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## Morphological and pathogenic variability in different isolates of *Fusarium oxysporum* f. sp. *cumini*

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### Abstract

Wilt of cumin incited by *Fusarium oxysporum* f. sp. *cumini* is one of the important disease and a big constraint in successful cultivation. Therefore, in the present study, 7 isolates of *Fusarium oxysporum* f. sp. *cumini* collected from different cumin growing areas of Rajasthan were assessed for morphological and pathogenic variations. The different isolates produced sickle shaped macro conidia and spores of varying width, length having variable number of septa. The isolate I<sub>2</sub> produced highest length and number of septa in macro conidia. The length of macro conidia varied from 22.77 µm in isolate I<sub>3</sub> to 29.65 µm in isolate I<sub>2</sub> whereas, micro conidia length varied from 7.54 µm in I<sub>4</sub> to 11.53 µm in I<sub>6</sub>. In study of pathogenic variability, among different isolate, the isolate I<sub>1</sub> proved most virulent as it showed disease symptoms earliest whereas, isolate I<sub>5</sub> proved least virulent.

**Keywords:** Cumin, Wilt, *Fusarium oxysporum* f. sp. *cumini*, Variability

### Introduction

A spice is substance of plant origin, primarily from various parts of the plant such as dried seed, fruit, root or bark which is used in very small quantities as a food additive for flavor, color or as a preservative. It is used for flavoring the food products but in addition it is also used in preservation of food and provision of nutritional and health benefits (Nazeem, 1995)<sup>[12]</sup>. Cumin (*Cuminum cyminum* L.) locally known as 'zeera' is an important seed spice and one of the earliest known minor spices used by mankind. It belongs to family umbelliferae (apiaceae) and believed to have originated from Egypt and Syria (Edison *et al.* 1989)<sup>[3]</sup>. Cumin have good antioxidant potential and this spice can be used to produce novel natural antioxidants as well as flavoring agents that can be used in various food products (Nadeem and Riaz, 2012)<sup>[11]</sup>. Seeds have pleasant aroma and spicy taste due to presence of aromatic alcohol 'cuminal' (cuminaldehyde). The crop suffers from many diseases such as wilt (*Fusarium oxysporum* f.sp. *cumini*), blight (*Aternaria burnsii*), powdery mildew (*Erysiphe polygoni*) and some other diseases. Among these wilt is an important disease of cumin with incidence up to 25.7% but may be 60% in some cases. Wilt is an important disease of cumin with incidence up to 25.7% but may be 60% in some cases. Losses in yield up to 25% have been reported from North Gujarat and up to 60% from Rajasthan (Khare *et al.* 2014; Lodha and Mawar, 2014; Dange, 1995)<sup>[5, 6, 1]</sup>. Mathur and Mathur (1956)<sup>[8]</sup> reported wilt of cumin from Rajasthan and identified the causal organism to be *Fusarium oxysporum* (Schl.) Snyder and Hansen. In this context an investigation was planned to isolate, identify pathogen from infected plants of cumin and to study the variability in *F. oxysporum* f.sp. *cumini*.

### Materials and Methods

#### Variation and variability

Single spore cultures were raised from isolates of different collections of *Fusarium oxysporum* f. sp. *cumini* on 2 per cent Potato Dextrose Agar slant. A total 6 isolates obtained on isolation from the surveyed district and a local isolate from Agronomy farm of SKNCOA, Jobner were used in present study. Isolates were transferred separately on 2 per cent PDA in petridishes to study in detail for their discernible characters on the basis of cultural characters such as the amount and color of aerial mycelium (Maerz and Paul, 1950)<sup>[7]</sup>, colony growth and pigmentation of substratum.

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### The isolates were coded as

Code	Place of collection of isolates
I <sub>1</sub>	Jalore
I <sub>2</sub>	Nagaur
I <sub>3</sub>	Jodhpur
I <sub>4</sub>	Pali
I <sub>5</sub>	Sirohi
I <sub>6</sub>	Ajmer
I <sub>7</sub>	Agronomy farm S.K.N. COA, Jobner

### Morphological variability

To detect morphological variability in conidia formed by different isolates, purified culture of each isolate was grown in PDA containing plate. From 7-day old culture of each isolate, 2 mm diameter bits were cut from periphery of the growth with the help of a sterilized cork borer. One such bit was transferred aseptically with the help of sterilized inoculation needle to inoculate one PDA plate. Four petridishes each inoculated with 1 bit of the same isolate constituted 4 replications for that isolate. The inoculated petridishes were incubated at 25±1 °C temperature. On 7th day of incubation, spores were collected from culture in each petridish according to the following procedure.

From each petridish, constituting one replication, 3 bits (each of 2 mm diameters) were cut with the help of sterilized cork borer from random points on peripheral growth. Three bits were shaken in 5 ml water for 10 minutes on a horizontal shaker. The harvested spores were stained with dilute solution of cotton blue and examined microscopically. For each isolate, 10 macro- and 10 micro-conidia from the total number of spores separated out from 3 bits per replication were separately examined for their size, shape and septation. Thus, 60 macro- and micro-conidia from each isolates were examined for their size, shape and septation.

### Pathogenic variability

Cumin cultivar RZ-19 was raised in 30 cm earthen pots. The pots were surface sterilized with 2 per cent formalin solution. After 24 hrs of sterilization, each pot was filled with sterilized soil (Soil: FYM = 3:1) sterilized at 1.045 kg / cm<sup>2</sup> for one hour for three consecutive days. These pots were inoculated with fungus inoculum (@ 10 per cent w/w soil) multiplied on

sorghum grain medium. Five pots were sown with fifteen surface sterilized seeds in each pot that were treated as five replications for each of the isolates. Disease incidence was recorded regularly commencing after 15 days till 60 days of sowing and per cent incidence was calculated as under:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

### Result and Discussion

#### Morphological variability

##### Macro-conidia

All the isolates produced sickle shaped macro-conidia. Among different isolates, isolate I<sub>3</sub> and I<sub>6</sub> were similar and produced spores of minimum length and isolate I<sub>2</sub> produced spores of maximum length as compared to other isolates (Table 1).

The different isolates produced spores of varying width and the isolate I<sub>4</sub> showed significantly maximum width of spores (5.20 µm) than all other isolates. The isolate I<sub>5</sub> showed minimum width of spore (3.25 µm) that was similar with isolate I<sub>1</sub> and significantly lower than all other isolates.

All the isolates showed wide variability in number of septa present. The isolate I<sub>1</sub> produced significantly lowest number (2.50) of septa while isolate I<sub>2</sub> produced significantly highest (3.80) number of septa than all other isolates.

##### Micro-conidia

All the isolates formed ovoid micro-conidia. The isolate I<sub>4</sub> gave minimum length (7.54 µm) whereas maximum length of micro-conidia (11.53µm) was found in isolate I<sub>6</sub>, (Table 1). The different isolates produced spores of varying width and the isolate I<sub>6</sub> showed significantly maximum width of spores (4.10 µm) that was similar with isolate I<sub>3</sub>. The isolate I<sub>1</sub> showed minimum width of spore (2.90 µm) than all other isolates.

The isolate I<sub>1</sub>, I<sub>3</sub>, I<sub>5</sub> and I<sub>6</sub> did not differ significantly so far as number of septa is concerned. However, the isolates I<sub>3</sub>, I<sub>5</sub> and I<sub>6</sub> produced significantly lower number of septa than I<sub>4</sub> that produced highest number of septa amongst all isolates.

**Table 1:** Morphological variations in macro and micro conidia of different isolates of *F. oxysporum* f. sp. *cumini* on PDA medium

Isolates	Macro-conidia			Micro-conidia		
	Length µm	Width µm	No. of septa	Length µm	Width µm	No. of septa
I <sub>1</sub>	27.50	3.90	2.50	9.50	2.90	0.87
I <sub>2</sub>	29.65	4.50	3.80	10.65	3.75	0.92
I <sub>3</sub>	22.77	4.35	3.65	10.72	4.02	0.80
I <sub>4</sub>	28.30	5.20	3.50	7.54	3.94	0.97
I <sub>5</sub>	26.45	3.25	2.98	9.42	3.50	0.75
I <sub>6</sub>	24.10	5.00	3.15	11.53	4.10	0.77
I <sub>7</sub>	25.75	4.25	3.75	10.25	3.15	0.90

### Pathogenic variability

The data regarding the time taken by different isolates to produce first symptom of disease and per cent incidence (Table 2) revealed that isolate I<sub>1</sub> was found highly virulent as it showed 86.66 per cent wilt incidence on 19<sup>th</sup> day of inoculation when no other isolate was able to produce disease symptoms. On 21<sup>th</sup> day of inoculation, in addition to isolate I<sub>1</sub>, the isolate I<sub>2</sub> also showed disease symptoms with disease

incidence (81.66%). The isolate I<sub>5</sub> proved least virulent as plants inoculated with this isolate show disease symptoms up to 30<sup>th</sup> day of inoculation and showed lowest disease incidence as compared to other isolates. On 34<sup>th</sup> day of inoculation, the isolate I<sub>1</sub> remaining at par with isolates I<sub>2</sub> exhibited significantly highest disease incidence as compared to isolates I<sub>3</sub> I<sub>4</sub>, I<sub>5</sub> and over I<sub>6</sub> and I<sub>7</sub>.

**Table 2:** Pathogenic variability among the isolates of *F. oxysporum* f. sp. *Cumini*

Isolates	Interval between inoculation and 1st appearance of disease (days)	Wilt disease incidence (%)
I <sub>1</sub>	19	86.66(68.58)*
I <sub>2</sub>	21	81.66(64.64)
I <sub>3</sub>	24	66.66(54.73)
I <sub>4</sub>	27	46.66(43.08)
I <sub>5</sub>	30	33.33(35.26)
I <sub>6</sub>	32	79.99(63.43)
I <sub>7</sub>	34	73.33(58.91)
	S.Em+	2.05
	CD (p=0.05)	6.10
	CV (%)	6.11

\*\* Values in parenthesis are angular transformed values

The different isolates produced sickle shaped macro conidia and spores of varying width, length having variable number of septa. Thus, isolates of the pathogen showed great variation in morphological characters as reported by Mathur and Prasad (1967) [9]. Some isolates ceased sporulation on continuous culturing but regained it on passing through the host.

Among different isolate, the isolate I<sub>1</sub> proved most virulent as it showed disease symptoms earliest whereas, isolate I<sub>5</sub> proved least virulent. Mehta *et al.* (2013) [10] studied ten isolates of *Fusarium oxysporum* f.sp. *cumini* for their discernible characters such as the colour of mycelium, pigmentation of substrate, type and amount of mycelia growth, virulence and nutritional requirement. All the isolates were found to be pathogenic though they differed in the percent disease incidence. Deshwal and Kumari (2012) [2] reported phylogenetic relationships among fungal isolates of *Fusarium oxysporum* f.sp. *cumini*, collected from different regions of Rajasthan correlates with variation exist at pathogenicity.

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