 1000ml of distilled water), Oat meal agar media (OMA) (rolled oats: 30g, agar: 15g, 1000ml of distilled water), Czapek Dox agar media (CDA) (Sucrose: 30g, NaNo3: 2g, K2HPo4: 1g, MgSo4: 1g, KCl: 0.5g, FeSo4: 0.01g, Agar: 20g, 1000ml of distilled water), Malt Extract Agar media (MEA) (malt extract: 25g, agar: 20g, 1000ml of distilled water) and V8 juice agar media. The colony diameter (cm) was measured at 48, 72, 96, 144 and 168 hours after incubation. The experiment was laid out in completely randomized design with three replications. The colony diameter was measured from all the four sides.

RESULTS AND DISCUSSION

Survey and isolation of *Fusarium* spp.

Fusarium dry root rot of chickpea was observed in all the ten locations surveyed and the disease incidence ranged from 11.22 to 52.63% in all the crop stages (Table.1). The maximum dry root rot incidence was recorded in Kavalapur field 1 (52.63%). This was followed by Poosaripalayam, Kavalapur field 2 and Palus. These locations are recorded with dry root rot incidence of 48.41, 45.6, 38.2%, respectively. The minimum dry root rot incidence was recorded in Miraj (11.22%). The maximum incidence of *Fusarium* dry root rot with 52.63% in Natukalpalayam may be due to presence of higher amount of inoculum in soil or monocropping of chickpea repeatedly in same location. A positive correlation of inoculum density and disease incidence have been applied to several soil borne diseases caused by *Fusarium spp*. Marios and Mitchell (1980) has shown that the higher inoculum density of

50,000 chlamydospores per pot resulted in 44% of crown rot incidence in tomato under glass house condition, whereas pot with minimum inoculum of 500 chlamydospores per pot resulted in 33% disease incidence only. Ebbels (1975) reported that the cotton dry root rot caused by *F. oxysporum* f sp. *vasinfectum* builds up steadily due to monocropping in Tanzania.

Pathogenicity test

The plants inoculated with pathogen have shown typical symptoms of yellowing of leaves, drooping and dry root roting (Plate 2) and the characteristic symptom of vascular discoloration was also observed. Re-isolated the pathogen, which was similar to that of the original culture and thus Koch's postulates were proved. In our study, initial symptom of yellowing was observed as early as 5 DAI in twenty-five days old seedling. This may be due to aggressiveness of pathogen or susceptibility stage of the plant. Cumagun *et al*. (2010) proposed that the stage or age of the plant may affect the pathogenicity of *F. oxysporum*. In their experiment, symptom was observed on 7 DAI in both 7 days and 30 days old bottle gourd seedlings. However, there is a significant difference in disease severity caused by *F. oxysporum* among 7 days and 30 days old bottle gourd seedlings.

Cultural and morphological characterization

The results given in Table 2 showed that there is a significant difference among ten isolates with respect to colony characters, pigmentation and colony margin. A white flattened mycelium and reddish pigmentation with smooth margin was observed in FO1. White fluffy mycelial growth with yellowish pigmentation was observed in FO2. FO3 isolate have shown white raised outer growth with pale yellowish pigmentation and smooth margin. Pale yellowish cottony growth and yellowish pigmentation with smooth margin was observed in FO4 isolate. FO5 have shown white raised fluffy mycelial growth with smooth margin. FO6 isolate have shown white sparse mycelial growth with smooth margin and without any pigmentation. Pinkish mycelial growth and pinkish pigmentation was observed in FO8 isolate. The isolate FO9 have shown yellowish fluffy growth without any pigmentation. Whitish mycelial growth without any pigmentation and with smooth margin was observed in FO10 isolate. The front and back view of ten *Fusarium* isolates plates have shown in the Plate 3. Chandran and Kumar (2012) while studying variability of *F. oxysporum*, they found three isolates as white cottony growth, six as white sparse growth, two as white fluffy and three as dense growth. Similar type of results was also observed by Gogoi*et al*., (2017). The

colony diameter (cm) of ten *Fusarium* sp isolates was observed up to seven days (Table 3). On Potato Dextrose Agar medium, the colony diameter ranged from 6.7 to 9.0 cm. The highest colony diameter of 9.0cm was observed in FO 2 and FO 7 followed by FO5 (8.2cm), FO8 (7.7cm), FO9 (7.4cm). The minimum colony diameter was observed in FO1 with 6.1cm. This fast colony growth of the isolates FO2 and FO7 might be due to their virulent nature. Chavan*et al*., (2011) recorded cultural and morphological variability of eight different isolates of *F.oxysporum* causing dry root rot in Patchouli. They found that the *Fusarium* isolates with good mycelial growth of 90.00 mm showed abundant sporulation whereas isolate with mycelial growth of 84.00 mm showed moderate sporulation.

Table 1: Survey at different regions of Sangli district.

S.No	Isolate	Location	Latitude(˚N)	Longitude(˚E)	Crop stage	*PDI (%)
1.	FO1	Miraj	11.008948	76.934065	Flowering stage	11.2^{i} (19.55)
2.	FO2	Kavalapur 1	10.6220	77.0670	Harvesting stage	52.63^{a} (46.51)
3.	FO3	Kavalapur 2	10.6619	77.0081	Harvesting stage	45.6^{c} (42.48)
4.	FO4	Kasabe digraj	10.7360	76.9641	Harvesting stage	33.2^{e} (35.18)
5.	FO5	Islampur	10.5620	76.9211	Flowering stage	19.62^{h} (26.29)
6.	FO6	Ashta	10.5821	76.9343	Vegetative stage	28.3^{f} (32.14)
7.	FO7	Palus	10.9698	76.8598	Harvesting stage	38.2^{d} (38.17)
8.	FO8	Bedag	11.2321	77.1067	Harvesting stage	27.2^{f} (31.44)
9.	FO9	Bhilawadi	11.0062	76.9333	Vegetative stage	48.41^{b} (44.09)
10.	FO10	Tasgaon	10.9581	77.1019	Flowering stage	21.3^{g} (27.49)
SED						0.22
CD (0.05)						0.793

*Mean of three replications; Values in parenthesis are arcsine transformed In a column, means followed by a Table.2 Colony characteristics of *Fusarium* isolates on PDA

Table 2: Colony characteristics of *Fusarium* isolates on PDA.

S.No	Isolate	Colony characters[a]	Pigmentation[b]	Colony margin
1.	FO1	White flatten mycelial growth	Whitish	Smooth margin
2.	FO2	White fluffy mycelium	Whitish	Irregular margin
3.	FO3	White raised mycelial growth on the outer border of colony	Whitish	Smooth margin
4.	FO4	Pale yellowish cottony mycelial growth	Yellowish	Smooth margin
5.	FO5	White raised fluffy growth	Light yellow	Smooth margin
6.	FO6	White sparse mycelial growth	Whitish	Smooth margin
7.	FO7	White cottony growth	Whitish	Smooth margin
8.	FO8	Pinkish mycelial growth	Pinkish	Irregular margin
9.	FO9	Yellowish fluffy mycelium	Whitish	Irregular margin
10.	FO10	Whitish mycelium with raised border	Whitish	Smooth margin

aColony characters were determined by observing the upper surface of the colony

bPigmentation were determined by observing the lower surface of the colony

Table 3: Colony diameter (cm) of *Fusarium* isolates on PDA for 7 days of incubation.

S.No	Isolates	*Colony diameter (cm)					
		48 hrs	**72 hrs**	**96 hrs**	**120 hrs**	**144 hrs**	**168 hrs**
1.	FO1	2.6^{e}	3.1^{f}	4.1^{i}	5.2^{g}	5.6^{f}	6.1^{h}
2.	FO2	2.7^{d}	4.2^{c}	6.2^{b}	8.1^{a}	8.6^{a}	9.0^{a}
3.	FO3	2.6^{e}	3.4^{e}	4.3^{h}	5.6^{f}	6.1^{e}	6.7^{g}
4.	FO4	2.1^{f}	3.2^{f}	4.6^{g}	5.6^{f}	6.6^{d}	7.1^{ef}
5.	FO5	3.5^{b}	4.6^{b}	5.6^{d}	6.7^{d}	7.7^{b}	8.2^{b}
6.	FO6	2.7^{de}	3.6^{d}	5.1^{e}	6.1^{e}	6.6^{d}	$7.2d^{e}$
7.	FO7	3.1^{c}	4.1^{c}	6.3^{b}	7.6^{b}	8.4^{a}	9.0^{a}
8.	FO8	4.1^{a}	5.6^{a}	6.6^{a}	7.1^{c}	7.5^{b}	7.7^{c}
9.	FO9	3.2^{c}	4.6^{b}	5.8^{c}	6.6^{d}	7.1^{c}	7.4^{d}
10.	FO10	2.6^{e}	3.6^{d}	4.9^{f}	6.1^{e}	6.6^{d}	6.9^{fg}
SEm(±)		0.03	0.04	0.21	0.23	0.10	0.76
CD (0.05)		0.10	0.17	0.20	0.23	0.36	0.56

*values are mean of three replications

In column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 4: Morphological characteristics of *Fusarium* isolates on PDA.

S.No	Isolate	Spore size (µm)		Septations		Shape		Colour
		Micro conidia (L X B)	Macro conidia (L X B)	Micro conidia	Macro conidia	Micro conidia	Macro conidia	
1.	FO1	(8.158 – 11.109) X (3.453 – 4.405)	(13.471 – 21.440) X (4.604 – 5.122)	0	1 -2	Elongated oval	Elongated, curved with blunt ends	Hyaline
2.	FO2	(8.984 – 10.127) X (2.807 – 4.005)	(13.927 – 22.768) X (4.604 – 4.883)	0	3 - 4	Elongated oval	Sickle shaped	Hyaline
3.	FO3	(8.718 – 11.449) X (3.311 – 4.441)	(14.368 – 19.2) X (3.883 – 6.45)	0	3 - 4	Elongated oval	Sickle shaped	Hyaline
4.	FO4	(8.857 – 11.955) X (2.2884 – 4.205)	(13.483 – 16.00) X (4.000 – 5.215)	0	1 - 2	Elongated oval	Elongated, curved with bluntends	Hyaline
5.	FO5	(10.511 – 14.843) X (3.124 – 5.200)	(19.349 – 33.970) X (3.256 – 4.94)	0	3 - 4	Elongated oval	Sickle shaped with narrow tip	Hyaline
6.	FO6	(8.718 – 10.332) X (3.162 – 4.561)	(13.220 – 22.768) X (3.720 – 4.205)	0	1 - 3	Round to oval	Sickle shaped with blunt ends	Hyaline
7.	FO7	(10.560 – 12.265) X (3.538 – 3.842)	(17.839-23.923) X (4.870 – 5.385)	0	2 - 3	Elongated oval	Sickle shaped	Hyaline
8.	FO8	(8.352 – 11.207) X (3.418 – 6.512)	(14.403 – 19.251) X (4.079 – 5.492)	0	1 - 2	Elongated oval	Elongated, curved with bluntends	Hyaline
9.	FO9	(9.159 – 11.705) X (4.317 – 5.492)	(19.042 – 31.902) X (2.332 – 4.079)	0	3 - 4	Elongated oval	Sickle shaped	Hyaline
10.	FO10	(10.024 – 12.758) X (2.332 – 3.795)	(17.533 – 32.423) X (2.778 – 4.299)	0	3	Oval	Sickle shaped with pointed tip	Hyaline

Table 5a: Colony diameter of *Fusarium* isolate FO2 for 7 days of incubation on different media.

Media	*Colony diameter (cm)					
	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
PDA	2.7^{c}	4.2^{a}	6.2^{a}	8.1^{a}	8.6^{a}	9.0^{a}
PSA	2.6^{c}	4.3^{a}	6.1^{a}	7.6^{b}	8.6^{a}	9.0^{a}
CPA	3.4^{a}	3.9^{b}	4.6^{b}	5.1^{d}	5.9^{c}	6.4^{cd}
OMA	3.0^{b}	3.7^{c}	4.4^{c}	5.2^{d}	5.8^{c}	6.6^{c}
V8	2.7^{c}	4.0^{b}	4.6^{b}	5.8^{c}	6.3^{b}	7.2^{b}
MEA	1.8^{d}	2.7^{d}	3.6^{d}	4.4^{e}	5.1^{d}	6.2^{d}
SEm(±)	0.04	0.04	0.05	0.09	0.06	0.10
CD (0.05)	0.132	0.136	0.194	0.322	0.229	0.369

*values are mean of three replications

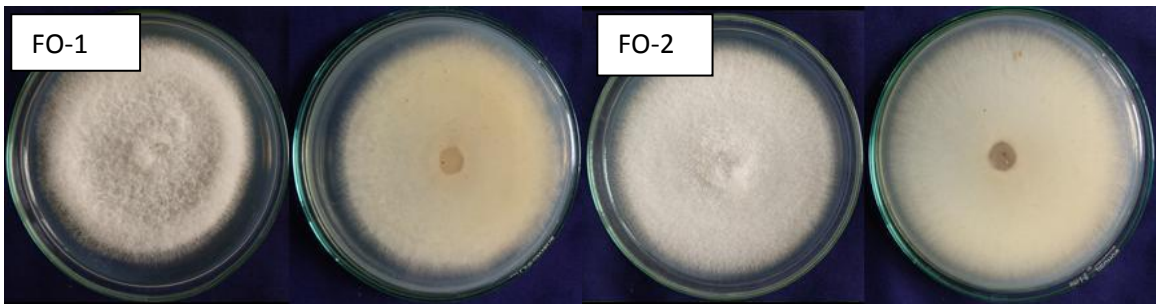
In column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 5b: Colony diameter of *Fusarium* isolate FO7 for 7 days of incubation.

Media	*Colony diameter (cm)					
	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
PDA	3.1^{a}	4.1^{b}	6.3^{a}	7.6^{b}	8.4^{a}	9.0^{a}
PSA	3.2^{a}	4.6^{a}	6.2^{a}	8.2^{a}	8.7^{a}	9.0^{a}
CPA	2.4^{c}	3.6^{c}	4.7^{b}	5.9^{c}	7.5^{b}	8.8^{a}
OMA	2.6^{b}	3.7^{c}	4.6^{b}	5.7^{c}	7.7^{b}	8.0^{b}
V8	2.1^{d}	3.1^{d}	4.4^{c}	5.7^{c}	6.2^{c}	6.5^{d}
MEA	1.4^{e}	3.2^{d}	4.2^{d}	5.3^{d}	6.5^{c}	7.6^{c}
SEm(±)	0.03	0.05	0.03	0.06	0.10	0.11
CD (0.05)	0.124	0.182	0.128	0.207	0.376	0.408

*values are mean of three replications

In column, means followed by a common letter are not significantly different at the 5% level by DMRT



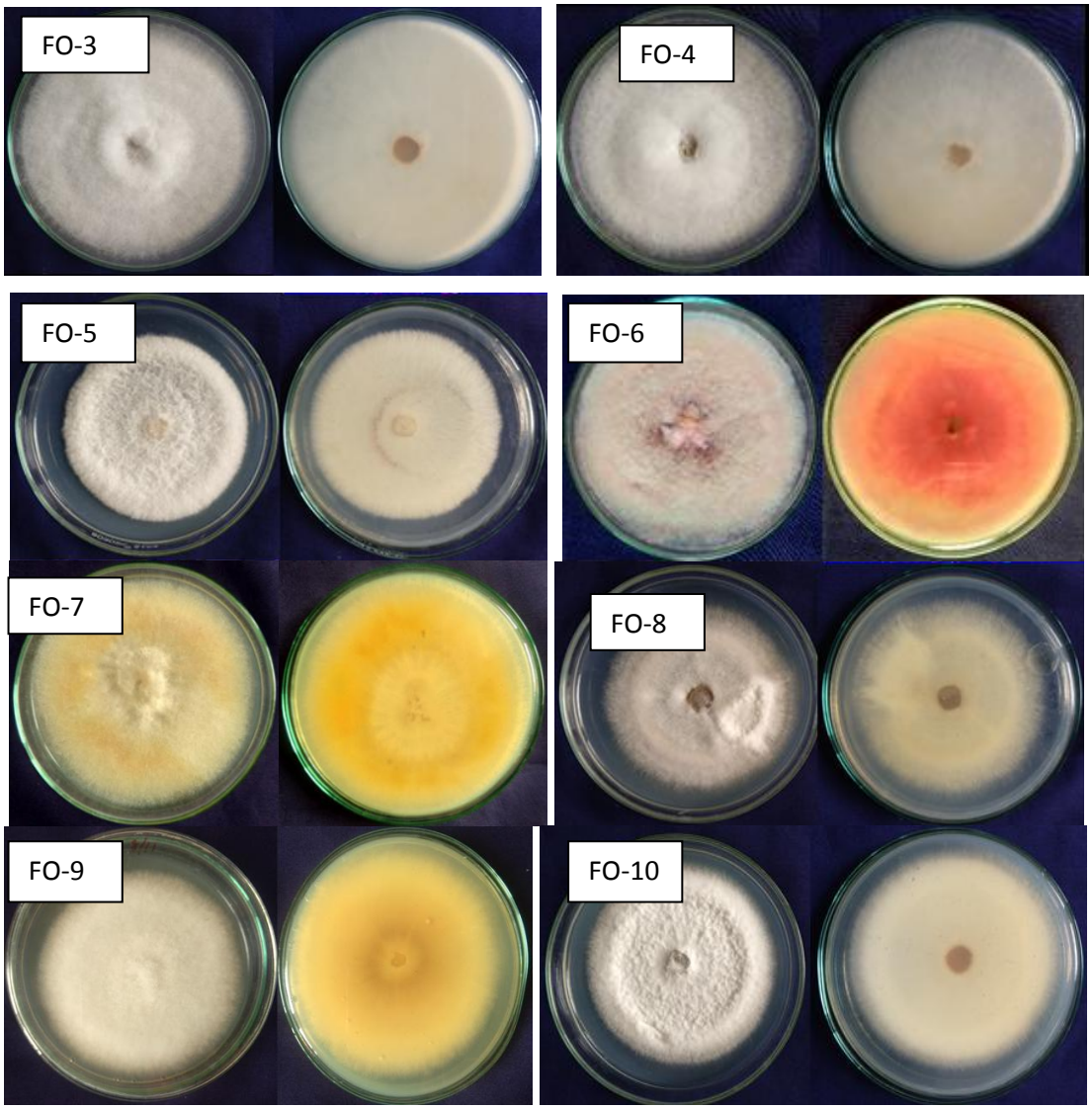
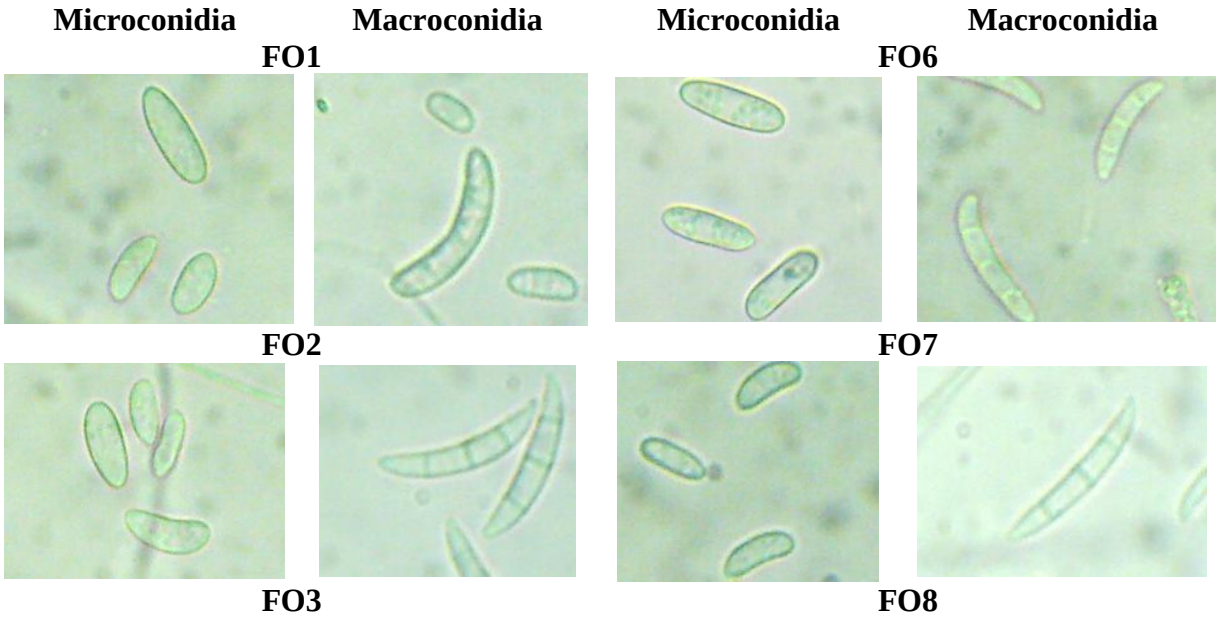


Plate.1: Pure culture of *Fusarium* isolates on PDA.



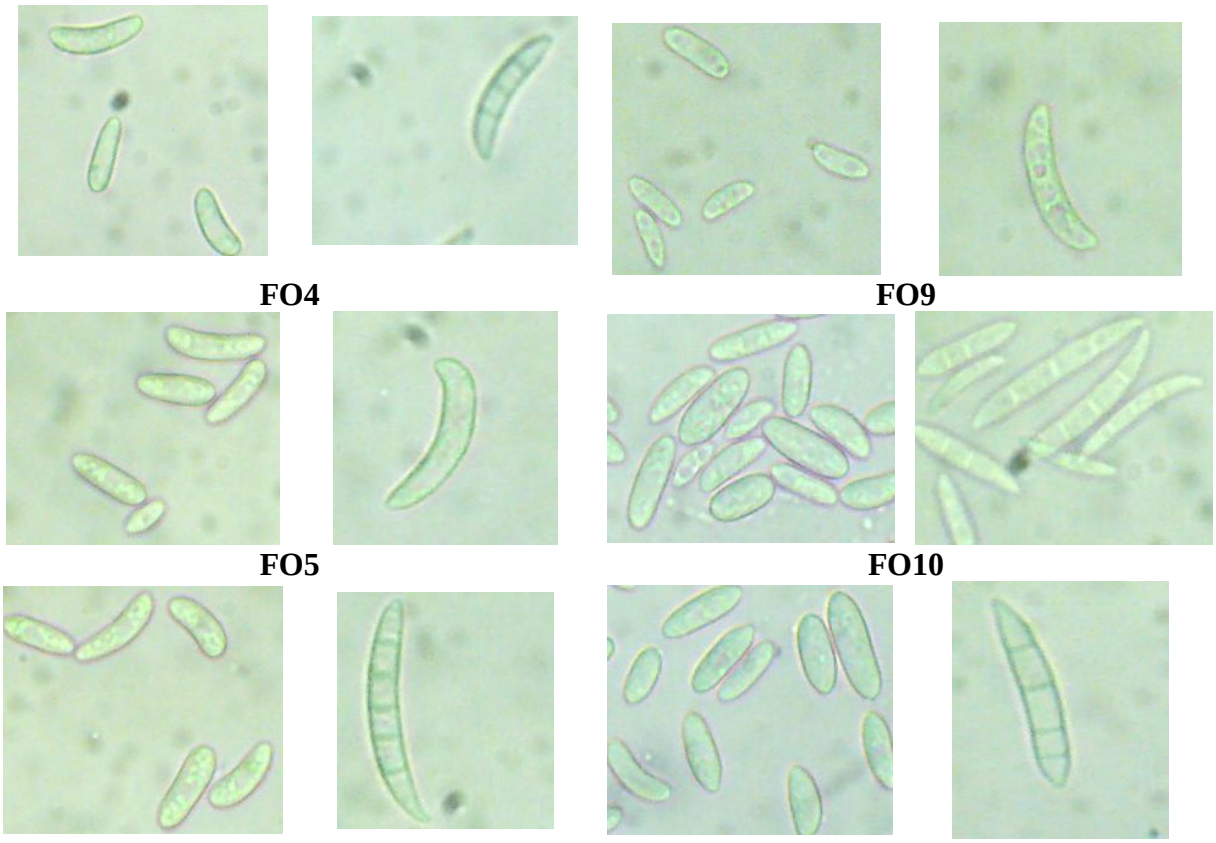


Plate.2 Photomicrograph showing micro and macroconidia of *Fusarium* isolates (40X).

Plate.3: (a nd b) Photomicrograph showing terminal and intercalary chlamydospore (40X).

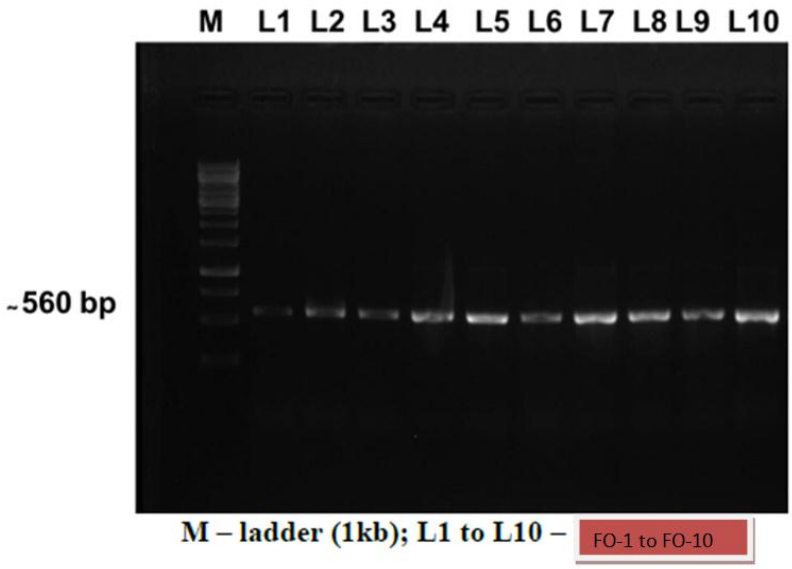


Plate.4: Molecular characterisation of *Fusarium* isolates.

Fusarium spp produced two types of asexual spores *viz,* microconidia and macroconidia and a resting spore namely chlamydospores in 15-20 days old cultures. The images of micro and macroconidia of all isolates were given in Plate 4. Chlamydospores were observed in end of the mycelium as terminal chlamydospores (Plate 5a) and also in the middle as intercalary chlamydospores (Plate 5b) in chain. The size, shape and number of septation in micro and macroconidia of all the ten isolates were given in Table 4. There is no septation in microconidia and 2–4 septations in macroconidia. The shape of microconidia is elongated oval and hyaline. The shape of macroconidia is varied for different isolates. The isolates FO1, FO4 and FO8 had elongated and curved macroconidia with blunt ends. Whereas, the isolates FO2, FO3, FO7 and FO9 have sickle shaped macroconidia. The other isolates namely FO5 and FO10 have sickle shaped with pointed tips. The size of microconidia ranged from (9.2-11.8) x (3.2-4.6)µm and macroconidia is (15.7-24.4) x (3.8 – 5.0)µm.The maximum size of microconidia was observed in isolates FO5 (10.511 – 14.843) x (3.124 – 5.200) µm which is followed by FO10 (10.024 – 12.758) x (2.332 – 3.795)µm, FO7 (10.560 – 12.265) x (3.538 – 3.842)µm, FO4 (8.857 – 11.955) x (2.2884 – 4.205)µm and minimum size was observed in FO2 (8.984 – 10.127) x (2.807 – 4.005)µm. The maximum size of macroconidia was observed in isolate FO5 (19.349 – 33.970) x (3.256 – 4.94)µm which is followed by FO10 (17.533 – 32.423) x (2.778 – 4.299)µm, FO9 (19.042 – 31.902) x (2.332 – 4.079)µm, FO7 (17.839- 23.923) x (4.870 – 5.385)µm and minimum size of macroconidia was observed in FO4 (13.483 – 16.00) x (4.000 – 5.215) µm. Gogoi *et al*.(2017) observed that the size of micro and macroconidia of *Fusarium oxysporum* ranges from (3-4 x 1-2) to (9-10 x 1-3) µm

and (26.42 – 32.46) x (3.24 – 4.74) µm to (17.39 – 23.17) x (2.91 – 4.51) µm respectively. Chandran *et al*. (2012) studied the morphological variability of *F. oxysporum*. They observed 3-5 and 0-1 septations in macro and microconidia respectively, which is similar to our results. They also given that the macroconidia are sickle shaped with blunt ends and microconidia areround to oval shaped. Results obtained in our observation is in agreement with the work mentioned above.

Molecular characterization

The fungal pathogen associated with chickpea dry root rot was confirmed using universal ITS primers. The PCR amplification of ITS region of *Fusarium spp.,* yielded an amplicon size of approximately 560 base pairs (Plate 6). The sequence analysis of FO2 and FO7 has shown 99% and 98% similarity with *Fusarium oxysporum*, respectively. The amplicon of FO2 and FO7 was sequenced and submitted to Gene bank. These results are in agreement with the findings of Sreegayathri *et al*., (2018) who reported that the PCR amplification of ITS region of *Fusarium oxysporum* yielded approximately 550 to 570 bp amplicon

Growth rate on different media

The growth rate of two virulent isolates FO2 and FO7 on different media was studied seven days after incubation (Table 5a and 5b). The mycelial growth of isolates FO2 and FO7 differ statistically in different media with mean value ranged from 6.2to 9.0 cm and 6.5 to 9.0 cm at room temperature, respectively. For the isolate FO2, the maximum growth of 9 cm was observed in Potato Dextrose Agar media (PDA) which is on par with Potato Sucrose agar (PSA) media (9.0cm) and it is followed by V8 (7.2cm), OMA (6.6cm) and CPA (6.4cm) whereas minimum growth of 6.2 cm was observed in MEA. For the isolate FO7, maximum growth of 9 cm was recorded in PSA which is on par with PDA (9.0cm) and it is followed by CPA (8.8cm), OMA (8.0cm) and MEA (7.6cm) whereas minimum growth of 6.5cm was recorded in V8 juice agar media. Similar type of work was done by (Mezzomo *et al*., 2018). This maximum rate of mycelial growth in PDA and PSA medium might be due to greater utilization of carbohydrate source by the *Fusarium* sp. Silva and Teixeira (2012) state that carbohydrate-richmedia such as PDA and PSA can induce the production of conidia of *F. oxysporum*. Lazarotto *et al*. (2013) also suggested PDA and PSA medium for the mass production of macroconidia of *F. chlamydosporum*. The results of the present study concluded that *Fusarium oxysporum*, a fungus was associated with chickpea dry root rot disease. The identity of the fungus was confirmed through cultural, morphological and

molecular characterization through sequence comparison of ITS region using BLAST search. The variations were observed in cultural and morphological characters among *F.oxysporum* isolates. The culture media such as PDA and PSA supported the growth of fungus.

REFERENCES

1. Chandran, M Ravi, and M Reddi Kumar. "Studies on Cultural, Morphological Variability in Isolates of Fusarium Oxysporum (Mart.) Sacc., Incitant of Dry Root-Rot of Citrus", 2012; 6(2): 152–62.
2. Chavan, Sreedevi S, Yashoda R Hegde, and S K Prashanthi. "Morphological and Molecular Variability in Fusarium Oxysporum Causing Dry root rot of Patchouli", 2011; 64(3): 258–60.
3. Cumagun, C.J.R., Aguirre, J.A., Relevante, C.A. and Balatero, C.H., Pathogenicity and aggressiveness of Fusarium oxysporum Schl. in bottle gourd and chickpea. Plant Protection Science, 2010; 46(2): 51-58.
4. Ebbels, D.L., Fusarium dry root rot of cotton: a review, with special reference to Tanzania. Cotton Growing Review, 1975; 52(4): 295-339.
5. Gogoi, Monuj, D.K. Sarmah, and S. Ali. "Cultural and Morphological Variations of Fusarium Oxysporum (Mart.) Sacc. Causing Root Rot of Patchouli in Assam, India." International Journal of Current Microbiology and Applied Sciences, 2017; 6(11): 1889–1901.https://doi.org/10.20546/ijcmas.2017.611.225.
6. Lazarotto, M.; Mezzomo, R.; Maciel, C. G.; Finger, G.; Muniz, M.F.B. Mycelia growth and sporulation of *Fusarium chlamydosporum* species complex under different culture conditions. *Revista Ciências. Agrárias*, 2014; 57(1): 35-40.
7. Marois, J. J., & Mitchell, D. J., Effects of fumigation and fungal antagonists on the relationships of inoculum density to infection incidence and disease severity in Fusarium crown rot of tomato. Phytopathology, 1981; 71(2): 167-170.
8. Mezzomo, Ricardo, Jéssica Mengue Rolim, Tales Poletto, Marília Boff De Oliveira, Marília Lazarotto, and Marlove Fátima Brião Muniz. "Mycelial Growth and Sporulation of Fusarium Spp. Pathogenic to Ilex Paraguariensis in Different Culture Media and under Exposure to Different Light Levels." *Scientia Agraria*, 2018; 19(1): 14–19. https://doi.org/10.5380/rsa.v19i1.55844.
9. Silva, J. L.; Teixeira, R. N. V. Esporulação e crescimento micelial de *Fusarium oxysporum* em diferentes meios de cultura e regimes de luminosidade. *Revista Agro@mbiente On-line*, 2012; 6(1): 47-52.

10. Sreegayathri, E., G. Karthikeyan, L. Rajendran, A. Shanthi, and R. Ram Jegathesh. "Effect of Root Knot Nematode (Meloidogyne Incognita) Infestation on Severity of Dry root rot (Fusarium Oxysporum) in Chickpea and Its Management." *Madras Agricultural Journal*, 2018; 105(10–12). https://doi.org/10.29321/maj.2018.000207.
11. Tamilselvi, N. A., L. Pugalendhi, and T. Raguchander. "Exploiting Cucurbitaceous Species as Rootstocks for Management of Fusarium Dry root rot (*Fusarium Oxysporum*) in Chickpea." *Australian Journal of Crop Science*, 2016; 10(10): 1460–65. https://doi.org/10.21475/ajcs.2016.10.10.p7750.