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Characterization and variability of *Fusarium oxysporum* f. sp. *melongenae* (Schlecht) Mutuo and Ishigami from wilting eggplants in Marathwada region of Maharashtra

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Abstract
Identification and characterization of fungal pathogens associated with wilting of eggplants (*Solanum* spp.) is a necessary precondition for efficient disease management and for the development of durable host resistance in eggplant cultivars. A study was carried out to identify and differentiate *Fusarium* isolates from eggplant fields in Marathwada region of Maharashtra. Twenty four isolates of *Fusarium oxysporum* f. sp. *melonagenae* (Schlecht) Mutuo and Ishigami., incitant of wilt of eggplants were studied for its Pathogenic, cultural and morphological variability and characterization. The radial growth of isolates viz FOM A3, FOM B5, FOM H7, FOM J10, FOM L14, FOM N16, FOM O20 and FOM P22 were more than 70 mm after 6 days of inoculation and considered as fast growing category. Among all the isolates of FOM P22 isolate showed significant variation in radial growth (85 mm) on PDA medium at 6 DAI. In present study, cultural and morphological variability of the entire 8 highly pathogenic test isolates viz., FOM A3, FOM B5, FOM H7, FOM J10, FOM L14, FOM N16, FOM O20 and FOM P22 of *F. O. melongenae* were attempted. The isolates have shown mycelium color ranging from Milk White (FOM A3), Purple (FOM B5), Pink (FOM H7), Light Purple (FOM J10), Classy Pink (FOM L14), Buff (FOM N16), Brown (FOM O20) and Baby Pink (FOM P22). The size of macro conidia ranged from 22.40 - 31.83 x 3.89-5.52µm and the size of micro conidia ranged from 5.92 - 9.34 × 2.61- 4.15µm. The number of septa in macro conidia and in micro conidia is 2-5 and 0-1, respectively and conidia are hyaline. The macro conidia is sickle shaped with blunt end and micro conidia is round to oval shaped. Intercalary and terminal chlamydospores were observed in all the FOM. Isolates and significant variation was noticed in cultural characters of eight different isolates with respective mycelium colour, substrate colour, sporulation etc.

Keywords: Characterization, variability, *Fusarium oxysporum* f. sp. *melongenae*

Introduction
Vegetables play an important role in human nutrition and health by providing minerals, micronutrients, vitamins, antioxidants and dietary fiber. Vegetables cultivation is significant part of the national agriculture economy, especially in developing world. Brinjal (*Solonum melongenae*) also known as eggplant is a common and popular vegetables crop grown in the subtropics and tropics. Its belong to family 'Solanacae*'* it is one of the widely used vegetables crops by most of the people and is popular in many countries viz. central south and south east Asia, some parts of Africa and central America (Harish *et al.*, 2011) [10] It is grown in 691,000 hectares with production of eight production of eight to nine million tonnes (equivalent to one quarter of global production), which makes India the second largest producer of brinjal in the world. In India, brinjal is cultivated in 729 hectares with a total production of about 12616 million tonnes. The major brinjal producing states in India are Andhra Pradesh, Maharashtra, Karnataka, Orissa, Madhya Pradesh and West Bengal (IKISAN 2018).

Fusarium oxysporum f.sp. *melongenae* (FOM)
Brinjal is susceptible to many diseases like *verticillium* wilt (*Verticillium dahliae*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *melongenae*) and bacterial wilt (*Ralstonia solanacearum*) (Kalloo and Berg, 1993) [13] and (Sihachakr *et al*., 1994). *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *melongenae*, is a major constraint in brinjal production in India. The disease is widely distributed in tropical, subtropical and some warm temperate regions of the world. The pathogen is difficult to control since it is soil-borne and has a wide host-range, including several hundred species representing 44 families of plants. Infection is through root-to-root transmission, movement of soil and dissemination by farm implements, and insect

transmission. A combination of high temperature and poor drainage favor development of the disease which causes 75 to 81% yield loss during summer in India (Das and Chattopadhyay, 1953; Rai *et al*., 1975; Rao *et al*., 1976) [5, 22, 24].

Fusarium wilt in brinjal is being managed by application of bactericides, copper fungicides and by crop rotation, with no adequate control. Once the disease develops and wilt symptoms appear in the field, application of bactericides and copper fungicides has no effect on the bacterium. Crop rotation is not a viable control method, as; the bacteria can persist indefinitely in infested fields (Jaworski and Morton, 1964; Sonoda, 1978) [11]. In the absence of effective chemicals and bactericides for managing this disease, emphasis is laid on developing brinjal (brinjal) cultivars with resistance to *Fusarium oxysporum*. Though resistance to *Fusarium* wilt has been studied in several crops, especially tomato, there is little published work on *Fusarium* wilt resistance in brinjal (Chaudhary and Sharma, 2000; Zakir Hussain *et al*., 2005; Mondal *et al*., 2013) [4, 34, 17].

Wilt-associated pathogens cause severe eggplant losses in terms of quality and quantity Arumeru district. The causal pathogens of these wilts have not been identified. The fungal species verticillium and fusarium are know to be some of the most common pathogens that cause wilts on egg plant. Symptoms of wilt caused by these species or unidentified bacteria are frequently similar and cannot be relied upon for diagnostic purposes. Lack of information on pathogen identity and the characteristic symptoms associated with each fungal pathogen has hindered proper management of eggplants wilt. Morphological, cultural and pathological characterisation of the fungal pathogens associated with wilting in eggplants is a necessary precondition for developing efficient disease management strategies or host resistance in eggplant cultivars. Genetic variability should ideally correlate with phenotypic manifestation of the pathogen and virulence (Rozlinah & Sariah, 2006) [26].

Materials and Methods

A roving field Survey was conducted during field survey (Kharif, 2018-19 in major brinjal growing areas of Marathwada region. In each district, three to four tashils were selected and in each tashils four villages were chosen based on prevalence of disease and totally 24 samples from were collected. The pathogen was isolated from the roots of infected brinjal by tissue segment method (Rangaswami and Mahadevan, 1999) [23] on Fusarium specific medium.

Applying tissue segment method, the 24 isolates of *Fusarium oxysporum* were isolated aseptically from the infected eggplant root and stem. Naturally wilted eggplant specimens collected during field survey (Kharif, 2018-19) were subjected to tissue isolation (Tuite, 1969) [32] on autoclaved and cooled Potato Dextrose Agar (PDA) medium. Infected xylem tissues were surface sterilized with 1 per cent aqueous Sodium hypochlorite (NaOCl) solution for 2-3 minutes and then washed by giving three sequential changes with sterile distilled water in Petri plates. Later, these root / stem bits were inoculated aseptically on autoclaved and cooled PDA medium in sterilized Petri plates under Laminar-air-flow and incubated in BOD incubator at 28±2ºC temperature for five days. Sub-culturing was done from the developing colonies by single spore isolation. Axenic culture of the pathogen was obtained by single hyphal tip method and maintained on PDA throughout the present investigation and maintained at 4ºC for future use.

The pathogen isolates were mainly identified on the basis of cultural and morphological characters as Fusarium spp. (Subramanian, 1971) [30]. The pure cultures of different isolates of Fusarium spp. were maintained on PDA slants. Different isolates of Fusarium were multiplied on sand maize medium and added to soil in pots at 7.5 percent (W/W) by soil infestation following the procedure given by Haware (1980. Based on the disease incidence, the virulent isolate was identified and used in further studies. SMM was prepared by mixing 100 g Maize grain flour, 50 g sand, 50 ml distilled water in 250 ml conical flask and sterilized at 15 psi for one hr for three consecutive days. Flasks were subsequently inoculated with 5mm diameter discs of culture of the test fungus and incubated at 28+2ºC

Soil mixture without inoculum served as control. Each pot was planted with brinjal seedlings and symptoms development was observed. Seedling mortality percent was recorded at 43 DAS. Based on percent mortality of seedlings, virulent isolate was identified.

Identification of *Fusarium oxysporum*

Morphological characteristics of cultures were studied by microscopic observation under compound microscope at different magnifications and growth characteristics like colony color, texture, growth, pigmentations, hyphae / mycelial color and sclerotium color were observed in Petri plates. Fusarium species were identified according to Nelson *et al*. (1981)

Morphological studies were conducted by taking a small amount of pure culture using a sterile needle and transferred onto a clean glass slide. The culture was taken from four positions of the culture plate, two at right angle to each other, one from very close to the inoculation point and another mid point of radius. Total three culture plates of each isolate were used for the morphological studies after 7, 15, 25 days after incubation at 28+2ºC. The culture was stained with 0.1% lacto phenol cotton blue and observed for the micro conidia, macro conidia and chlamydospores using a compound microscope. Micro Conidial size was measured with the help of an ocular micrometer after calibrating the microscope using seven day old culture of each isolate which was replicated thrice with 100 conidia in each replication. Length and breadth of 100 macro conidia for each of three replications of all the isolates were measured using 15 days old culture. The mode of chlamydospore production *viz*., solitary, pairs, chains and location were observed using 25 day old culture. Cultural characteristics *viz*; the substrate pigmentation, color, mycelia appearence and nature of mycelium of the different isolates were observed on PDA cultures after 7-8 days of inoculation. The radial growth of pathogen was recorded up to 16 days after inoculation.

Characterization of Fusarium isolates from eggplant

The characterization of *Fusarium* isolates into species aggregates was made on the basis of cultural and morphological characters analysis (Seifert, 1996) [27]. The mycelial colony appearance of F. oxysporum isolates were cottony, thin flat to fluffy, thread like spreading at periphery and looking wet. Growth of the colony was delicate and wooly to cottony becoming felted and wrinkles in mature cultures. The color of the colonies varied from creamy white to white while some isolates produced pinkish violet pigments in colonies after 15 to 20 days of incubation at 28ºC on PDA medium.

Pathogenicity test

Pathogenicity test of 24 isolates of *Fusarium* were proved by water culture technique as well as by sick soil method.

Results and discussion

Identification of Pathogen

On the basis of *Fusarium* wilt disease symptoms expressed (both on naturally and artificially diseased) on eggplants, pathogenicity test and cultural and morphological characteristics the test pathogen was identified and Out of 24 isolates of FOM collected from various Agro-climatic zones of Marathwada region of Maharashtra, during *Kharif* 2018-19 and only 8 isolates were carried forward for further cultural and morphological studies on basis of high virulence seen amongst them. (Table No.1)

Table 1: Characteristics of *Fusarium oxysporum.* f. sp. *melongenae* grown on PDA

Colony/Propagule Character	Shape	Size	Septation	Color
Colony	Luxuriant cottony growth	Radial growth 28.6mm after 72 hours at 28±1 ^{0}C	….	Initially white, turns pinkish later in some cases
Mycelium	Smooth	….	Septate	White, pinkish
Pigmentation		……	…	Pinkish, purplish
Conidiophore	Sparsely branched	……	….	….
Microconidia	Oval	5.92 - 9.34 × 2.61- 4.15µm	Aseptate/ Monoseptate	Hyaline.
Macroconidia	Sickle shaped	22.40 - 31.83 x 3.89-5.52µm	Septate (3-5 septa)	Hyaline.
Chlamidospores		3.50 – 9.50 x 4.50-12.00 µm	Aseptate	Thick

Abundant micro conidia were noticed on 3rd day and macro conidia were observed on 12th day after inoculation reported by Booth (1971) [2].

A total of 24 isolates of *Fusarium* representing 8 districts distributed under marathwada region of Maharashtra state were obtained and assigned them the nomenclature as detailed in the following Table.No:2

Table 2: Isolates of *Fusarium oxysporum.* f. sp. *melongenae* collected from different regions of Marathwada

S. No	Isolate code	Districts	Tahsil
1	FOM A1	Aurangabad	Gangapur
2	FOM A2	Aurangabad	Sillod
3	FOM A3	Aurangabad	Soegaon
4	FOM B4	Beed	Ashti
5	FOM B5	Beed	Ambajogai
6	FOM B6	Beed	Parali Vaijnath
7	FOM H7	Hingoli	Sengaon
8	FOM H8	Hingoli	Basmat
9	FOM H9	Hingoli	Kalamnuri
10	FOM J10	Jalna	Badnapur
11	FOM J11	Jalna	Ghasawangi
12	FOM J12	Jalna	Jaffrabad
13	FOM L13	Latur	Ahmadpur
14	FOM L14	Latur	Chakur
15	FOM L15	Latur	Udgir
16	FOM N16	Nanded	Kinwat
17	FOM N17	Nanded	Biloli
18	FOM N18	Nanded	Deglur
19	FOM O19	Osmanabad	Bhoom
20	FOM O20	Osmanabad	Tuljapur
21	FOM O21	Osmanabad	Kalamb
22	FOM P22	Parbhani	Gangakhed
23	FOM P23	Parbhani	Jintur
24	FOM P24	Parbhani	Sailu

Variability in pathogen isolates

In present study, cultural and morphological variability of all the 8 highly pathogenic test isolates *viz*., FOM A3, FOM B5, FOM H7, FOM J10, FOM L14, FOM N16, FOM O20 and FOM P22 of *F. O.melongenae* were attempted.

Level of pathogenicity

On the basis of wilt incidence, level of pathogenicity categorized into four group *viz*., non-pathogenic (00%), weakly pathogenic (0.1 to 20%), moderately pathogenic (> 20 to 50%) and highly pathogenic (> 50%).

Among them only one isolates *viz*., FOM B4 was non-pathogenic. Twelve isolates were included in moderately pathogenic level *viz* FOM A2,FOM B6,FOM H8,FOM J11,FOM J12,FOM L13,FOM L15,FOM N17,FOM N18,FOM O21,FOM P23 and FOM P24. Eight isolates *viz*., FOM A3, FOM B5, FOM H7, FOM J10, FOM L14, FOM N16, FOM O20 and FOM P22were highly pathogenic and those were carried further for studies (Table no-3).

Table 3: Grouping of *Fusarium oxysporum*. f. sp. *melongenae* isolates based on level of virulence

S. No.	Level of Virulence	Range of wilt incidence (%)	No. of Isolates	Frequency (%)	Isolates
1	Non Pathogenic	0	1	4.17	FOM B4
2	Weakly Pathogenic	Up to 20%	3	12.50	FOM A1, FOM H9 and FOM O19
3	Moderately Pathogenic	> 20 to 50%	12	50.00	FOM A2, FOM B6, FOM H8, FOM J11, FOM J12, FOM L13, FOM L15, FOM N17, FOM N18, FOM O21, FOM P23 and FOM P24
4	Highly Pathogenic	> 50%	8	33.33	FOM A3, FOM B5, FOM H7, FOM J10, FOM L14, FOM N16, FOM O20 and FOM P22

Pathogenic variability of the test isolates of *Fusarium oxysporum*

By applying water culture technique, all 24 isolates of FOM were tested for pathogenic variability. The results (Table no-4) revealed that only one isolate *viz* FOM B4 was found avirulent and rest of other 23 isolates were virulent.

By applying sick soil method in pot culture, all 24 isolates of FOM were tested for pathogenic variability. The results (Table No-5) revealed that only one isolate *viz*., FOM B4 was found avirulent and 12 isolates were moderately virulent and rest of eight isolates were highly virulent.

Table 4: Pathogenic variability among the test isolates of *Fusarium oxysporum.* f.sp. *melongenae* (water culture technique)

S. No.	Isolates	Wilt / Mortality percentage		Level of virulence
		5th day	7th day	
1	FOM A1	53.33	60.00	Highly Virulent
2	FOM A2	100.00	100.00	Highly Virulent
3	FOM A3	100.00	100.00	Highly Virulent
4	FOM B4	0.00	0.00	avirulent
5	FOM B5	66.67	66.67	Highly Virulent
6	FOM B6	100.00	100.00	Highly Virulent
7	FOM H7	100.00	100.00	Highly Virulent
8	FOM H8	50.00	63.33	Highly Virulent
9	FOM H9	63.33	66.67	Highly Virulent
10	FOM J10	56.67	60.00	Highly Virulent
11	FOM J11	100.00	100.00	Highly Virulent
12	FOM J12	43.33	66.67	Highly Virulent
13	FOM L13	53.33	60.00	Highly Virulent
14	FOM L14	56.76	76.00	Highly Virulent
15	FOM L15	60.00	66.67	Highly Virulent
16	FOM N16	53.33	66.67	Highly Virulent
17	FOM N17	53.33	63.33	Highly Virulent
18	FOM N18	100.00	100.00	Highly Virulent
19	FOM O19	43.33	60.00	Highly Virulent
20	FOM O20	100.00	100.00	Highly Virulent
21	FOM O21	100.00	100.00	Highly Virulent
22	FOM P22	56.67	60.00	Highly Virulent
23	FOM P23	53.33	63.33	Highly Virulent
24	FOM P24	56.67	63.33	Highly Virulent

*****Mean of three replications, PRE: Pre-emergence, POE: Post-emergence
A: Avirulent, WV: Weakly Virulent,
MV: Moderately Virulent and HV: Highly Virulent

Table 5: Pathogenic variability among the test isolates of *Fusarium oxysporum*. f.sp. *melongenae* (Pot culture technique)

S. No.	Isolates	PRE mortality* (%)	Av. POE mortality / wilting* (%) DAS				Reactions
			30	60	90	120	
1	FOM A1	0.00	0.00	7.00	10.00	20.00	WV
2	FOM A2	0.00	0.00	18.00	26.00	34.00	MV
3	FOM A3	0.00	0.00	42.00	68.00	100.00	HV
4	FOM B4	0.00	0.00	0.00	0.00	0.00	AV
5	FOM B5	0.00	0.00	32.50	59.18	100.00	HV
6	FOM B6	0.00	0.00	18.00	26.00	34.00	MV
7	FOM H7	4.00	0.00	40.00	70.00	90.00	HV
8	FOM H8	0.00	0.00	18.00	26.00	34.00	MV
9	FOM H9	0.00	0.00	10.00	18.00	20.00	WV
10	FOM J10	3.00	0.00	25.00	58.00	100.00	HV
11	FOM J11	0.00	0.00	24.00	32.00	40.00	MV
12	FOM J12	0.00	0.00	15.00	26.00	34.00	MV
13	FOM L13	0.00	0.00	25.00	40.00	50.00	MV
14	FOM L14	2.00	0.00	28.00	43.00	100.00	HV
15	FOM L15	0.00	0.00	18.00	26.00	34.00	MV
16	FOM N16	1.00	0.00	38.00	64.00	100.00	HV

17	FOM N17	0.00	0.00	16.00	32.00	44.00	MV
18	FOM N18	0.00	0.00	25.00	36.00	48.00	MV
19	FOM O19	0.00	0.00	0.00	16.00	18.00	WV
20	FOM O20	5.00	10.00	30.00	60.00	80.00	HV
21	FOM O21	0.00	0.00	20.00	26.00	44.00	MV
22	FOM P22	4.00	0.00	39.58	68.75	100.00	HV
23	FOM P23	0.00	0.00	18.00	26.00	34.00	MV
24	FOM P24	0.00	0.00	16.00	27.00	40.00	MV

PRE: Pre-emergence, POE: Post-emergence
A: Avirulent, WV: Weakly Virulent,
MV: Moderately Virulent, HV: Highly Virulent

Frequency of FOM isolates was varied on the base on level of virulence. Avirulent group had 4.17% frequency where as weakly virulent group shown 12.50% and Moderately virulent group shown highest frequency *i.e.* 50.00%. However highly virulent leveled 8 isolates had 33.33% frequency (Table No-5)

Cultural variability among FOM isolates

Based on radial growth, the *Fusarium oxysporum.* f. sp. *melongenae* isolates were characterized as fast growing, moderately growing and slow growing. The growth of 24 isolates of *Fusarium oxysporum.* f.sp. *melongenae* was observed up to 16 days. The radial growth of isolates *viz* FOM A3, FOM B5, FOM H7, FOM J10, FOM L14, FOM N16, FOM O20 and FOM P22 were more than 70 mm after 6 days of inoculation and considered as fast growing category (Table 6).

Table 6: Radial growth (in mm) of Fusarium oxysporum isolates on PDA

S. No	Isolates	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI	14 DAI	16 DAI	Score
1	FOM A1	7	20	31	45	52	75	80	90	+
2	FOM A2	12	25	42	51	75	90	-	-	++
3	FOM A3	18	42	72	90	-	-	-	-	+++
4	FOM B4	6	15	21	42	50	79	83	90	+
5	FOM B5	19	43	62	90	-	-	-	-	+++
6	FOM B6	13	23	44	59	71	90	-	-	++
7	FOM H7	16	42	78	90	-	-	-	-	+++
8	FOM H8	9	19	46	57	72	90	-	-	++
9	FOM H9	8	19	23	49	55	73	85	90	+
10	FOM J10	18	45	72	90	-	-	-	-	+++
11	FOM J11	10	21	48	54	78	90	-	-	++
12	FOM J12	11	24	38	43	57	70	83	88	+
13	FOM L13	10	27	30	42	59	78	84	82	+
14	FOM L14	17	44	79	90	-	-	-	-	+++
15	FOM L15	12	24	48	53	77	90	-	-	++
16	FOM N16	14	45	73	90	-	-	-	-	+++
17	FOM N17	12	20	37	41	59	72	88	90	+
18	FOM N18	10	24	48	53	77	90	-	-	++
19	FOM O19	9	17	26	42	51	79	85	86	+
20	FOM O20	18	46	72	90	-	-	-	-	+++
21	FOM O21	9	16	42	51	73	90	-	-	++
22	FOM P22	19	44	85	90	-	-	-	-	+++
23	FOM P23	11	24	44	51	72	90	-	-	++
24	FOM P24	12	21	48	53	77	90	-	-	++

+: Fast growing, ++: Moderately growing, +++: Slow growing, DAI: Days after inoculation

Among all the isolates of *Fusarium oxysporum.* f.sp. *melongenae* FOM P22 isolate showed significant variation in radial growth (90 mm) at 8DAI.Where as the radial growth of isolates FOM A2,FOM B6,FOM H8,FOM J11,FOM L15,FOM N18,FOM O21,FOM P23 and FOM P24 were between 51 to 59 mm at 8 DAI and considered as moderate growth category. The remaining all isolates of pathogen viz FOM A1, FOM B4, FOM H9, FOM J12, FOM L13, FOM N17 and FOM O19 grown less than 50 mm even after 8 days of inoculation and they were grouped under slow growth category (Table 6).

The results obtained on cultural characteristics *viz*., mycelial colour, colony appearance, growth speed, colony shape, margin, sporulation and pigmentation *etc.* in respect of 8 test isolates of FOM grown on PDA were observed. (Table No.7)

Table 7: Cultural variability among the test isolates of *Fusarium oxysporum.*

S. No.	Parameters	Isolates (District Location) / Characteristics							
		FOM A3 (Aurangabad)	FOM B5 (Beed)	FOM H7 (Hingoli)	FOM J10 (Jalna)	FOM L14 (Latur)	FOM N16 (Nanded)	FOM O20 (Osmanabad)	FOM P22 (Parbhani)
1	Colour	Milk White	Purple	Pink	Light purple	Classy Pink	Buff	Brown	Baby Pink
2	Mycelial Appearance	Luxuriant, appressed, felted and fluffy	Scanty, partially appressed and	Luxuriant, appressed and fluffy	Luxuriant, partially appressed and	Scanty, partially appressed and	Luxuriant, partially appressed and	Scanty, partially appressed and fibrous	Luxuriant, appressed, felted and

			fibrous		fibrous	fibrous	fibrous		fluffy
3	Growth speed	Fast	Medium	Fast	Fast	Slow	Fast	Slow	Fast
4	Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
5	Colony margin	Non-Serrated, smooth	Serrated	Serrated	Serrated	Non-Serrated, smooth	Serrated	Serrated	Non-Serrated, smooth
6	Sporulation	++++	+++	++++	+++	++++	+++	++++	++++
7	Substrate Pigmentation	Vatican White	Crushed purple	Mulberry purple	Mulberry Purple	Pearly purple	Cameo White	Mustard Brown	Dark Pink

Sporulation: ++++ = Excellent, +++ = Good,

Mycelial colour

Observation for mycelium colors were taken and found distinct differences among different isolates. The isolates have shown mycelium color ranging from Milk White (FOM A3), Purple(FOM B5), Pink(FOM H7), Light Purple(FOM J10), Classy Pink(FOM L14), Buff(FOM N16), Brown(FOM O20) and Baby Pink (FOM P22). (Table No.7)

In a similar study, Madhukeshwara (2000) [14] studied cultural variability among six isolates of F. udum causing wilt of pigeon pea. All the isolates varied with each other in terms of growth, mycelium, pigmentation and sporulation. Most of the isolates produced cottony white raised mycelium, pale yellow to dusky red colour pigmentation and moderate to profuse sporulation on PDA medium.

Champawat and Pathak (1989) [3] studied cultural variability among nine isolates (I1 to I9) of *F. oxysporum* f.sp. *cumini* causing wilt of cumin. The isolates I2, I3 and I8, I9 showed white mycelial and different colours of substrate pigmentation on PDA media. Isolate I3 produced light yellow coloured substrate pigmentation on PDA medium.

Substrate colour

The substrate colour was observed from the bottom side of Petri dish based on the colony pigmentation. Seven day after inoculation the cultures were assigned to Vatican white, Crushed purple, Mulberry Purple, Mulberry Purple, Pearly Purple, Cameo White, Mustard Brown, and Dark Pink Colour by Aurangabad (FOM A3), Beed (FOM B5), Hingoli, (FOM H7), Jalna (FOM J10), Latur (FOM L14), Nanded (FOM N16), Osmanabad (O20) and Parbhani (FOM P22) Respectively. (Table 7).

Colony growth rate

On the basis colony growth rate, the test isolates were categorized as fast growing, moderate growing and slow growing. In fast growing category, the five isolates *viz*., FOM A3, FOM H7, FOM J10, FOM N16 and FOM P22.Where as in medium / moderate growing category isolate was FOM B5 as well as in slow growing category. However two isolates FOM L1, FOM O20 were included in slow growing category. (Table no.7).

Colony shape and margin

On the basis of colony shape (circular) and colony margin (non-serrated, smooth or serrated), the test isolates were categorized into two groups. The group I included about three isolates with circular colony and non-serrated with smooth margin, which were FOM A3, FOM L14 and FOM P22 whereas the group II included the isolates with circular colony and serrated margin, which contained rest of the five isolates *viz*., FOM B5, FOM H7, FOM J10, FOM N16, and FOM O20 (Table No.7).

Sporulation

The sporulation induced by the test isolates varied from Good (+++) to excellent (++++). However, it was excellent (++++) in about five isolates *viz*., FOM A3, FOM H7, FOM L14, FOM O20 and FOM P22 where as it was good (+++) with three isolates *viz*, FOM B5, FOM J10 and FOM N16. (Table No.7).

Micro-conidia were abundant, one septate, oval to ellipsoidal in shape hyaline and borne on short and plump monophialides and measured 5.92 - 9.34 × 2.61- 4.15µm. Macro-conidia were produced in sporodochium, thin walled, delicate, 3 to 5 septate, cylindrical, straight to curved, sickle shaped pointed at both ends and measured 22.40 - 31.83 x 3.89-5.52µm (Table No-8). Chlamydospores were formed in old culture, which were spherical, 1 celled, smooth and thick walled, terminal or intercalary produced singly or in pairs and measured 3.50 – 9.50 x 4.50-12.00 µm (Table No-8). These results were in agreement with Gupta *et al*. (2010) [9] and reported that morphological studies revealed that *F. oxysporum* produced three types of spores. i.e. micro-conidia, macro-conidia and chlamydospores. However, the results are in line with the findings of Leslie & Summerell (2006) [12] Nelson *et al*. (1994) [19], Alexopoulus *et al*. (1996) [1] and Sultana *et al.* (2014) [31].

Morphological variability among *Fusarium oxysporum* isolates

Results (Table No: 8) revealed that all the 8 test isolates of FOM exhibited a wide range of variability in respect of number of septa.

Septation

Results (Table.No:8) revealed least variability was observed among the test isolates in respect of septa on the micro-conidia. Among the test isolates, septation was ranged from 0 to 1. Isolates FOM B5and FOM O20 were recorded 0-1 septation, whereas isolates FOM A3, FOM H7, FOM J10, FOM L14, FOM N16, FOM O20 and FOM P 22 were more or less oval without septation.

Variability among the test isolates in respect of septa on the macro-conidia were distinct. Among the test isolates, septation was ranged from 1-2 to 3-4.Of the test isolates, FOM B5 recorded maximum (2-5) septation, followed by the isolate *viz*., FOM A3(2-4), FOM J10 and FOM N16 (2-3), FOM H7, FOM O20 (1-4), FOM L14and FOM P 22 (1-3). (Table No.8)

Conidial size

The results (Table.No.8) revealed that all the ten isolates exhibited a wide range of variability in respect of size of micro-conidia and macro-conidia. Average size of micro-conidia of the test isolates was ranged from 9.34 x 4.15µm (FOM O20) to5.92 x 2.61 µm (FOM P 22). However, maximum micro-conidial size (9.34 x 4.15) was recorded in isolate FOM O20. This was followed by the isolates *viz.*, FOM N16 (9.02 x 4.10), FOM J10 (8.74 x 3.92), FOM A3 (8.62 x 3.70), FOM B5 (7.41 x 3.10), FOM H7 (6.55 x 2.73), FOM L14 (6.32 x 2.81) and FOM P 22 (5.92 x 2.61).

Average size of macro-conidia of the test isolates was ranged from 31.83 x 3.89 µm (FOM N16) to 22.40 x 4.62 µm (FOM P22). However, maximum macro-conidial size (31.83 x 3.89), was recorded in isolate in FOM N16. This was followed by the isolates *viz.*, FOM O20(30.86 x 5.52), FOM A3(28.60 x 4.30), FOM J10(28.29 x 4.10), FOM L14(26.22 x 4.30), FOM B5(25.63 x 4.40) & FOM H7(23.20 x 3.90). FOM P 22(22.40 x 4.62).

Madhukeshwara (2000) [14] reported the size of micro and macro conidia were 6 – 8 x 2 – 3µm and 19 – 26 x 3.5µm respectively, in most of the isolates. Desai *et al*. (2003) [6] observed that the size of micro and macro conidia of *F. oxysporum* f.sp. *ricini* ranged from 5.25 – 14.00 x 3.50 – 7.00 µm and 17.5 – 70.00 x 3.50 – 5.25 µm, respectively. The isolates which were highly virulent produced abundant sporulation, while moderately virulent isolates were having poor sporulation.

Chlamydospores

The chlamydospores were observed 10 days old culture of all isolates they were either terminal or intercalary and formed singly or in pair but rarely in chain. Terminal Chlamydospores were prominent in isolates FOM O20, and FOM P22. They were of variable in size and their dimension ranged from 3.51- 12.21µm. Chlamydospores dimension was maximum in isolates FOM O20, and FOM P22. (9.52-12.21 µm) followed by FOM N16, FOM J10, FOM B5, FOM L14, FOM A3 and, were as it was observed minimum in isolate FOM H7 (3.51-4.52µm). Table No: 8.

Table 8: Morphological variability among the test isolates of *Fusarium oxysporum.* f.sp. *melongenae*

S. No.	Isolates	Micro-conidia		Macro conidia		Chlamydospores
		Av. Size (µm) Length x Breadth	Septation (No.)	Av. Size (µm) Length x Breadth	Septation (No.)	Diametere (µm)
1	FOM A3	8.62 x 3.70	0	28.60 x 4.30	2 to 4	5.50-7.51
2	FOM B5	7.41 x 3.10	0-1	25.63 x 4.40	2 to 5	7.50-8.53
3	FOM H7	6.55 x 2.73	0	23.20 x 3.90	1 to 4	3.51-4.52
4	FOM J10	8.74 x 3.92	0	28.29 x 4.10	2 to 3	7.50-10.50
5	FOM L14	6.32 x 2.81	0	26.22 x 4.30	1 to 3	6.50-8.52
6	FOM N16	9.02 x 4.10	0	31.83 x 3.89	2 to 3	7.51-9.51
7	FOM O20	9.34 x 4.15	0- 1	30.86 x 5.52	1 to 4	9.52-11.54
8	FOM P 22	5.92 x 2.61	0	22.40 x 4.62	1 to 3	8.51-12.21

Morphology of the pathogen in respect of septate mycelium, macro conidia and micro conidia and their dimensions are reported in present studies in conformity with Booth (1971) [2], Ravichandran and Reddy Kumar (2012), Madhusudhan.P. *et al*., (2010) [15] who reported three distinct types of conidial characteristics, morphology, pigmentation and size of macro-conidia of different isolates of Fusarium spp.

In the presents study the significant variation was noticed in cultural characters of eight different isolates with respective mycelium colour, substrate colour, sporulation etc. These results are in agreement with earlier reports of Gupta *et al*. (1986) [9], Shubatrivedi and Gurha S N (2007) [28], Madhusudan *et al*., (2010) [15], Ravichandran and Reddy (2012), Sreeja, S. J. (2014) [29], Ghante P.H, (2017) [8], Padvi *et al.* (2018) [21], Mwaniki. P.K. *et al.* (2018) [18], Desai A G *et al.* (2018), Nirmaladevi, D (2012) [20], was observed existence of wide variation among different isolates of Fusarium spp.

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