

## Original Research Article

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## Variability in Morphological and Cultural Characters of Different Isolates of *Fusarium oxysporum* f.sp. *lini*

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#### Keywords

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Thirty-five isolates of *Fusarium oxysporum* f.sp. *lini* were isolated from available diseases plant samples. Morphological studies of these isolates revealed that macroconidia were straight; spindle as well as sickle shaped. In macroconidia, average length varied from 11.26  $\mu\text{m}$  in Foli-9 to 38.33  $\mu\text{m}$  in Foli-13 with 1-5 septa and average breadth varied from 1.95  $\mu\text{m}$  in Foli-22 to 3.16  $\mu\text{m}$  in Foli-13. In microconidia, average length varied from 4.45  $\mu\text{m}$  in Foli-9 to 11.19  $\mu\text{m}$  in Foli-13 with 0-1 septa and average breadth of varied from 1.75  $\mu\text{m}$  in Foli-2 to 3.01  $\mu\text{m}$  in Foli-29. Six isolates showed cottony white, 12 isolates showed pale white and 17 isolates showed purplish white growth of culture. Eleven isolates were fast growing, 21 isolates were medium growing and 3 isolates were slow growing.

### Introduction

*Linum usitatissimum* L. commonly known as linseed or flaxseed is an ancient oilseed and fiber crop. India, it is grown mainly for seed, used for extracting oil and it is such a valuable crop that every part of the plant has specific economic importance (Gill, 1967). Among the fungal diseases of linseed, wilt is a major constraint responsible for low production and productivity along with rust and powdery mildew (Kishore *et al.*, 2011).

Linseed wilt incited by *Fusarium oxysporum* f.sp. *lini* (Bolly) Snyder and Hansen was first reported by Luggar (1890) from Minnesota, USA. Bolley and Manns (1932) isolated the

*Fusarium oxysporum* f.sp. *lini* (Bolly) from disease infected seeds for the first time by growing it on glucose agar medium *in vitro* in petriplates. Kommedahl *et al.*, (1970) identified that isolates of *Fusarium oxysporum* f.sp. *lini* vary in: 1) morphology, with respect to the amount and type of sporulation, production of different types of conidia, size and number of septa and pigment production on growth media; 2) physiology, rate and type of growth on substrates and in host; 3) environmental preferences, antibiotic capabilities; and 4) pathogenicity. Therefore, it is considered an ideal pathogen to demonstrate diversity within a species.

## Materials and Methods

### Collection, isolation and purification of *Fusarium oxysporum* f.sp. *lini*

Linseed plants naturally infected and showing typical wilt symptoms were collected from farmer's fields from 29 locations in district Kangra and Mandi of Himachal Pradesh and brought to the laboratory. At each field, three observations on total number of linseed plants in 1 m<sup>2</sup> area and total wilted plants in the sampling area were recorded to calculate per cent disease incidence of wilt in the field by using formula (Mayee and Datar, 1986) as detailed below.

**Per cent Disease Incidence** = Number of plants infected by wilt / Total number of plants x 100

Wilt incidence of five fields at each location was recorded to calculate the average disease incidence of the location. Linseed plants showing typical wilting symptoms were collected in separate paper bags and brought to the laboratory for isolation of *Fusarium oxysporum* f.sp. *lini* from the diseased plant parts. A total of 25 isolates were obtained from the isolation of diseased plant samples of these 29 locations. Some additional samples were procured from the other major linseed growing parts of India. The roots and stems of infected plants were washed in tap water to remove adhering soil particles, if any and root bark was removed before isolation to avoid contamination.

The roots and stems were split open and small bits (size 2.5cm) were cut with sterilized sharp blade. These bits were then disinfected with 0.1% solution of mercuric chloride for one or two minutes, then washed thoroughly in sterile distilled water thrice to remove the traces of mercuric chloride, dried in sterile blotter paper and aseptically transferred on

PDA in petriplate, and incubated at 25 ± 2°C for a week. Fungus growth in plate was examined and then sub-cultured on PDA slants. By frequent subculturing, it was purified and maintained on PDA slants for further studies. A total of 35 isolates of pathogen were isolated from available samples and were designated as Foli-1 to Foli-35.

The pure culture of fungus was obtained by adopting single spore techniques as described by Choi *et al.*, (1999). On the basis of cultural and morphological characteristics i.e. size of micro and macro conidia, number of septa, colony colour, colony growth and pigmentation etc., the test pathogen was identified as *Fusarium oxysporum* f.sp. *lini* by comparing with the monograph of *Fusarium oxysporum* f.sp. *lini* given by Booth (1971) and described by Burgess *et al.*, (1994).

### Morphological and cultural characteristics

Thirty-five isolates of *Fusarium oxysporum* f.sp. *lini* were further studied for their morphological characters *viz.*, size of macroconidia, microconidia & septation, 5mm diameter disc of mycelia of each isolates were taken from the actively growing culture and placed upside down centrally on 90 mm petridish containing solidified PDA medium and the inoculated plates were incubated at 25 ± 2° C for 9 days. Each plate was replicated three times. To record size and septation of macroconidia & microconidia a clear slide was prepared from 9 days old culture, stained with cotton blue and observed under the calibrated compound microscope. The length and breadth of twenty macroconidia and microconidia for each of three replications were measured. Cultural characteristics such as colony colour, pigmentation and colony diameter after 9 days of inoculation were also recorded for all the 35 isolates.

## Results and Discussion

The data in the Table 1 represents that wilted plant samples were collected from six linseed growing blocks of district Kangra *viz.*, Rait, Kangra, Bhawarna, Nagrota Bagwan, Sulah and Baijnath. The average disease incidence of the 5 locations in block Rait varied from 8.22 to 35.00 per cent, at 2 locations of block Knagra average disease incidence was recorded 32.29 and 40.75 per cent, at 3 locations of block Nagrota Bagwan average disease incidence varied from 11.30 to 42.00 per cent and at 4 locations of block Baijnath it varied between 12.15 to 28.95 per cent.

Whereas at single locations of block Bhawarna and Sullah the average disease incidence was recorded 10.25 and 32.55 per cent. Sixteen isolates were obtained from the isolation of these samples and designated as: Foli-1 to Foli-16. Similarly, disease samples were also collected from the linseed fields of Chauntra and Drang blocks of district Mandi and the average disease incidence in these blocks was recorded 9.50 to 22.45 per cent and 7.36 to 48.20 per cent, respectively.

Nine isolates were obtained from the isolation of diseased roots and other plant parts collected from linseed fields of these two blocks of district Mandi and designated as: Foli-17 to Foli-25. Table 1 also shows occurrence of *Fusarium oxysporum* f.sp. *lini* in wilted plant samples procured from other ten linseed growing parts of India i.e. Raipur (Chhattisgarh), Faizabad (Uttar Pradesh), Gorakhpur (Uttar Pradesh), Kanpur (Uttar Pradesh), Kota (Rajasthan), Kaul (Haryana), Ludhiana (Punjab), Gurdaspur (Punjab), Mukeriyana (Punjab) and Jammu (Jammu & Kashmir). Ten isolates were obtained from the isolation of diseased roots and other plant parts of linseed and designated as: Foli-26 to Foli-35.

## Variability in morphological characters

Morphological studies revealed variation in size of microconidia, macroconidia and number of septa among thirty five isolates of *Fusarium oxysporum* f.sp. *lini*. The results presented in Table 2 indicate that all isolates of *Fusarium oxysporum* f.sp. *lini* used in study varied significantly in their morphological and cultural characteristics on PDA (Plate a). Macroconidia were straight; spindle as well as sickle shaped. Table 2 showed variation in size of macroconidia. The average length of macroconidia varied from 11.26  $\mu\text{m}$  in Foli-9 to 38.33  $\mu\text{m}$  in Foli-13. The average breadth of macroconidia varied from 1.95  $\mu\text{m}$  in Foli-22 to 3.16  $\mu\text{m}$  in Foli-13. While, number of septa in macroconidia varied from 1-5. Based on the length of macroconidia, the isolates were grouped into two categories as large (above 30  $\mu\text{m}$ ) and small (up to 30  $\mu\text{m}$ ) macroconidia as given by Dubey *et al.*, (2010). Four Isolates Foli-11, Foli-13, Foli-20, & Foli-30 showed above 30  $\mu\text{m}$  length of conidia and categorized as large macroconidia and remaining 31 isolate showed up to 30 length of conidia and categorized as small macroconidia. Table 2 also shows that variation was also recorded in size of microconidia. The average length of microconidia varied from 4.45  $\mu\text{m}$  in Foli-9 to 11.19  $\mu\text{m}$  in Foli-13. The average breadth of microconidia varied from 1.75  $\mu\text{m}$  in Foli-2 to 3.01  $\mu\text{m}$  in Foli-29. The number of septa varied from 0-1. In similar studies Saharan and Mehta (2002) stated that both micro and macroconidia were produced by isolates of *Fusarium oxysporum* f.sp. *lini*.

The average size of microconidia ranged from 4.8 - 14.4 x 2.2 - 4.8  $\mu\text{m}$  and in macroconidia the average size ranged from 21.0 - 53.0 x 2.4 - 5.6  $\mu\text{m}$ . Kriplani *et al.*, (2018) studied cultural, morphological and pathogenic variability of *Fusarium oxysporum* f.sp. *pisi*.

The mycelia colour of the isolates varied from white to light pink, purple and pale yellow colour. The radial growth of the isolates ranged from 5.4 cm to 8.9 cm at 8 days. The size of macroconidia ranged from 11.6 x 3.1 to 25.2 x 6.2  $\mu\text{m}$  and size of microconidia ranged from 3.02 x 2.1  $\mu\text{m}$  to 9.2 x 5.6  $\mu\text{m}$ .

The number of septation of macroconidia was mostly 2-3 & microconidia in most of the isolates were having no septum. Dubey *et al.*, (2010) observed isolates of *F. oxysporum* f.sp. *ciceris* to vary with respect to their conidia size. Microconidia varied from 5.1-12.8 x 2.5-5.0  $\mu\text{m}$  in size, whereas macroconidia were from 16.5-37.9 x 4.0 x 5.9  $\mu\text{m}$  with 1-5 septations most commonly with 2-3 septate conidia. Gupta *et al.*, (2011) noticed morphological variation among isolates of *F. oxysporum* f.sp. *pisi*.

The size of microconidia varied from 3.16 x 3.16  $\mu\text{m}$  (isolate I19) to 9.13 x 5.44  $\mu\text{m}$  (isolate I7) whereas macroconidial size varied from 11.77 x 3.16  $\mu\text{m}$  (isolate I19) to 24.60 x 5.91  $\mu\text{m}$  (isolate I7). All isolates formed chlamydospores on PDA medium except isolate I2. Chlamydospores size varied from 6.85 x 6.15  $\mu\text{m}$  (isolate I4) to 13.70 x 10.18  $\mu\text{m}$  (isolate I5).

### Variability in cultural characteristics

Table 3 shows that the colony colour of the 35 isolates varied from cottony white to purplish white. Six isolates *viz.*, Foli-1, Foli-9, Foli-10, Foli-12, Foli-21 & Foli-27 showed cottony white growth. 12 isolate *viz.*, Foli-3, Foli-8, Foli-13, Foli-15, Foli-16, Foli-18, Foli-22, Foli-23, Foli-29, Foli-31, Foli-33 & Foli-34 showed pale white and 17 isolates *viz.*, Foli-2, Foli-4, Foli-5, Foli-6, Foli-7, Foli-11, Foli-14, Foli-17, Foli-19, Foli-20, Foli-24, Foli-25, Foli-26, Foli-28, Foli-30, Foli-32 & Foli-34 showed purplish white growth of culture. The isolates also varied in their pigmentation from

without any pigmentation in 9 isolates *viz.*, Foli-3, Foli-4, Foli-12, Foli-13, Foli-15, Foli-21, Foli-27, Foli-31 & Foli-35 to light pinkish in 6 isolates *viz.*, Foli-1, Foli-5, Foli-7, Foli-17, Foli-20 & Foli-34, purplish in 17 isolates *viz.*, Foli-2, Foli-6, Foli-9, Foli-10, Foli-14, Foli-16, Foli-18, Foli-19, Foli-22, Foli-23, Foli-24, Foli-26, Foli-28, Foli-29, Foli-30, Foli-32 & Foli-33 and brownish in 3 isolates *viz.*, Foli-8, Foli-11 & Foli-25 (Plate b). Similarly, Nath *et al.*, (2017) observed that *Fusarium oxysporum* f.sp. *ciceris* exhibited variations in colony characteristics such as color, shape, margin and texture. Colony colors were purplish white, whitish orange, creamy white, cottony white. While, Prameela *et al.*, (2005) observed variation in substrate pigmentation in isolates of *F. oxysporum* f.sp. *carthami*.

The colony diameter of the isolates after 9 days of incubation showed that the fastest growing isolates were Foli-11, Foli-25 and Foli-30 with 90 mm of growth. All the isolates were grouped in to 3 groups i.e. fast growing (above 80 mm growth), medium growing (70-80 mm growth) and slow growing (below 70 mm growth) as described by Wagh (2009). Eleven isolates *viz.*, Foli-2, Foli-6, Foli-10, Foli-11, Foli-13, Foli-25, Foli-29, Foli-30, Foli-32, Foli-33 and Foli-35 were fast growing. While 21 isolates *viz.*, Foli-1, Foli-3, Foli-4, Foli-5, Foli-7, Foli-8, Foli-9, Foli-12, Foli-14, Foli-15, Foli-16, Foli-17, Foli-19, Foli-20, Foli-21, Foli-22, Foli-23, Foli-24, Foli-28, Foli-30 & Foli-34 were medium growing and 3 isolates *viz.*,

Foli-18, Foli-26 and Foli-27 were slow growing. Wagh (2009) also observed variability in growth among different isolates of *Fusarium oxysporum* f.sp. *lini*. In their study one isolate was recorded as fast growing (82.00 mm) while remaining 5 isolates showed moderate mycelial growth on PDA ranging from 71.60 mm to 78.10 mm

**Table.1** Occurrence of *Fusarium oxysporum* f.sp. *lini* in the plant samples collected from different linseed fields of Himachal Pradesh and procured from other linseed growing parts of India

Sr. no.	Location of sample collection	Name of Isolate	Average disease incidence (%) at the location
	District Kangra:-		
1.	Parei (Block Rait)	Foli-1	35.00
2.	Rait (Block Rait)	Foli-2	08.22
3.	Rajol (Block Rait)	Foli-3	25.15
4.	Parsail (Block Rait)	Foli-4	19.62
5.	Shahpur (Block Rait)	Foli-5	12.50
6.	Kangra (Block Kangra)	Foli-6	32.29
7.	Daulatpur (Block Kangra)	Foli-7	40.75
8.	Nagri (Block Bhawarna)	Foli-8	10.25
9.	Rajiana (Block Nagrota Bagwan)	Foli-9	42.00
10.	Tikri (Block Nagrota Bagwan)	Foli-10	11.30
11.	Malan (Block Nagrota Bagwan)	Foli-11	34.21
12.	Palampur (Block Baijnath)	Foli-12	28.95
13.	Utrala (Block Baijnath)	Foli-13	24.69
14.	Mathrehar (Block Baijnath)	Foli-14	15.50
15.	Baijnath (Block Baijnath)	Foli-15	12.15
16.	Garla (Block Sullah)	Foli-16	32.55
	<b>District Mandi:-</b>		
17.	Ahjoo (Block Chauntra)	Foli-17	22.45
18.	Ladhruhin (Block Chauntra)	Foli-18	09.50
19.	Chauntra (Block Chauntra)	Foli-19	14.34
20.	Joginder Nagar(Block Drang)	Foli-20	15.25
21.	Harabag (Block Drang)	Foli-21	48.20
22.	Bhararoo (Block Drang)	Foli-22	07.36
23.	Masoli (Block Drang)	Foli-23	43.88
24.	Machial (Block Drang)	Foli-24	15.75
25.	Balh (Block Drang)	Foli-25	13.60
	<b>Location of samples procured from other parts of India</b>		
26.	Raipur (Chhattisgarh)	Foli-26	-
27.	Faizabad (Uttar Pradesh)	Foli-27	-
28.	Gorakhpur (Uttar Pradesh)	Foli-28	-
29.	Ludhiana (Punjab)	Foli-29	-
30.	Kanpur (Uttar Pradesh)	Foli-30	-
31.	Kaul (Haryana)	Foli-31	-
32.	Kota (Rajasthan)	Foli-32	-
33.	Jammu (Jammu & Kashmir)	Foli-33	-
34.	Gurdaspur (Punjab)	Foli-34	-
35.	Mukeriyana (Punjab)	Foli-35	-

**Table.4.3** Variability in morphological characters of different isolates of *Fusarium oxysporum* f.sp. *lini*

Isolate	Average size of conidia					
	Macroconidia			Microconidia		
	(L)* (µm)	(B)* (µm)	No. of septa	(L) (µm)	(B) (µm)	No. of septa
<b>Foli-1</b>	13.44	2.25	1-2	5.52	2.34	0
<b>Foli-2</b>	15.81	2.56	1-2	4.89	1.88	0
<b>Foli-3</b>	16.94	2.10	1-3	9.20	2.64	0-1
<b>Foli-4</b>	15.64	3.10	1-3	8.53	2.95	0-1
<b>Foli-5</b>	16.22	2.95	2-3	5.29	2.59	0
<b>Foli-6</b>	29.53	2.62	2-4	5.40	1.95	0
<b>Foli-7</b>	19.25	1.98	2-4	6.33	1.99	0
<b>Foli-8</b>	14.96	2.59	1-2	4.56	2.11	0
<b>Foli-9</b>	11.26	2.14	1-2	4.45	2.96	0
<b>Foli-10</b>	24.61	2.81	1-4	11.05	2.86	0-1
<b>Foli-11</b>	31.31	2.57	1-3	7.84	2.55	0-1
<b>Foli-12</b>	15.19	2.22	1-3	5.60	2.94	0
<b>Foli-13</b>	38.33	3.16	3-5	11.19	2.36	1-1
<b>Foli-14</b>	17.85	2.51	1-3	7.51	2.73	0-1
<b>Foli-15</b>	18.45	2.19	2-3	6.26	2.82	0-1
<b>Foli-16</b>	18.12	2.47	1-4	8.49	2.61	0-1
<b>Foli-17</b>	23.05	3.02	2-4	8.12	1.95	0-1
<b>Foli-18</b>	16.49	2.53	1-3	6.47	2.77	0
<b>Foli-19</b>	12.34	2.91	3-4	10.01	2.12	0-1
<b>Foli-20</b>	36.90	2.42	1-3	5.33	2.25	0-1
<b>Foli-21</b>	23.23	2.81	1-3	4.51	2.13	0
<b>Foli-22</b>	19.67	1.95	2-3	7.26	2.80	0-1
<b>Foli-23</b>	17.94	2.01	2-4	6.44	2.51	0-1
<b>Foli-24</b>	16.55	2.31	1-3	7.55	2.95	0-1
<b>Foli-25</b>	17.02	1.99	1-3	6.14	2.22	0
<b>Foli-26</b>	15.29	2.34	1-3	6.07	1.94	0
<b>Foli-27</b>	12.21	2.72	1-2	4.81	1.75	0
<b>Foli-28</b>	16.30	2.95	1-4	7.12	2.75	0-1
<b>Foli-29</b>	17.62	2.31	2-4	8.82	3.01	0-1
<b>Foli-30</b>	34.29	2.79	2-4	9.88	2.74	0-1
<b>Foli-31</b>	14.66	2.81	1-3	6.11	2.87	0-1
<b>Foli-32</b>	19.37	2.76	2-4	7.45	1.97	0-1
<b>Foli-33</b>	19.78	2.53	2-4	8.02	2.32	0-1
<b>Foli-34</b>	28.95	2.64	1-4	5.98	2.21	0
<b>Foli-35</b>	17.20	2.25	1-4	7.43	2.69	0-1

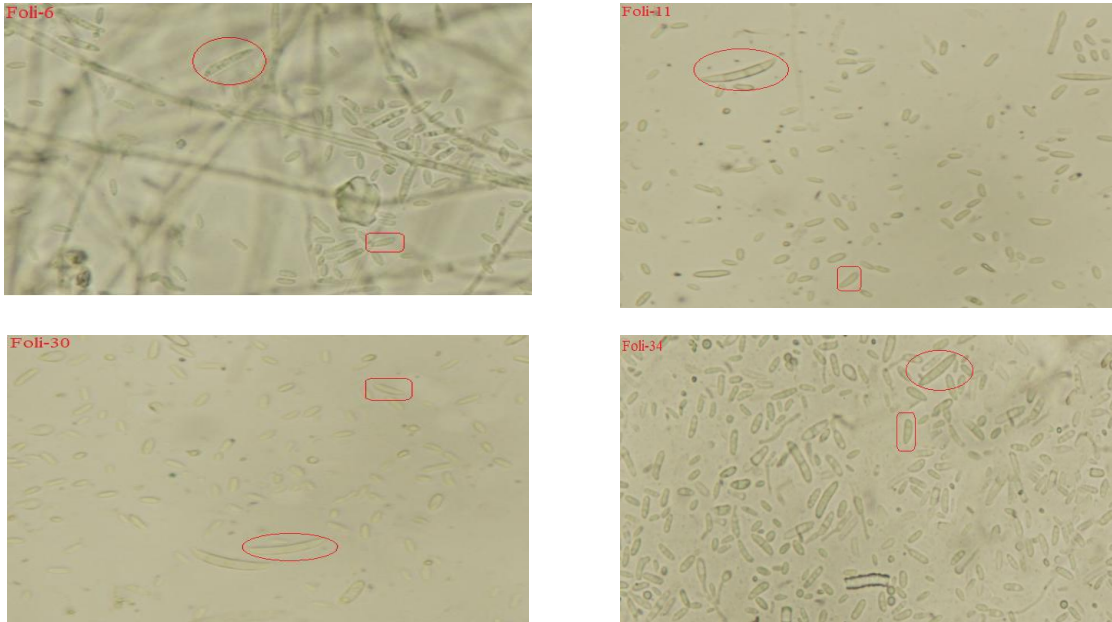
L\*= Length; B\*= Breadth



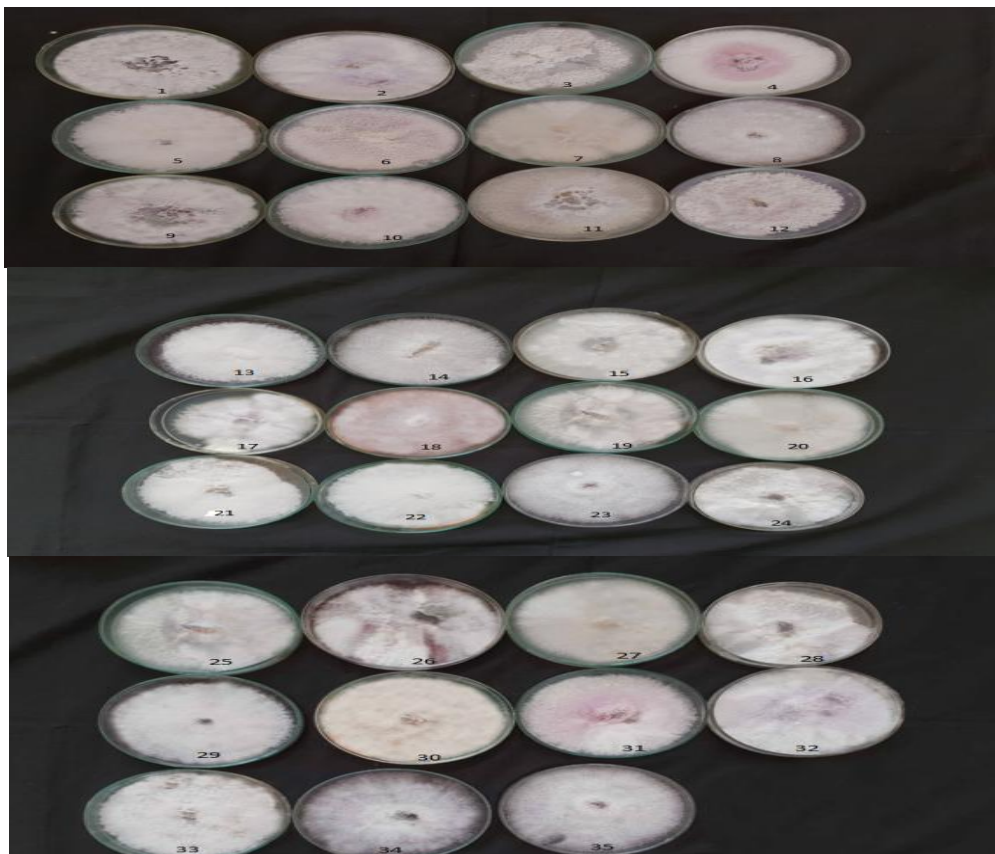
**Table.3** Variability in cultural characters of different isolates of *Fusarium oxysporum* f.sp. *lini*

<b>Isolate</b>	<b>Colony colour</b>	<b>Pigmentation</b>	<b>Colony diameter (mm) at 9 DAI</b>
<b>Foli-1</b>	Cottony white	Light Pink	78
<b>Foli-2</b>	Purplish White	Purplish	81
<b>Foli-3</b>	Pale White	Absent	79
<b>Foli-4</b>	Purplish white	Absent	75
<b>Foli-5</b>	Purplish white	Light Pink	79
<b>Foli-6</b>	Purplish white	Purplish	88
<b>Foli-7</b>	Purplish white	Light Pink	77
<b>Foli-8</b>	Pale White	Brownish	72
<b>Foli-9</b>	Cottony white	Purplish	73
<b>Foli-10</b>	Cottony white	Purplish	84
<b>Foli-11</b>	Purplish White	Brownish	90
<b>Foli-12</b>	Cottony white	Absent	76
<b>Foli-13</b>	Pale White	Absent	83
<b>Foli-14</b>	Purplish white	Purplish	76
<b>Foli-15</b>	Pale White	Absent	73
<b>Foli-16</b>	Pale White	Purplish	75
<b>Foli-17</b>	Purplish White	Light Pink	79
<b>Foli-18</b>	Pale white	Purplish	68
<b>Foli-19</b>	Purplish white	Purplish	79
<b>Foli-20</b>	Purplish White	Light Pink	75
<b>Foli-21</b>	Cottony white	Absent	73
<b>Foli-22</b>	Pale White	Purplish	76
<b>Foli-23</b>	Pale White	Purplish	74
<b>Foli-24</b>	Purplish White	Purplish	77
<b>Foli-25</b>	Purplish white	Brownish	90
<b>Foli-26</b>	Purplish white	Purplish	69
<b>Foli-27</b>	Cottony White	Absent	68
<b>Foli-28</b>	Purplish White	Purplish	72
<b>Foli-29</b>	Pale White	Purplish	89
<b>Foli-30</b>	Purplish White	Purplish	90
<b>Foli-31</b>	Pale White	Absent	78
<b>Foli-32</b>	Purplish white	Purplish	81
<b>Foli-33</b>	Pale White	Purplish	87
<b>Foli-34</b>	Pale White	Light Pink	80
<b>Foli-35</b>	Purplish white	Absent	82

**Plate.a** Microscopic view of conidia of different isolates of *Fusarium oxysporum* f.sp. *lini* (macroconidia in circle & microconidia in rectangle)



**Plate.b** Different Isolates of *Fusarium oxysporum* f.sp. *lini* (Foli-1 to Foli-35)





In conclusion Kommedahl *et al.*, (1970) identified that isolates of *Fusarium oxysporum* f.sp. *lini* vary in: 1) morphology, with respect to the amount and type of sporulation, production of different types of conidia, size and number of septa and pigment production on growth media; 2) physiology, rate and type of growth on substrates and in host; 3) environmental preferences, antibiotic capabilities; and 4) pathogenicity. Therefore, it is considered an ideal pathogen to demonstrate diversity within a species. During the study it was concluded that all the 35 isolates of *Fusarium oxysporum* f.sp. *lini* exhibited considerable variations in cultural and morphological characters. In macroconidia, average length varied from 11.26 µm in Foli-9 to 38.33 µm in Foli-13 with 1-5 septa and average breadth varied from 1.95 µm in Foli-22 to 3.16 µm in Foli-13. In microconidia, average length varied from 4.45 µm in Foli-9 to 11.19 µm in Foli-13 with 0-1 septa and average breadth of varied from 1.75 µm in Foli-2 to 3.01 µm in Foli-29. Six isolates showed cottony white, 12 isolates showed pale white and 17 isolates showed purplish white growth of culture. It was also concluded that the colony diameter ranged from 68.00 to 90.00 mm at 25 ± 2° C for 9 days and fastest growing isolates were Foli-11, Foli-25 and Foli-30 with 90 mm of growth. Eleven isolates were fast growing, 21 isolates were medium growing and 3 isolates were slow growing.

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