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Morphological and pathogenic variability in *Alternaria alternata* causing Alternaria blight of fennel

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Abstract
Alternaria blight of fennel, caused by Alternaria alternata, is one of the major diseases of fennel. Studies were conducted to compare the morphological and pathogenic variability among 15 isolates of Alternaria alternata isolated from fennel, were collected from different locations of Sirohi, Pali, Jodhpur, Dausa and Tonk districts of Rajasthan. All the isolates showed variation in their morphological characters i.e. colony color and shape; conidial number, size, width, length, shape and septation on PDA. Among these, isolates i.e. JF_1 and TF_1 showed excellent sporulation with maximum colony diameter 90.0 mm and 90.0 mm, respectively. These isolates show variation in their spore length and width also. All the isolates were virulent on the tested variety of fennel for virulence. Among these isolates, JF_1 was the most virulent causing maximum disease intensity (80.37%) followed by TF_1 (78.18%), JF_3 (76.24%), SF_3 (72.65%) and DF_3 (72.60%) while minimum disease intensity was recorded with DF_2 (45.25%).

Keywords: Fennel, Alternaria blight, Alternaria alternata, morphological, pathogenic, variability

Introduction
India known as the land of spices, is the largest producer, consumer and exporter of spices in the world. Seed spices are very important in human health and have a crucial role in Indian cuisine. Fennel (*Foeniculum vulgare* Mill.) belongs to the family *Apiaceae* is an annual aromatic herb. The fennel seeds are used in curing diseases like cholera, bile disturbances, nervous disorders, constipation, dysentery and diarrhea. In India, the seeds are also used for mastication and chewing either alone or with betel leaves (Agarwal *et al*. 2001) [1]. The major fennel growing belt spreads from arid to semi-arid regions covering large area in Rajasthan and Gujarat. During 2019-20 total area under the crop in India is about 82731 hectares with production of 139760 tonnes (Anonymous, 2021) [2]. In Rajasthan, during 2019-20 it occupies an area of 26250 hectares with an annual production of 25620 tonnes (Anonymous, 2021) [2].
It is mainly cultivated in the districts of Sirohi, Jodhpur, Nagour, Tonk, Dausa and Pali and to a limited extent in Bharatpur, Kota and Ajmer. Fennel is attacked by a number of diseases. Among these diseases, *Alternaria* blight of fennel caused by *Alternaria alternata* (Fr.) Keissler is a serious bottleneck in fennel producing areas. It causes loss of different magnitudes. More than 50% of the inspected fields of fennel showed Alternaria blight symptoms with an incidence ranging from 30 to 100% in Italy (Infantino *et al.* 2009) [3]. Use of resistant varieties is one of the important alternatives to overcome this problem and for breeding resistant varieties, knowledge of variability in the pathogen is most essential. Very little information on morpho and pathogenic variability in *Alternaria alternata* of fennel is available negligible from India. Keeping in view the variations in disease intensity of different areas, the studies have been conducted to ascertain the cultural, morphological and pathogenic variations among different isolates of *A. alternata* from different areas of Rajasthan.

Materials and Methods
Survey and collection
Survey of major fennel growing areas of Sirohi, Pali, Jodhpur, Dausa and Tonk districts of Rajasthan was conducted at seed formation stage of the crop, to record *Alternaria* blight intensity and to collect disease samples. The diseased samples collected during survey were brought to the laboratory in paper bags for further studies.

Isolation, purification and identification
To ascertain the variability among the isolates of *A. alternata*, cultural and morphological studies were conducted on potato dextrose agar (PDA) medium.

Isolations were made from infected samples of plants showing typical symptoms of *Alternaria* blight. Fifteen isolates of *A. alternata* isolated from fennel, different locations of Sirohi, Pali, Jodhpur, Dausa and Tonk districts of Rajasthan and designated as SF (Sirohi fennel isolate), PF (Pali), JF (Jodhpur), DF (Dausa) and TF (Tonk), respectively. Small pieces of the leaves and stems of fennel plant were cut from the diseased portion along with some healthy tissues, surface sterilized for 1-2 minutes in 0.1 per cent mercuric chloride solution followed by three washings with sterilized distilled water. These bits were transferred aseptically to PDA in Petri dishes separately. Incubation was done at 25 ± 1 ºC for 7 days. Sub-cultures from un-contaminated peripheral growth were made on PDA slants.

For purification of the fungus, single spore technique was used. After sporulation, conidial suspension was made in sterile water and the dilution was adjusted such that in one loop full, 20-25 conidia could be counted under low power objective of microscope. One such loop full was mixed with 25 ml melted and sterilized agar (2%) and poured in sterile Petri dishes. After 12 hours of incubation at 25 ±1 ºC, the single germinating conidium was cut with the help of dummy objective and transferred to PDA slants. They were subsequently allowed to grow and sporulate. Mono conidial culture established in this way was maintained by periodical transfer on PDA slants. After purification, fungus was allowed to sporulate. The sporulating pure culture of each isolate was identified on the basis of morphological characters.

Cultural and morphological variability

Single spore cultures of different isolates established and maintained on potato dextrose agar (PDA) were studied for their cultural and morphological characters. Ten day old culture of each isolate was inoculated (5 mm diameter disc) separately on PDA and incubated at 25±1^{0}C. After 7 day of incubation, radial growth of fungal mycelium, colony characters of each isolate such as size of conidia *i.e.* length, width and number of septation were recorded. The size of conidia was measured using ocular and stage micrometer.

Pathogenic variability

To test the pathogenic variability among the 15 isolates, spore suspension of each isolate were prepared in sterilized distilled water separately by blending 10 days old fungal culture in pestle and mortar and filtered through cheese cloth, spore suspension was further diluted to 1x10^5 spores/ml and three months old fennel plant were separately inoculated with each isolate and observations on disease intensity were recorded after 15 days of inoculation.

Randomly selected five plants from each field were rated as per following description and per cent disease intensity (PDI) was calculated by using the formula of Wheeler (1969) [9].

S. No	Description	Grade
1	No incidence/ Healthy	0
2	Symptoms on leaf tip and leaves only	1
3	Symptoms on leaves and petiole	2
4	Symptoms on leaves, petiole and stem	3
5	Symptoms on leaves, petiole stem and inflorescence	4
6	Symptoms on leaves, stem, inflorescence including Seed	5

$$PDI = \frac{\text{Sum of numerical disease rating x 100}}{\text{No. of plants assessed x Maximum disease rating}}$$

Results and Discussion

Survey and collection

A roving survey was conducted during *Rabi* 2013-14 and 2014-15 in five major fennel growing districts of Rajasthan *viz.*, Sirohi (63.66%), Pali (61.00%), Jodhpur (83.00%), Dausa (66%) and Tonk (69.66%). During the survey, discussions were held with the farmers concerned, regarding occurrence and incidence of the disease.

Isolation, purification and identification

Isolation of the pathogen from diseased plants of fennel was done on Potato Dextrose Agar medium (PDA). After seven days of incubation at 25 ±1 ^{0}C, growth of fungus was developed. Pure culture of the pathogen, obtained by single sporing on PDA, yielded *Alternaria* sp. The hyphae were branched, septate and olive to dark brown in colour. Conidiophores were dark brown, single or in small groups, simple or branched. Conidia were light olive to dark brown with 3 to 8 transverse septa along with many longitudinal septa. Shape of conidia varied from obclavate to ovoid or ellipsoidal with a short cylindrical beak of 2 to 5 µm thickness. Size of 100 conidia along with beak varied from 19-62 X 9-18 µm in size. On the basis of measurement and other morphological characters, the pathogen was identified as *Alternaria alternata* (Fr.) Keissler. The identity was further confirmed from ITCC, Division of Plant Pathology, IARI, New Delhi with ID. NO. 9256.13.

Cultural variability

The cultural and morphological variability such as shape, colour and size of colony and colour and size of conidia were recorded in 15 isolates of *A. alternata* by growing them on PDA. The results showed that isolates of *A. alternata* differ in their colony characters, colony diameter and sporulation (Table-1). Among the 15 isolates, four isolates *i.e.* JF_1, TF_1 JF_3 and DF_1 showed excellent sporulation with a colony diameter 90.0 mm, 90.0 mm, 88.9 mm, and 88.7 mm, respectively. SF_3, JF_2, DF_3, PF_1, SF_1 and TF_3 isolates showed very good sporulation with a colony diameter 88.5 mm, 87.6 mm, 87.0 mm, 86.0 mm, 85.0 mm and 85.0 mm, respectively. PF_3, TF_3, SF_2, & PF_2 isolates showed moderate sporulation with 85.0 mm, 80.0 mm, 79.0 mm and 75.0 mm diameter of colony, respectively and isolate DF_2 showed poor sporulation with very loose dark cottony growth of mycelium (54.0 mm dia.).

Morphological variability

Morphological observations recorded on each isolate revealed that isolates vary in their spore length and width. In general, spore length and width was found in between 25.80 to 49.27µm and 8.14 to 24.27µm, respectively. Whereas number of horizontal and vertical septa varied between 4 to 8 and 2 to 5, respectively (Table 2). The JF_1 isolate showed maximum

length of spore (49.27 µm). The maximum width of spores (24.27 µm) was observed in JF_3 isolate. The maximum horizontal septa (8) obtained in SF_1, JF_1 and TF_2 isolates. Whereas minimum horizontal septa (4) were observed in PF_2, JF_2 and TF_2 isolates, respectively. Vertical septa were maximum (5) in JF_7 isolate followed by SF_1, SF_3, JF_3 and TF_1 isolates and minimum (2) in PF_2, JF_2, DF_2 and TF_3 isolates.

Pathogenic variability

All the isolates tested were found pathogenic to fennel and varied in disease intensity (Table- 3). Mean per cent disease intensity was highest (74.93%) with Jodhpur isolates (JF_1 to JF_3) followed by Tonk isolates (TF_1 to TF_3) (66.64%) and Sirohi isolates (SF_1 to SF_3) (64.02%). Among these isolates JF_1 (Borunda, Jodhpur) was observed to be most virulent and produced maximum (80.37%) disease intensity whereas DF_2 isolate was found less virulent (45.28%). Minimum per cent disease intensity (58.71%) was observed with Pali isolates. The overall mean disease intensity of five districts was 64.16 per cent.

Cultural, morphological and pathogenic variability among isolates of different species of *Alternaria* have also been reported by several workers (Martinzer *et al.* 2002; Sharma and Pandey, 2012; Kumar *et al.* 2003; Tetarwal *et al.* 2008 and Shekhawat *et al*. 2013) [5, 6, 4, 8, 7].

Table 1: Colony diameter and sporulation of *A. alternata* isolates on PDA after 7 days of incubation at 25 ± 1 ^{0}C

S. No.	Isolate No.	Colony characters	Colony diameter (mm)	Sporulation
1	SF1	Black colored centre with blackish white periphery	85.00	***
2	SF2	Brownish centre with blackish green growth with white cottony periphery	79.00	**
3	SF3	Dark Black centre, with greenish white periphery	88.50	***
4	PF1	Black centre and concentric rings	86.00	***
5	PF2	Black white centre and light green whitish periphery	75.00	**
6	PF3	Brownish centre with grayish black growth	85.00	**
7	JF1	Circular, velvety greenish black growth	90.00	****
8	JF2	Brown centre and greenish velvety growth	87.60	***
9	JF3	Dark black centre with white cottony growth.	88.90	****
10	DF1	Black centre with grayish colony	88.70	****
11	DF2	Blackish growth with whitish periphery	54.00	*
12	DF3	Black centre with light greenish growth	87.00	***
13	TF1	Circular, dark green centre with greenish black growth and white periphery	90.00	****
14	TF2	Brown centre, cottony growth with yellowish white periphery	80.00	**
15	TF3	Black colored centre with blackish white periphery	85.00	***

*Poor, **moderate, ***very good, ****excellent

Table 2: Variation in conidial size of *Alternaria alternata* isolates

S. No.	Isolate No.	Length X width	Number of septation	
		(µ m)	Horizontal	Vertical
1.	Sf_1	37.65 X 14.50	8	4
2.	Sf_2	29.10 X 11.50	5	3
3.	Sf_3	42.00 X 16.50	7	4
4.	Pf_1	30.27 X 14.30	5	3
5.	Pf_2	25.85 X 9.12	4	2
6.	Pf_3	34.00 X 14.30	5	3
7.	Jf_1	49.27 X 24.21	8	5
8.	Jf_2	28.25 X 8.14	4	2
9.	Jf_3	46.27 X 24.27	7	4
10.	Df_1	32.27 X 12.30	6	3
11.	Df_2	25.80 X 8.15	5	2
12.	Df_3	35.60 X 11.50	6	3
13.	Tf_1	48.22 X 21.50	7	4
14.	Tf_2	47.20 X 22.35	8	3
15.	Tf_3	29.10 X 8.15	4	2

Table 3: Pathogenic variability of different isolates of *A. alternata*

S. No.	Isolate No.	Village	Tehsil	District	Per cent disease intensity
1.	SF_1	Arthwara	Abu road	Sirohi	57.22
2.	SF_2	Posalia	Abu road	Sirohi	62.20
3.	SF_3	Abu road	Abu road	Sirohi	72.65
				Mean	**64.02**
4.	PF_1	Dujana	Sumerpur	Pali	63.20
5.	PF_2	Malyawas	Sumerpur	Pali	50.80
6.	PF_3	Sumerpur	Sumerpur	Pali	62.15
				Mean	**58.71**
7.	JF_1	Borunda	Bilara	Jodhpur	80.37
8.	JF_2	Kettu	Bilara	Jodhpur	68.25

9.	JF_3	Bilara	Bilara	Jodhpur	76.24
				Mean	**74.93**
10.	DF_1	Mandawri	Lalsot	Dausa	68.22
11.	DF_2	Todia	Lalsot	Dausa	45.28
12.	DF_3	Lalsot	Lalsot	Dausa	72.60
				Mean	**62.03**
13.	TF_1	Kakodiya	Deoli	Tonk	78.18
14.	TF_2	Rajmahal	Deoli	Tonk	62.60
15.	TF_3	Deoli	Deoli	Tonk	59.55
				Mean	**66.64**
		Overall Mean			64.16

Conclusion

The results on cultural, morphological and pathogenic variability among different isolates of *A. alternata*, collected from different locations in five districts of Rajasthan showed that isolates differed in their colony characters, diameter and sporulation. Isolates from JF_1, JF_3, DF_1 and TF_1 showed excellent sporulation with a colony diameter 90.00 mm, 88.90 mm, 88.70 mm and 90.00 mm, respectively. In general, spore length and width was found in between 25.80 to 49.27µm and 8.14 to 24.27µm respectively, Number of horizontal and vertical septa varied between 4 to 8 and 2 to 5, respectively. All the isolates were pathogenic to fennel and produced characteristic symptoms of disease. Mean per cent disease intensity was highest with Jodhpur isolates followed by Tonk isolates.

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