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Kishor Madhukar Wankhede
Ph.D scholar, Plant Pathology Section, College of Agriculture, Nagpur, Dr. PDKV, Akola, Maharashtra, India

Dr. RW Ingle
Head & Professor (CAS) (Rt), Plant Pathology Section, College of Agriculture, Nagpur, Maharashtra, India

Dr. SB Bramahnkar
Professor (CAS), Plant Pathology Section, College of Agriculture, Nagpur, Maharashtra, India

Jagdish M Parbat
Assistant Professor, Plant Pathology Section, College of Agriculture, Nagpur, Maharashtra, India

Ganesh G Chaware
Senior Research Fellow (SRF), ICAR- Agricultural Technology Application Research Institute, College of Agriculture Campus, Shivajinagar, Pune, Maharashtra, India

Corresponding Author:
Kishor Madhukar Wankhede
Ph.D scholar, Plant Pathology Section, College of Agriculture, Nagpur, Dr. PDKV, Akola, Maharashtra, India

Survey and variability among *Fusarium oxysporum* f. sp. *lini* isolates inducing wilt disease of linseed in Vidarbha growing regions of Maharashtra

Kishor Madhukar Wankhede, RW Ingle, SB Bramahnkar, Jagdish M Parbat and Ganesh G Chaware

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Abstract
Linseed is very rich in oil and the oil and protein percent in seed of linseed varies from 37.8 to 43.2% and 20.00 to 24.8% respectively and after extracting oil the refuse called seed cake is a well-known fattening food for cattles. The crop is susceptible to wilt, a devastating soil-borne disease induced by the fungus *Fusarium oxysporum* f.sp. *lini*. These investigations were deemed necessary due to the possible danger of linseed wilt caused by *Fusarium oxysporum* f.sp. *lini*, and as a result, they were started for the evaluation of the disease occurrence, characterization, and differences in the morphological, cultural behavior of collected isolates of the pathogen. Understanding cultural, morphological variation of *F. oxysporum* isolates is of highest importance, which has not been before explored in Vidarbha regions of Maharashtra. 13 places of Eight districts viz Akola, Nagpur, Wardha, Bhandhara, Gondia, Amravati, Gadchiroli and Chandrapur of Maharashtra state were surveyed throughout the rabi season of continues two years (2021-2022 and 2022-2023). 11 samples of FOL were procured from different states of India with help of AICRP, Nagpur. Wilt incidence in different districts varied from 15.63 to 35.19 percent. Maximum per cent disease incidence 39.50 was recorded in AICRP Nagpur district. In total 24 isolates of pathogen (FOL) were covered. All collected isolates were showed variations in color of colony, morphology and pigmentation response. Isolates produced appressed flat, partially appressed, partially flat appressed, mycelium growth, mycelial colour in all isolates varied from white, dull white, cottony white, dark white. Radial growth of all isolates at 7 DAI, total 8 isolates were moderate growing (50 - 70 mm), total 17 isolates were fast growing (more than 70 mm). In length and breadth maximum average size was recorded in the isolate Fol-4 (25.13 × 6.24 µm**)** length and breadth in macroconidia, numbers of septa observe varied from between 2-7 in all isolates while in microconidia maximum average size was recorded in the isolate Fol - 17 (10.34 x 2.35 µm) no sepatation was found in all isolates.

Keywords: Cultural and morphological variability, diseases incidencee, survey *Fusarium oxysporum* f. sp. *lini*

1. Introduction
Linseed is one of the oldest crop cultivated for its seeds and fiber. The two products of seed are linseed oil and linseed meal. Flax (*Linum usitatissimum*) is also one of the richest dietary sources of α-linolenic acid (ALA) and is a good source of soluble fiber mucilage in human nutrition. Linseed is very rich in oil and the oil and protein percent in seed of linseed varies from 37.8 to 43.2% and 20.00 to 24.8% respectively. During 2023-24, a total of 1.67 lakh tonnes of linseed was produced in India from an area of 2.39 lakh ha with an average productivity of 698 kg/ha. In terms of productivity, Rajasthan stands first (1072 kg/ha) followed by Bihar (851 kg/ha) and Nagaland (771 kg/ha) (Linseed, Annual report 2023-24). Among the fungal diseases of linseed, Alternaria blight, Powdery Mildew, Rust, Wilt is a major constraint responsible for low production and productivity along with rust and powdery mildew (Kishore *et al.* 2011). The crop loss due to linseed wilt incited by *Fusarium oxysporum* f. sp. *lini* is the most destructive disease (Sattar and Hafiz (1952) reported losses by wilt disease in linseed crop up to 80 % under favourable conditions. Linseed wilt incited by *Fusarium oxysporum* f.sp. *lini* (Bolly) Snyder and Hansen was first reported by Luggar (1890) from Minnesota, USA. In India, disease was first reported by Pearl (1923) in Madhya Pardesh. Flax wilt is caused by the soil- and seed-borne fungus *Fusarium oxysporum* f.

sp.*lini* (Bolley) Snyd. and Hans. This pathogen invades the roots, mainly through root hairs, develops in xylem vessels and becomes systemic. Disease symptoms may be obvious during the early seedling stage or may appear later. The pathogen infects the plant through the roots and colonizes the vascular system, leading to wilting, stunting, and ultimately, plant death. The disease spreads through soil-borne inoculum, including infected plant debris and soil particles. The mycelium is septate, branched, and intracellular. The pathogen produces branched, hyaline, and short conidiophores that give rise to hyaline and micro and macro conidia. The conidia are both micro- and macro-conidia. Favorable environmental conditions, such as high soil moisture and warm temperatures, promote disease development and progression. The pathogen with its high saprophytic ability can survive in soil for a long period during which it may have to go through different environmental stresses and biological competition which may lead to the existence of physiologic races. The most effective and practical method of control worldwide is to use fungicides (Gupta *et. al*., 1988) [18] or resistant cultivars. However, the effectiveness of host resistances is curtailed by the occurrence of pathogenic races in *F. oxysporium* f.sp. *lini* (Jimenez-Gasco *et.al*., 2004) [8]. Researchers from all across the world suggested creating *F. oxysporum* as a global model for the knowledge of fungal virulence. The various isolates of *Fusarium* have been classed on the basis of their nutritional demands, temperature, sensitivity to chemicals (Khare et al 1980) [9] and morphology and aggressiveness. Increased attention is being paid by the scientists to develop wilt resistant varieties for large scale cultivation of Linseed and appropriate management of the disease. Therefore, accurate and rapid identification of pathogen is necessary. Identification of pathogenic races would be done by use of different reaction to selected host genotypes. To reduce the effects caused by the variability in the pathogen it is essential to know the genetic nature and pathogenic types of isolates prevailing in linseed growing areas of India. Hence, the present investigation will help to understand the variability in the pathogen which will be useful for its exploitation in wilt resistance breeding and further used for development of area specific resistant varieties of linseed. The present study was conducted to correlate cultural and morphological variability in *F.oxysporum* isolates collected from major linseed growing areas of India. The study will help to know the prevalence and distribution of more virulent isolates of wilt pathogens in the country. The identified virulent isolates will be used for screening of breeding material of linseed and the obtained resistant lines will be supplied to linseed breeders to develop wilt resistant linseed varieties with high yielding potential. The introduction of wilt resistant linseed cultivars will help to reduce losses caused by wilt disease and thus enhance the linseed production in the country.

2. Materials and Methods

Survey and collection of samples

During two year Rabi cropping season, 2021-2022 and 2022-23 at 8 districts *viz.*, Akola, Nagpur, Wardha, Bhandhara, Gondia, Amaravati, Gadchiroli, Chandrapur surveyed for the assessment of disease incidence. Linseed plants naturally infected and showing typical wilt symptoms were collected from farmer's fields from different locations in different district of Maharashtra and brought to the laboratory. At each field, three observations on total number of linseed plants in l m^2 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) [11] total number of plants per spot as [(number of diseased plants/total number of plants) x 100]. Samples were placed into paper bags and proper labeled to indicate location, sample number and date of collection. The samples collected during inspection were brought to Plant Pathology Section, College of Agriculture, Nagpur

Isolation and purification of pathogen

Isolation of *Fusarium oxysporum* f. sp. *lini* isolates collected from different locations, from wilted linseed plant roots/stem were made on nutritional artificial media (PDA). The causal organisms were isolated from root of plant that affected by wilt disease. 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 24 isolates of pathogen were isolated from available samples. Isolation was made and the isolation culture was purified by following single spore isolation techniques (Choi 1999). A little bit of fungal growth with spore placed was in a test tube containing 5 ml of sterilized water and shaken well to prepare 60 suspension of spore. A dilute spore suspension was poured on water agar media. Petri dishes to form a very thin layer on it and spores allowed settling down on the agar surface. Settled spores were separated out from each other, selected under the microscope. The position of single spore was marked help of plastic rod on glass surface under low power. The plates were taken to the isolation chamber and cut of agar from marked areas were cut of the help of dummy cutter in Petri dish and transferred to Petri dishes containing sterilized PDA medium. After proper growth of fungus obtained by single spore culture regular sub-culturing was done to check contamination, till pure cultures were obtained. The culture was then transferred into the incubator at 25±2 ºC temperature and growth observed after four days.

Morphological and cultural characteristics

The isolates of Fol collected from different linseed growing states of India were studied for their morphological characterization. All 24 isolates were grown on potato dextrose agar (HiMedia, Mumbai, India) medium for morphological studies comprising viz., size of macroconidia, microconidia & septation of all the isolates, 5 mm 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. The spore dimensions such as length and width of both the microconidia and macroconidia were measured through a

calibrated compound microscope (Procam software) for 50 spores of each isolate and the mean was recorded as final dimension. Septation of the macroconidia was also recorded.

The isolates were also cultured in liquid media in 250 ml flask containing 100 ml of potato dextrose broth (PDB). These flasks were incubated at 25±2 °C for 15 days. The colony characters, sporulation and pigmentation of all the isolates were also recorded.

3. Results and Discussion

Survey and Collection of Samples

Roving survey was carried out during *rabi* 2021-22 and 2022-23 at 13 different locations among Vidarbha region of the Maharashtra contained diverged zones. The random survey was conducted covering 13 number of field in the Eight districts of Maharashtra *viz*., Akola, Nagpur, Wardha, Bhandhara, Gondia, Amaravati, Gadchiroli, Chandrapur during rabi season 2021-22 and 2022-23. Date of first appearance of disease and final disease incidence was recorded. Incidence of wilt was recorded during the course of investigation. Highest wilt incidence recorded at AICRP, Nagpur district 34.16 percentage ranged from (33.14 and 35.19 %) in 2021-22 and 2022-23 repectively followed by ARS Sonapur, Gadchiroli 31.28 % (30.00 and 32.56 %), Alewahi, Chandrapur 30.49 % (32.53 and 28.45%) and Nagbhir, Chandrapur 28.90% (30.15 and 27.65%) in two successive years. In lowest wilt incidence recorded in Lakhani, Bhandhara district 17.90% (15.63 and 20.18%) followed by Rengatur, Nagpur 22.12% (20.50 and 23.75%), Barshitakli, Akola 22.20% (24.30 and 20.11%), Kvk Hiwara, Gondhia 22.89% (20.80 and 24.98%) in two successive years (Table- 1), (plate 1 and 2). Moderate disease incidence recorded at Shiwapur, Nagpur 27.11 % (26.00 and 28.23 %), Aptur, Nagpur 26.62 % (27.60 and 25.64 %).

The incidence of linseed wilt was recorded in four district Akola, Chandrapur, Wardha, Nagpur (Linseed annual report 2023-24). The incidence of linseed was recorded in Bhandhara, Gondia, Gadchiroli, Amaravati (Linseed annual report 2022-23). Similar finding incidence of linseed wilt was recorded in three districts like Latur, Beed and Osmanabad (Ranganath, 2016).

Variability in morphological characteristics

All isolates of *Fusarium oxysporum* f.sp. *lini* differed in their morphological characteristics when grown on PDA. Morphological studies revealed variation in size of microconidia, macroconidia and number of septa among twenty-four isolates of *Fusarium oxysporum* f.sp. *lini*. The results presented in Table 02 indicate that all isolates of *Fusarium oxysporum* f.sp. *lini* used in study varied significantly in their morphological and cultural characteristics on PDA.

(A) Macro conidia

Variation was observed in length of macroconidia. The average length of all twenty-four isolates varied from 6.98 µm to 32.98 µm and average breadth 1.27 µm to 8.01 µm. Minimum average size was recorded in the isolate Fol-9 (8.05 x 1.77 µm) while the maximum average size was recorded in the isolate Fol-3 (25.13 × 6.24 µm**)** length and breadth respectivly. Numbers of septa observe varied from between 2-7 in all isolates (Plate 3). In similar studies Saharan and Mehta (2002) [14] 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 µm and in macroconidia the average size ranged from 21.0 - 53.0 x 2.4 - 5.6 µm. Dubey *et al.* (2010) [15] 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 µm in size, whereas macroconidia were from 16.5 - 37.9 x 4.0 x 5.9 µm with 1-5 septations most commonly with 2-3 septate conidia. Similar finding were observed by other researchers to *viz.*, Gupta *et al.* (2011) [7], Kriplani *et al.* (2018) [10].

(B) Micro Conidia

Variation was observed in length of microconidia. The average length of all twenty-four isolates varied from 2.20 µm to 10.34 µm and average breadth 0.97 µm to 2.35 µm. Minimum average size was recorded in the isolate Fol- 22 (2.20 x 0.97 µm) while maximum average size was recorded in the isolate Fol - 17 (10.34 x 2.35 µm). Number of septa in microconidia were 0 in all isolates (Plate 4). In similar studies Saharan and Mehta (2002) [14] 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 µm and in macroconidia the average size ranged from 21.0 - 53.0 x 2.4 - 5.6 µm. *Fusarium oxysporum* f.sp. *ricini* isolate of castor wilt (Desai *et al.* 2003) [4], *Fusarium oxysporum* f.sp. *carthami* isolate of Safflower wilt (Prameela *et al.* 2005) [12] and *Fusarium oxysporum* f.sp. *ciceri* isolate of Gram wilt (Trivedi and Gurha, 2007).

Variability in Cultural Characteristics

(A) Variability in mycelial appearance of *Fusarium oxysporum* f.sp. *lini* isolates

The *Fusarium oxysporum* f.sp.*lini* isolates selected for cultural studies showed variation on PDA medium in table 3, (Plate 5). The observation of mycelial colour were taken on 7th day after inoculation. The mycelium growth of *Fusarium oxysporum* f. sp. *lini* was observed varied from fluffy, appressed flat, partially appressed, partially flat appressed. fluffy growth was observed in isolates FOL 1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24 appressed flat in FOL4, 17, FOL 5 partially appressed, FOL 23 partially flat appressed. The mycelial colour in all isolates varied from white, dull white, cottony white, Dark White. In Isolates FOL 1, 4, 6, 8, 9, 12, 16, 17, 20, 21, 22, 24 dull white mycelial colour was observed. FOL 2, 5, 7, 10, 11, 13, 19, 23 White mycelial colour was observed, FOL 3 cottony white, FOL 14, 15 dark white.

Variation in colony pigmentation, mycelial growth, radial growth in *F. udum* isolates from different locations in India have been recorded by Chattopadhyay and Sen Gupta (1967); Jeswani *et al*. (1978); Gupta *et al*. (1998); Gaur and Sharma (1989); Rajendra and Patil (1992); Madhukeshwara and Seshadri (2001) [17] and Tiwari and Dhar (2011) [3, 18, 6, 13].

Variation in the radial growth rate of *Fusarium oxysporum* f.sp. *lini* isolates

On the basis of radial growth, the isolates of *F. oxysporum* f.sp.*lini* were categorized into 3 groups *viz*., fast growing (more than 70 mm), moderate growing (50 -70 mm) and slow growing (less than 50 mm) on PDA medium(Table 4), (Fig 1).

The result depicted in table 4 that out of 24 isolates seven isolates (FOL 1, FOL 6, FOL 9, FOL 12, FOL 16, FOL 21, FOL 22) were moderately growing. While remaining seventeen *viz.*, (FOL 2, FOL 3, FOL 4, FOL 5, FOL 7, FOL 8, FOL 10, FOL 11, FOL 13, FOL 14, FOL 15, FOL 17, FOL 18, FOL 19, FOL 20, FOL 23, FOL 24) were fast growing (Fig. 1). Similar variations in colony growth were recorded earlier by Prameela *et al.* (2005) [12] among the isolates of *Fusarium oxysporum* f.sp. *carthami*. Trivedi and Gurha (2007) grouped the isolates of *Fusarium oxysporum* f.sp. *ciceri* on the basis of mycelium growth i.e. fast, medium and slow. Similarly, Wagh *et al.* (2009) also observed variability in growth among different isolates of *Fusarium oxysporum* f.sp. *lini.*

(B) Variation in pigmentation of different isolates

The isolates varied in their pigmentation on 7th day after inoculationon PDA on reverse side of PDA plates. Light yellow to purple pigmentation was observed in isolates FOL 1, FOL 2, FOL 6, FOL 18, FOL 19, light yellow was observed in FOL 3, FOL 4, FOL 7, FOL 8, FOL 9, FOL 13, FOL 22, FOL 24, light orange to purple FOL 5, FOL 12, FOL 14, FOL 15, FOL 16, FOL 20, FOL 21, white was observed in FOL 11, FOL 17 while orange was observed in FOL 23, FOL 10.

The present results similar with the findings of Madhukeshwara and Seshadri (2001) [17] who studied the existence of variability among six isolates of *F. udum* with respect to morphological and cultural characteristics *i.e.* varied pigmentation was noticed from white to dusk red. Rangaswamy *et al.*, (2016) [9] studied that *F. udum* isolates were having considerable variation in substrate pigmentation for all the isolates.

Table 1: Percent disease incidence of linseed wilted field in different location of Vidarbha region

Sr. no	Sample Coding	District	Taluka	Village/ Center	Plant Growth stage	Percent Wilt incidence 2021-22	Percent Wilt incidence 2022-23	Mean
1	FOL 1	Akola	Telhara	Umri	Vegetative	26.04	24.65	25.34
2	FOL 2	Akola	Barshitakli	Bahirkhed	Vegetative	24.30	20.11	22.20
3	FOL 3	Nagpur	Nagpur	AICRP, Nagpur	Vegetative	33.14	35.19	34.16
4	FOL 4	Nagpur	Bhiwapur	Shiwapur	Vegetative	26.00	28.23	27.11
5	FOL 5	Nagpur	Umred	Aptur	Vegetative	27.60	25.64	26.62
6	FOL 6	Nagpur	Kuhi	Rengatur	Vegetative	20.50	23.75	22.12
7	FOL 7	Wardha	Samudrapur	Girad	Vegetative	22.42	25.67	24.04
8	FOL 8	Bhandhara	Lakhani	Deori	Vegetative	15.63	20.18	17.90
9	FOL 9	Gondia	Gondia	KVK Hiwara	Vegetative	20.80	24.98	22.89
10	FOL 10	Amaravati	Darayapur	Sanglud	Vegetative	27.27	27.23	25.75
11	FOL 11	Gadchiroli	Chamorshi	ARS Sonapur	Vegetative	30.00	32.56	31.28
12	FOL 12	Chandrapur	Nagbhir	Akapur	Vegetative	30.15	27.65	28.90
13	FOL 13	Chandrapur	Gondpimpiri	Alewahi	Vegetative	32.53	28.45	30.49

Plate 1: Survey at Amravati (Sanglud) district

Plate 2: Survey at Gadchiroli (Chamorshi) district

Table 2: Morphological characters (microscopic) of twenty-four isolates of *Fusarium oxysporum* f.sp. *lini*

Sr.no. o	Isolate	Mean		Size (µm) and septation of macroconidia		Mean		Size (µm) and septation of microconidia	
		Length	Breadth	Range	No.of.septa	Length	Breadth	Range	No.of.septa
1	FOL 1	14.25	3.27	13.92 - 16.35 × 2.95 × 3.97	2-4	3.45	1.50	3.22 - 3.94 × 1.35 - 1.58	0
2	FOL 2	12.76	2.98	12.24 - 15.65 × 2.61 × 3.67	2-3	4.27	1.85	3.29 - 5.05 × 1.15 - 2.03	0
3	FOL 3	25.13	6.24	17.61 - 32.98 × 5.25 - 7.56	2-7	7.38	1.70	6.15 - 8.48 × 1.60 - 1.76	0
4	FOL 4	11.21	3.72	10.27 - 13.38 × 2.34 - 4.12	2-4	4.87	1.17	4.40 - 5.15 × 2.15 - 2.75	0
5	FOL 5	9.52	2.01	8.97 - 10.26 × 1.98 - 2.30	2-4	3.05	1.91	2.37 - 3.16 × 1.37 - 2.21	0
6	FOL 6	8.67	3.17	6.98 -- 10.95 × 2.05 - 3.65	2-5	3.20	2.20	3.05 - 3.85 × 2.01 - 2.75	0
7	FOL 7	10.43	4.01	8.98 - 11.37 × 3.45 - 5.07	2-3	2.87	1.20	2.75 - 3.77 × 1.07 - 1. 87	0
8	FOL 8	11.64	3.08	9.78 - 12.97 × 2.65 - 3.98	2-4	3.25	2.00	3.17 - 4.07 × 1.97 - 2.19	0
9	FOL 9	8.05	1.77	8.65 - 9.85 × 1.85 - 2.17	2-3	2.45	1.27	2.29 - 3.15 × 0. 95 - 1.53	0
10	FOL 10	10.51	2.20	9.78 - 10.68 × 2.15 - 2.65	2-3	3.49	1.35	3.21 - 3.97 × 1.27 - 2.09	0
11	FOL 11	9.19	2.03	7.64 - 10.21 × 1.98- 2.82	2-3	4.95	2.11	4.25 - 5.03 × 2.09 - 2.17	0
12	FOL 12	11.97	1.65	11.35 - 12.67 × 1.53 - 1.98	2-4	3.12	1.97	2.15 - 3.37 × 1.45-2.56	0
13	FOL 13	9.89	2.63	8.85 - 10.90 × 1.27 - 2.87	2-3	2.50	1.35	2.01 - 3.19 × 0.97 - 1.95	0
14	FOL 14	24.05	2.81	18.10 - 31.25 × 2.23 - 3.26	2 - 5	8.24	1.46	7.23 - 9.04 × 1.40 - 1.50	0
15	FOL 15	15.09	4.56	12.90 - 17.55 × 3.85 - 5.25	2- 4	6.05	2.25	5.62- 6.51 × 2.04 × 2.57	0

16	FOL 16	11.04	2.40	10.63 - 12.98 × 2.08 - 3.58	2-3	3.10	1.48	2.5-5.67 × 0.97-2.09	0
17	FOL 17	20.50	2.11	14.14- 28.02× 1.81- 2.50	2 -7	10.34	2.35	7.98 - 12.10× 1.14- 1.22	0
18	FOL 18	9.68	2.12	8.75 - 10.15 × 1.95 - 2.34	2-3	3.35	1.63	2.5-5.67 × 0.97- 2.09	0
19	FOL 19	13.30	2.94	12.72 - 17.90 × 2.26 - 3.42	2- 5	6.15	2.02	6.07 - 6.38 × 1.92 - 2.05	0
20	FOL 20	16.19	5.04	13.25 - 19.85 × 4.65 - 8.01	2- 5	3.85	1.59	3.78 - 4.19 × 1.19 - 2.27	0
21	FOL 21	8.14	2.06	7.86 - 9.53 × 1.65 - 2.53	2	3.01	1.78	2.85 - 3.19 × 1.67 - 2.98	0
22	FOL 22	9.32	3.42	8.54 - 10.94 × 2.45 - 3.68	2-5	2.20	0.97	2.05 - 3.24 × 0.67 - 1.82	0
23	FOL 23	19.59	2.33	13.14 - 26.54 × 2.01 - 2.75	2 -- 5	8.97	1.95	7.92 - 10.04 × 1.78 - 2.08	0
24	FOL 24	24.22	2.70	17.86 - 29.51 × 2.04- 2.91	2- 6	9.04	1.90	6.92 - 10.82 × 1.75- 2.01	0
	SD±	5.2528				1.15947			

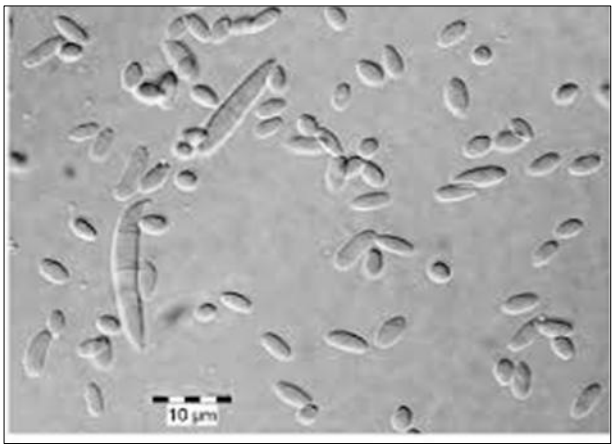

Plate 3: Magnification of microconidia at 40x × 10x = 400x

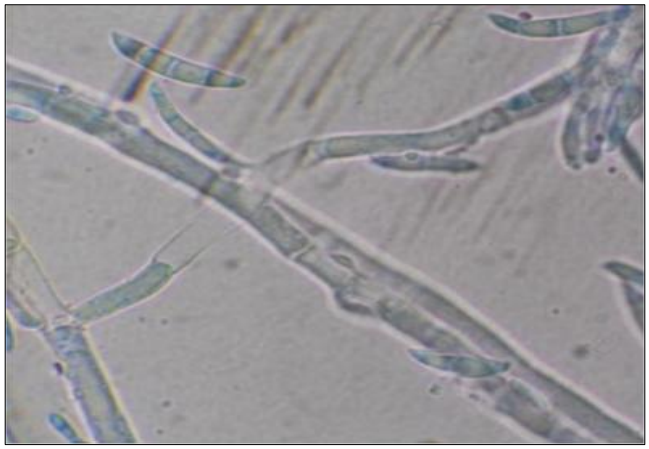

Plate 4: Magnification of Macroconidia at 100x × 10x = 1000x

Table 3: Cultural characterization of *Fusarium oxysporum* f.sp. *lini*

Code	Mycelium growth(mm)			Growth characters		
	3rd	5th	7th	Mycelium	Mycelial Colour	Pigmentation
FOL 1	34.60	38.42	55.08	Fluffy	Dull White	Light yellow to purple
FOL 2	45.65	60.29	80.00	Fluffy	White	Light yellow to purple
FOL 3	56.68	70.49	90.00	Fluffy	Cottony White	Light yellow
FOL 4	44.47	61.12	81.04	Appressed flat	Dull White	Light yellow
FOL 5	47.75	62.22	87.97	Partially appressed	White	Light orange to purple
FOL 6	35.15	42.50	64.04	Fluffy	Dull white	Light yellow to purple
FOL 7	44.31	60.12	80.04	Fluffy	White	Light yellow
FOL 8	24.57	48.80	88.37	Fluffy	Dull white	Light yellow
FOL 9	33.28	39.33	50.48	Fluffy	Dull white	light yellow
FOL10	37.74	46.40	71.20	Fluffy	White	orange
FOL11	43.71	45.26	78.33	Fluffy	White	White
FOL12	34.19	39.33	56.48	Fluffy	Dull white	light orange to purple
FOL13	45.60	53.28	76.01	Fluffy	White	light yellow
FOL14	49.90	63.28	90.00	Fluffy	Dark white	Light orange to purple
FOL15	45.00	50.17	90.00	Fluffy	Dark white	Light orange to purple
FOL16	22.98	35.18	56.90	Fluffy	Dull White	Light orange to purple
FOL17	49.65	64.29	90.00	Appressed Flat	Dull White	White
FOL18	47.68	55.49	79.00	Fluffy	Dark White	Light yellow to purple
FOL 19	50.71	62.26	88.33	Fluffy	White	Light yellow to purple
FOL 20	49.90	63.28	90.00	Fluffy	Dull White	Light orange to purple
FOL21	33.28	39.33	56.48	Fluffy	Dull White	Light orange to purple
FOL22	34.19	38.82	55.08	Fluffy	Dull White	Light yellow
FOL23	49.31	64.87	90.00	Partially Flat Appressed	White	orange
FOL24	48.60	58.28	82.01	Fluffy	Dull White	Light yellow

Table 4: Grouping of *Fusarium oxysporum* f.sp.*lini* isolates based on radial mycelial growth

Sr. No.	Radial growth	Radial growth 7DAI	*F. oxysporum* isolates
1	Slow growing	less than 50 mm	Nil
2	Moderate growing	50 - 70 mm	FOL 1, FOL 6, FOL 9, FOL 12, FOL 16, FOL 21, FOL 22 Total 7 isolates
3	Fast growing	more than 70 mm	FOL 2, FOL 3, FOL 4, FOL 5, FOL 7, FOL 8, FOL 10, FOL 11, FOL 13, FOL 14, FOL 15, FOL 17, FOL 18, FOL 19, FOL 20, FOL 23, FOL 24 Total 17 isolates

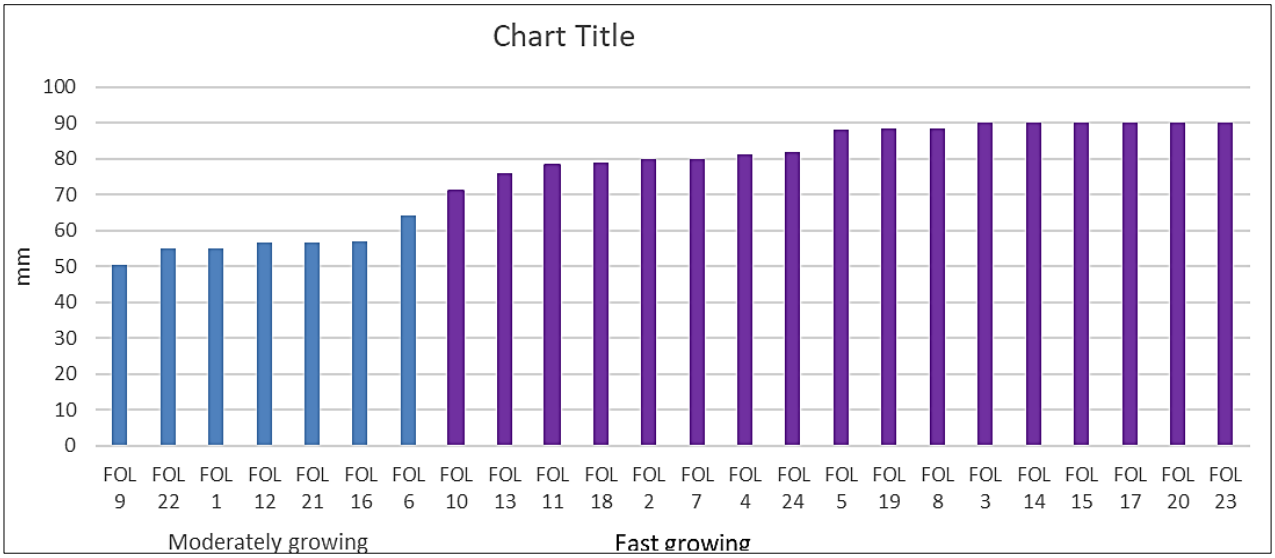

Fig 1: Grouping of *Fusarium oxysporum* f.sp.*lini* on radial mycelium growth rate at seven DAI (Days after inoculation)

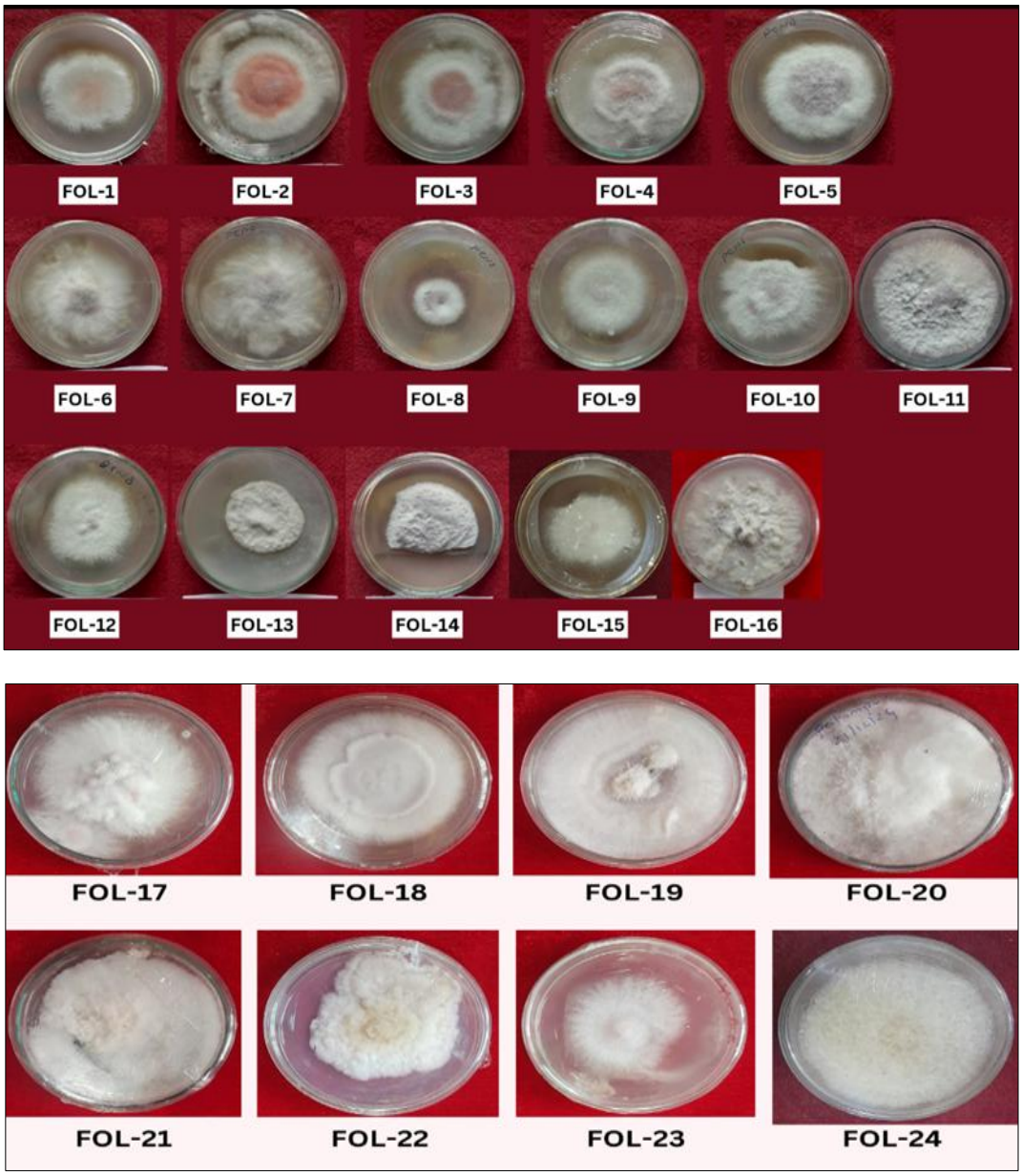

Plate 5: Mycelium growth of *Fusarium oxysporum* f.sp.*lini* isolates on PDA media

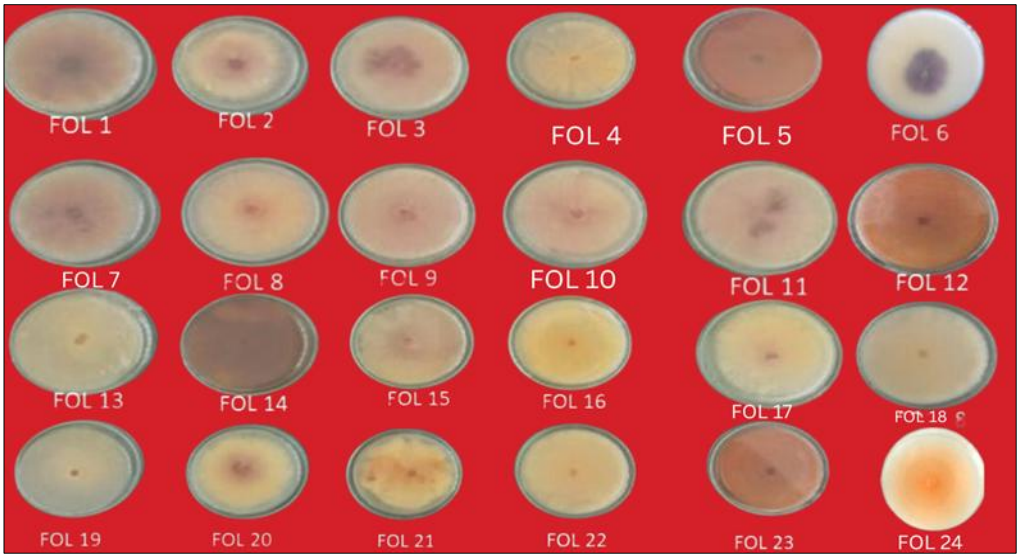

Plate 6: Pigmentation of *Fusarium oxysporum* f.sp.*lini* isolates on PDA media

4. Conclusion

Roving survey were carried out during *rabi* 2021-22 and 2022-23 season in major Linseed growing areas of Vidarbha region and revealed 17.90 to 34.16 mean per cent variation in wilt incidence. Wilt incidence was observed throughout the growth stage of linseed plants but more severe at vegetative, flowering and podding stage. Relatively, more wilt incidence was recorded in the area where linseed was grown over longer crop rotation. *Fusarium* infection at field level was confirmed by transversely cutting the wilted plants and blackening of vascular tissue was confirmed, prominent symptoms of linseed shepherd' s crook. Morphological study revealed high degree of variations among them macroconidia mean size range from length (8.05 - 25.13 µm) and breadth (2.01 - 6.24 µm). The mycelial growth on PDA was observed varied from fluffy, appressed flat, partially appressed, partially flat appressed mycelial growth with or without serrate margin and three types mycelial colour were observed white, dull white, cottony, dark white and Pigmentation light yellow to purple, light yellow, light orange to purple orange, White in different isolates of *F. oxysporum* f.sp. *lini* on PDA medium. Based on radial growth, isolates were categorized as fast growing (17 isolates), moderate growing (07 isolates).

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