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Short Communication

Fenugreek Leaf Blight Caused by *Alternaria alternata* in Semi-arid Conditions of Rajasthan

R.K. Solanki[1], Y.K. Sharma[2]*, R.K. Kakani[1] and R.S. Tomar[3]

[1]*ICAR-Central Arid Zone Research Institute, Jodhpur 342 003, India*

[2]*ICAR-National Research Centre on Seed Spices, Ajmer 305 006, India*

[3]*Junagadh Agricultural University, Junagadh 362 001, India*

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Fenugreek (*Trigonella foenum-graecum*) is an important seed spice crop cultivated in arid and semi-arid areas of western India in large. Fenugreek belongs to family leguminaceae and it has potential medicinal values. In India fenugreek is cultivated in states of Rajasthan, Madhya Pradesh, Gujarat, West Bengal, Uttaranchal and Haryana. Total acreage is of 1.67 lakh ha of which Rajasthan State have around 54% of national acreage (0.90 Lakh ha) (Spices Board, 2022). In Rajasthan, arid and semi-arid regions covering Bikaner, Jodhpur, Sikar, Nagaur and Jhunjhunu districts covers around 0.64 Lakh ha accounting around 70% of the state acreage under fenugreek. There are numerous diseases appearing in the crop causing economic losses. Alternaria leaf blight occurrence was first observed in seed (Rastogi *et al*., 1998) and in recent years 44 to 53% disease incidence was seen in the farmers field in Northern Plains of Uttar Pradesh (Singh *et al*., 2016), but its occurrence in western arid and semi arid parts of India is not reported. In the past few years, Alternaria leaf blight incidence has been noticed in the farmers field of Rajasthan. During survey in the adjoining areas of Ajmer in year 2013 field samples were collected showing circular to irregular dark brown lesions with grayish centre on leaves with black sporulation. These lesions gradually coalesced covering leaf margins causing withering and leaf dropping (Fig. 1). To confirm the pathogen standard methods were followed for morphological and molecular identification of the leaf blight causal agent in fenugreek; further field screening was also done to see the level of genetic resistance against leaf blight disease in fenugreek.

Pathogen was isolated from diseases plants after surface sterilization with 1% sodium hypochlorite and plated on potato dextrose agar (PDA); microbial colonies growth reached upto 90 mm diameter in 7 days at 25°C on PDA. Pure culture was morphologically identified at IARI, New Delhi. Fungal DNA was subjected to ITS regions amplification and molecular validation of the fungal species was done at Department of Biotechnology, Junagadh Agricultural University Junagadh, Gujarat (White *et al*., 1990; Iwen *et al*., 2002). Pathogenicity test were performed *in vitro* on detached leaves. To assess the level of genetic resistance in fenugreek genotypes, field trial was conducted in Randomized Block Design with 3 replications in year 2017-18. Natural incidence of the leaf blight disease was recorded using 0-4 scale (0: Healthy; 1: Small spot on leaf; 2: Spots covering entire leaf; 3: Spots on leaf and stem; 4: Spots on leaf, stem and pods) to calculate the percent disease index (PDI).

The pathogen isolation from diseased samples consistently showed presence of *Alternaria* fungus (Simmons, 2007), the pure culture was confirmed morphologically at Indian Agricultural Research Institute, New Delhi as *Alternaria alternata* (ITCC 9058.13), further the ITS region was amplified and on NCBI BLAST it showed near match to *Alternaria alternate* fungus (NCBI Gene Bank Accession No. KU862631). In past, no reports are available on molecular identification of the Alternaria leaf blight pathogen in fenugreek. *Alternaria alternata* pathogenicity tests were also done on fenugreek twice in detached leaf by spraying a conidial suspension (1×10^6 conidia ml^{-1} in sterile distilled water), obtained from 7-day old culture plates on to the healthy leaves of 3-months-old plants. Inoculated leaves and water-sprayed controls were incubated at room temperature. Symptoms started developing on the inoculated leaves after 4-5 days (Fig. 2). The pathogen was reisolated from the leaves

*E-mail: yksharma68@gmail.com

Fig. 1. Leaf blight symptoms on fenugreek leaves.

Fig. 2. Pathogenecity tests in detached leaf.

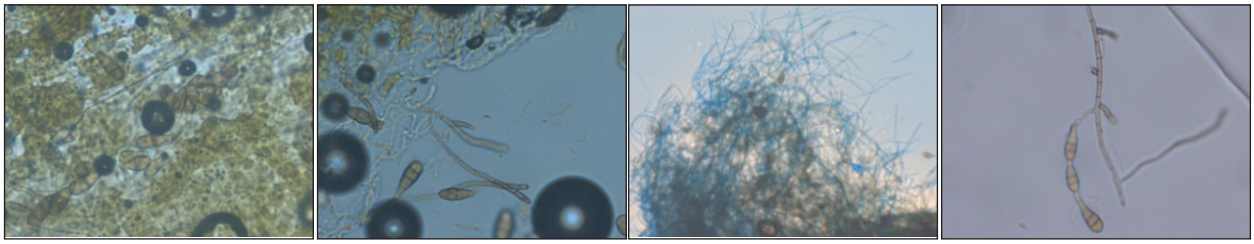

Fig. 3. Mycelium, condiophores and conidia in host cell and in culture.

of affected inoculated leaves to confirm Koch's postulates. The control plants did not exhibit symptoms. The pure culture of *A. alternata* was characterized morphologically using slide culture technique (Nagrale *et al*., 2013). Microscopic studies done on Olympus make Inverted Microscope showed that the mycelium was septate, irregularly branched. Mycelium width measured from 1.5 to 4.7 µ. Conidiophores arise singly or in cluster, straight, swollen at the apex measuring 27.3-110 µ long and 3.15-8.4 µ wide. Conidia born in chains and varied in shape, 1-3 longitudinal and 2-6 transverse septa were observed. The measured 20.80-96 µ in length and 8.2-16.5 µ in width. L: W ratio was calculated 3.49 for conidia and 9.82 for conidiophores (Fig 3; Table 1). *Alternaria* is a widespread pathogen affecting a wide range of hosts, and has been reported on seed spices like cumin, coriander and fennel (Uppal *et al*., 1938; Mathur, 1949; Joshi, 1955; Deshpande and Sehgal, 1964). Incidence of leaf blight on fenugreek was not observed in the arid and semi-arid regions of western India specifically Rajasthan. In the recent years the incidence is increasing, therefore development of leaf blight resistant varieties can help in management of the disease.

Table 1. Measurement of different morphological structures of A. alternata causing leaf blight of fenugreek (Slide culture technique)

Morphological structures		Measurement parameters		
		Length	Width	L:W ratio
Mycelium	Septate, irregularly branched	-	2.80 (1.5-4.7)	-
Conidia	Born in chains, varied in shape, 1-3 longitudinal and 2-6 transverse septa	47.10 (20.80-96.0)	13.50 (8.2-16.5)	3.49
Conidiophores	Arise singly or in cluster, straight, swollen at apex	42.25 (27.3-110)	4.30 (3.15-8.4)	9.82

Table 2. Alternaria leaf blight Incidence observed in fenugreek genotypes

Genotype	Mean *Alternaria leaf* blight PDI
B4-22-7-2-1	9.3 ± 0.6
B5-73-11	10.0 ± 2.3
IC-571706	10.7 ± 0.6
IC-585601	11.3 ± 1.3
AFg-5	11.3 ± 0.6
A3-31-2-7-(2)	11.3 ± 1.3
C4-2-14	11.3 ± 0.6
IC-572835	11.3 ± 0.6
B3-77-8-10	12.0 ± 0.001
B3-31-1-17	12.0 ± 1.1
IC-595313	12.0 ± 1.3
B5-5-6-1	12.0 ± 1.1
B5-33-20	12.0 ± 0.6
AFg-3	12.0 ± 1.1
C4-23-1	12.7 ± 2.4
IC-585599	12.7 ± 0.6
IC-595329	13.3 ± 0.6
IC-397265	14.0 ± 1.1
IC-418376	14.0 ± 0.001
IC-605357	15.3 ± 1.3
Mean	12.00 ± 1.7
CV	17.07
SE (d)	1.709

Field screening of 20 genotypes showed PDI ranging from 9.3 in genotype B4-22-7-2-1 to 15.3 in genotypes IC-605357. The mean incidence was 12.00%, no significant differences were observed in the genotypes for disease reaction, but none of the genotype showed resistant reaction (Table 2). It is suggested that large set of germplasm should be screened against the disease to identify potential genotypes for disease management. Leaf blight management can be done by cultural and chemical methods (Sharma *et al.*, 2019; Ambuse and Bhale, 2012) but genetic resistance is the best management practice.

The present study was first attempt to identify the pathogen causing Alternaria blight in fenugreek at molecular level; secondly screening of fenugreek genotypes is also first effort made in the region to identify resistant sources. Alternaria blight incidence in fenugreek significantly affects the economic yield in terms of leaves biomass. Green leaves are consumed as vegetable in Rajasthan and other parts of the country; hence management of the diseases is very important, chemical management is not the right approach as residues of the fungicide may be harmful for human body. The cultivar Afg-5 showed less incidence of leaf blight than the cultivar AFg-3. There is need to identify resistance genotypes for better management of the disease in the region and for other cultivated parts of the country.

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