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Study on cultural, morphological variability among the isolates of *Fusarium oxysporum* f. sp. *cumini*

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Abstract
Cumin (*Cuminum cyminum* L.) is an herbaceous, dicotyledonous annual plant, diploid (2n = 2x = 14) and allogam with hermaphrodite flowers. Cumin is extensively used in cuisines around the world. It is believed to be a native of the Mediterranean and near Eastern regions. The cultural and physical parameters of all these isolates were studied on potato dextrose agar medium. The different isolates of *F. oxysporum* f. sp. *cumini* (FOC-1, FOC-2, FOC-3, FOC-4, FOC-5, FOC-6, FOC-7, FOC-8, FOC-9, FOC-10, FOC-11, FOC-12, FOC-13, FOC-14 and FOC-15) were examined for their distinguishable characteristics such as radial growth, colony colour, colony appearance, colony texture, colony shape. Among four solid media, PDA medium was found most suitable for the growth of pathogen followed by, oat meal agar, corn meal agar and rose bengal agar, while rose bengal agar was found less growth of pathogen. The average maximum growth rate of mycelium was found in FOC-6 whereas FOC-11 showed minimum growth. The eight isolates (FOC-2, FOC-4, FOC-6, FOC-7, FOC-8, FOC-12, FOC-14 and FOC-15) showed fast growth rate whereas the five isolates (FOC-1, FOC-5, FOC-9, FOC-10 and FOC-13) showed moderate growth rate and FOC-3 and FOC-11 showed slow growth rate. Four isolates FOC-7, FOC-10, FOC-11 and FOC-15 showed appressed growth whereas, fluffy growth was shown by FOC-1, FOC-4, FOC-6, FOC-8, FOC-9, FOC-12 and FOC-14 isolate and FOC-2, FOC-3, FOC-5 and FOC-13 showed partially fluffy growth. Shape of the colony of isolates was found in round.

Keywords: *Cuminum cyminum*, variability, pathogen, Macroconidia, microconidia

Introduction
Cumin (*Cuminum cyminum* L.) is an herbaceous, dicotyledonous annual plant, diploid (2n = 2x = 14) and allogam with hermaphrodite flowers. Cumin is extensively used in cuisines around the world. It is believed to be a native of the Mediterranean and Near Eastern regions. It is mainly cultivated in India, Egypt, Libya, Iran, Pakistan and Mexico (Peter and Nybe, 2002) [9]. India is the largest producer in world under cumin cultivation 2021-22, approximately 10.36 lakh hectares of land in the yielded 7.25 lakh tonnes cumin seed (Anonymous, 2021-22) [1-2]. The pathogen produces abundant, cottony white mycelium in culture. Microconidia are scattered freely on the mycelium measuring 4.8-12.8 µm. Macroconidia constitute about 90% of the conidia produced, mostly 2-3 septate 34.44 × 3.28 µm. The chlamydospores are terminal or intercalary, spherical, smooth and measuring 8.2 µm in diameter on an average and survive in the soil for more than 10 years. (Lodha and Mawar, 2014) [5]. Keeping this background information in view, present study was undertaken to observe cultural variability among different isolates of *Fusarium oxysporum* f. sp. *cumini.*

Materials and Methods
Collection of diseased samples
Wilt infected cumin plant samples were collected from different cumin growing areas of Rajasthan *viz.*, Jodhpur, Barmer, Jalore, Nagour and Ajmer. These samples were carefully placed in paper bags, properly tagged and brought to laboratory for isolation and microscopic examinations.The typical wilt infected samples were collected from major cumin growing areas of Rajasthan during a survey of *Rabi* season 2021-22. One sample from each tehsil was selected on the basis of higher disease incidence in surveyed field. Isolations were made to study the cultural and morphological variations among the different isolates.

Growth characters on solid media

The different solid culture media was evaluated for obtaining maximum mycelial growth of the *F. oxysporum* f. sp. *cumini*. The experiment was laid out in a complete randomized design with three replications. Four solid culture media *viz.,* potato dextrose agar, rose bengal agar, corn-meal agar, and oat- meal agar used to compare the growth rate of test pathogen. The culture medium was prepared by the standardized methodand autoclaved at 121.6 °C 15 psi pressure for twenty minutes. Twenty ml of each media were poured in a 90 mm Petri dish. Each Petri plate was inoculated separately with uniform mycelia culture bits (5 mm) cut with the help of cork borer from young (5 days) vigorously growing culture were placed on the middle of each pre-poured medium and incubated at 25±1 °C (Dela Paz *et al*., 2006) [4]. The diameterof the growth of the fungus was measured after 48 and 96 h after inoculation.

The mycelial growth of test pathogen was measured after 7 days of inoculation. The percent growth inhibition (PGI) of each tested pathogen was calculated using the formula given by Vincent (1947) [10].

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

C = Mycelial growth of *F. oxysporum sp. cumini* in control (mm)

T = Mycelial growth of *F. oxysporum sp. cumini* in presence of antagonist (mm)

Result and Discussion

Cultural variability amongst fifteen isolates of *F. oxysporum* f. sp. *cumini*, collected from five districts of Rajasthan, were carried out in laboratory conditions. The cultural and physical parameters of all these isolates were studied on potato dextrose agar medium. The different isolates of *F. oxysporum* f. sp. *cumini* (FOC-1, FOC-2, FOC-3, FOC-4, FOC-5, FOC-6, FOC-7, FOC-8, FOC-9, FOC-10, FOC-11, FOC-12, FOC-13, FOC-14 and FOC-15) were examined for their distinguishable characteristics such as radial growth, colony colour, colony appearance, colony texture and colony shape (Table 1 and 2., fig- 1 and plate 1). Among four solid media, PDA medium was found most suitable for the growth of pathogen followed by, oat meal agar, corn meal agar and rose Bengal agar, while rose Bengal agar was found less growth of pathogen. The average maximum growth rate of mycelium was found in FOC-6 whereas FOC-11 showed minimum growth. Three isolates *viz.*, FOC-1, FOC-14 and FOC-15 produced white colour colony

Significant differences among fifteen isolates of *F. oxysporum* f. sp. *cumini* were observed on the basis of radial growth on PDA medium. The individual average radial growth of 15 isolates of *F. oxysporum* f. sp. *cumini* ranged from 61.67 to 90.00 mm. Maximum colony diameter of 90 mm was observed in isolate FOC-6 (90.00 mm) and while isolate FOC-11 showed the minimum radial growth and were rated as slow-growing as it gave 61.67 mm growth. Based on the observation, the colony colour of all the fifteen isolates was categorized as whitish, pinkish white, light white, light pink white and pinkish in appearance. Three isolates *viz.*, FOC-1, FOC-14 and FOC-15 produced white colour colony. Four isolates produced Pinkish white *i.e.* FOC-2, FOC-6, FOC-7 and FOC-9, one isolates FOC-3 produced a light whitish colony colour and FOC-4, FOC-10, FOC-11 and FOC-13 showed light pink white colour, FOC-5, FOC-8 and FOC-12 produced Pinkish colour. On the basis of colony growyh, all isolates were divided into three categories *viz.*, fast-growing, moderate growing and slow-growing. The Eight isolates (FOC-2, FOC-4, FOC-6, FOC-7, FOC-8, FOC-12, FOC-14 and FOC-15) showed fast growth rate whereas the five isolates (FOC-1, FOC-5, FOC-9, FOC-10 and FOC-13) showed moderate growth rate and FOC-3, FOC-5 and FOC-11 showed slow growth rate. All cultures of *F. oxysporum* F. sp. *cumini* showed three phenotypes (appressed, partially fluffy and fluffy) when grown on PDA medium. Four isolates FOC-7, FOC-10, FOC-11 and FOC-15 showed appressed growth whereas, Fluffy growth was shown by FOC-1, FOC-4, FOC-6, FOC-8, FOC-9, FOC-12 and FOC-14 isolate and FOC-2, FOC-3, FOC-5 and FOC-13 showed partially fluffy growth. Shape of the colony of isolates was found in round. The same finding was also reported by Chopada *et al*. (2015) [3] studied cultural and morphological variability among 10 isolates of *Fusarium oxysporum* from Gujarat and reported that all the isolates showed wide variations in respect of mycelia colour, mycelial growth, dry mycelial weight, sporulation, conidial size and formation of chlamydospores. The isolates produced moderate, profuse fluffy, thin flat to slight fluffy and submerged growth with white, yellow, light pink, dark pink, orange and purple-orange pigmentation. Mehta *et al.* (2013) [6] a total six isolates of *Fusarium oxysporum* f. sp. *cumini*, isolated from different cumin growing areas of Rajasthan, had cultural and morphological variability on different agar and broth media. Significant version in mycelial growth and conidia formation was observed on different media. Maximum mycelial growth (8.16 cm) and conidia formation was observed on Czapek Dox agar followed by PDA. Highest fungal biomass (246.87 mg) was observed with Czapek Dox broth followed by PDB (233.66mg). Moss and Smith (1984) [7] examined isolates of *F. oxysporum* and concluded that are very dynamic and exhibit high variation with respect to their cultural, morphological and pathogenic characters. Munde *et al*. (2020) [8] found the most suitable media were viz., Potato dextrose agar with maximum radial mycelial growth (89.66 mm). Followed by media Richard's agar (87.25 mm). The least mycelial growth was observed in Potato carrot agar (38.75 mm) and Malt extract (40.90 mm) Mycelium was pink in potato dextrose agar and Sabouraud's dextrose agar against *F. oxysporum* f. sp *gladioli.*

Table 1: Mycelial growth of different isolates of *Fusarium oxysporum* f. sp. *cumini* on various solid media

S. No.	Isolates	Mycelial growth (mm) on different media				
		Rose Bengal Agar	Corn Meal Agar	Oat Meal Agar	Potato Dextrose Agar	Mean growth
1.	FOC-1	45.00	58.67	61.67	76.00	60.34
2.	FOC-2	52.00	67.00	70.00	80.00	67.25
3.	FOC-3	40.67	49.00	50.00	70.67	52.59
4.	FOC-4	44.33	55.00	59.67	75.67	58.67
5.	FOC-5	43.67	54.33	57.00	74.67	57.42
6.	FOC-6	58.33	76.67	80.67	90.00	76.42
7.	FOC-7	50.00	65.00	66.00	79.00	65.00
8.	FOC-8	56.00	74.67	77.33	86.67	73.67
9.	FOC-9	48.67	63.00	64.67	78.67	63.75
10.	FOC-10	41.67	50.00	51.67	72.67	54.00
11.	FOC-11	40.00	46.33	46.67	61.67	48.92
12.	FOC-12	46.00	60.00	63.00	77.00	61.50
13.	FOC-13	42.00	52.67	55.00	74.00	55.92
14.	FOC-14	55.00	72.33	75.00	84.00	71.58
15.	FOC-15	54.00	70.00	72.00	82.00	69.50
	Average	47.82	60.04	63.36	77.51	
	S.Em (±)	0.77	0.93	0.66	0.98	
	C.D (p=0.05)	2.24	2.71	1.93	2.84	

Table 2: Colony characteristics of different isolates of *Fusarium oxysporum* f. sp. *cumini* on PDA medium

S.No.	Isolates	Mycelial growth (mm)	Colony colour	Colony appearance	Colony texture	Colony shape
1.	FOC-1	76.00*	Whitish	Moderate growing	Fluffy mycelium	Round
2.	FOC-2	80.00	Pinkish white	Fast growing	Partially fluffy	Round to irregular
3.	FOC-3	70.69	Light whitish	Slow growing	Partially fluffy	Round to irregular
4.	FOC-4	74.64	light pink white	Fast growing	Fluffy mycelium	Irregular
5.	FOC-5	74.20	Pinkish	Moderate growing	Partially fluffy	Round
6.	FOC-6	90.00	Pinkish- White	Fast growing	Fluffy mycelium	Round to irregular
7.	FOC-7	78.80	Pinkish white	Fast growing	Appressed	Round to irregular
8.	FOC-8	86.57	Pinkish	Fast growing	Fluffy mycelium	Round to irregular
9.	FOC-9	78.67	Pinkish white	Moderate growing	Fluffy mycelium	Round
10.	FOC-10	72.65	Light pink white	Moderate growing	Appressed	Round
11.	FOC-11	61.58	Light pink white	Slow growing	Appressed	Round to irregular
12.	FOC-12	77.00	Pinkish	Fast growing	Fluffy mycelium	Round
13.	FOC-13	74.00	Light pink white	Moderate growing	Partially fluffy	Round to irregular
14.	FOC-14	84.00	Whitish	Fast growing	Fluffy mycelium	Round
15.	FOC-15	82.00	Whitish	Fast growing	Appressed	Round

*Mean of three replication

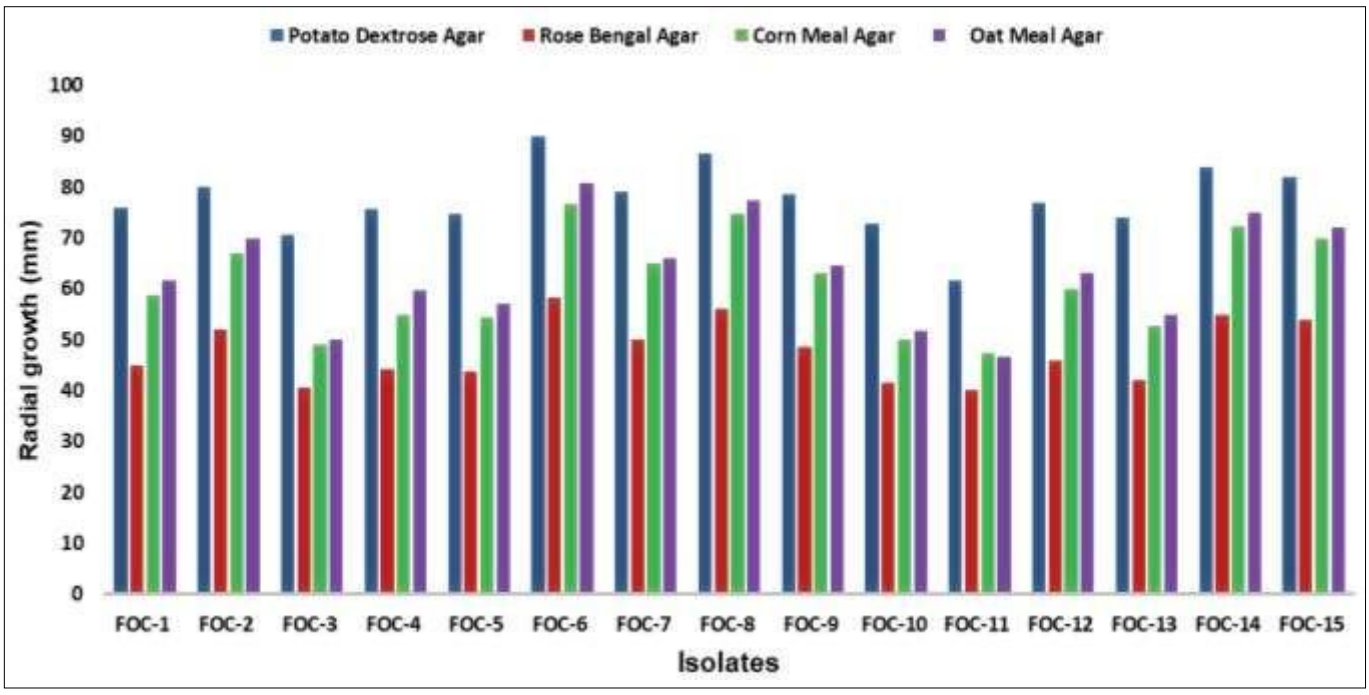

Fig 1: Mycelial growth of different isolates of *Fusarium oxysporum* f. sp. *cumini* on various solid media

	Rose Bengal Agar	Corn Meal Agar	Oat Meal Agar	Potato Dextrose Agar
FOC-1				
FOC-2				
FOC-3				
FOC-4				
FOC-5				
FOC-6				
FOC-7				

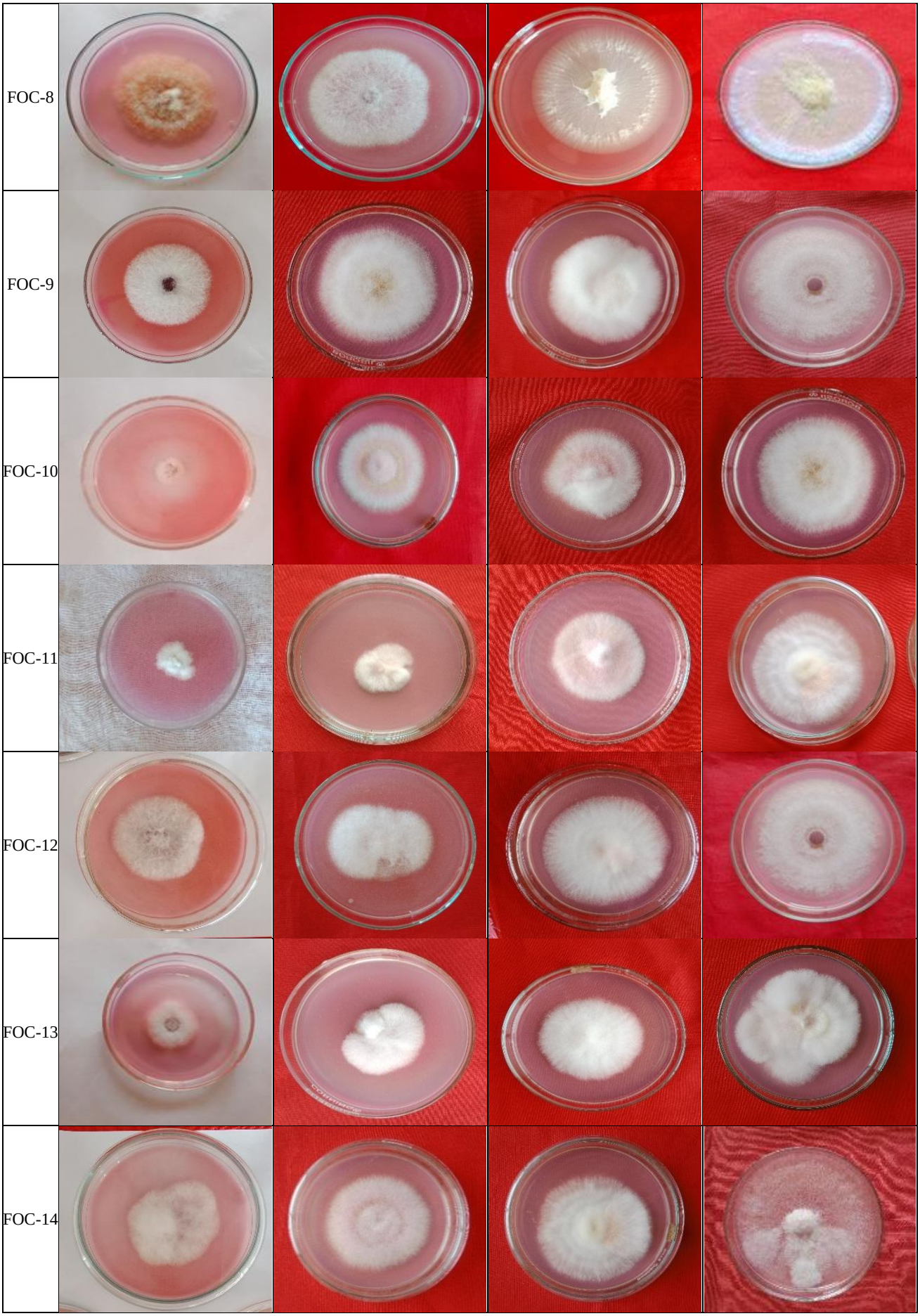
FOC-8
FOC-9
FOC-10
FOC-11
FOC-12
FOC-13
FOC-14

Plate 1: Mycelial growth of *Fusarium oxysporum* f. sp. *cumini* on various media

Conclusion

Out of Four solid media, potato dextrose agar medium was found most suitable for the growth of pathogen followed by, oat meal agar, corn meal agar and rose Bengal agar, while rose Bengal agar was found less growth of pathogen. The average growth rate of FOC-6 was maximum followed by FOC-8, FOC-14, FOC-15, FOC-2, FOC-7, FOC-9, FOC-12, FOC-4, FOC-5, FOC-13, FOC-10 and FOC-3 on all the tested media whereas FOC-11 showed minimum growth. Significant differences among fifteen isolates of *F. oxysporum* f. sp. *cumini* were observed on the basis of radial growth on PDA medium. The individual average radial growth of 15 isolates of *F. oxysporum* f. sp. *cumini* ranged from 61.67 to 90.00 mm. In the cultural studies the colony colour of all the fifteen isolates was categorized as whitish, pinkish white,light white, light pink white and pinkish in appearance. On the basis of colony growth, all isolates were divided into three categories *viz.*, fast-growing, moderate growing and slow-growing. The eight isolates (FOC-2, FOC-4, FOC-6, FOC-7, FOC-8, FOC-12, FOC-14 and FOC-15) showed fast growth rate whereas the five isolates (FOC-1, FOC-5, FOC-9, FOC-10 and FOC-13) showed moderate growth rate and FOC-3 and FOC-11 showed slow growth rate. All cultures of *F. oxysporum* f. sp. *cumini* showed three phenotypes (appressed, partially fluffy and fluffy) when grown on PDA medium.

Future scope

The present investigation has given new ideas related to wilt of cumin and also help to know about the different races and degree of virulent of pathogen which helpful for the management of wilt of cumin in different district of Rajasthan.

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