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Symptomatology, proving pathogenicity and standardization of inoculum density of *Fusarium oxysporum* causing wilt of fenugreek

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Abstract
Fenugreek (*Trigonella foenum graecum* L.) is an important seed spice, originated in South-Eastern Europe belonging to the family Fabaceae. Wilt of fenugreek caused by *Fusarium oxysporum*, exhibited symptoms like stunting of the plants, gradual yellowing of lower leaves, drooping, epinasty, drying of leaves, vascular discoloration and ultimately death of entire plant was noticed. In *Rhizoctonia solani* infected plants showes yellowing, drying of leaves, discolouration of roots and complete decaying of tap and lateral root system was noticed. Finally death of entire plants. The sclerotium wilt symptoms appeared as yellowing of lower leaves, stunted growth, finally death of plant. This is the new report of the Sclerotium wilt of fenugreek. *Fusarium oxysporum* colonies were cottony white to dark pink. Mycelium was septate and hyaline and produced both macro and microconidia. Macroconidia were moderately curved, stout, sickle shaped, usually 1-3 septate. *R. solani* produced black colour colony, dense, radiating luxurious mycelial growth and right angle branching.
S. rolfsii. produced the silky white, dense, radiating mycelial growth on potato dextrose agar medium. Initiation of sclerotial bodies were obtained from sixth day after inoculation. Pathogenicity test of *F. oxysporum* was observed for symptom expression. Death of the plant was observed at 28 days after sowing. In *Rhizoctonia solani* inoculated plants exhibited foliar yellowing and stunting of the plants at 12 days after sowing. Death of plants was noticed at 14 days after sowing. In *S. rolfsii* yellowing of lower leaves was noticed at 14 days after sowing and finally death of the plants was noticed at 18 days after sowing. The fungus was reisolated from infected plant tissue and compared with the original culture which yielded the *Fusarium oxysporum*, *Rhizoctonia solani* and *S. rolfsii* thus proving the pathogenicity following Koch's postulates respectively. A minimum of eight per cent inoculum was found to be optimum to cause the wilt disaese.

Keywords: Fenugreek, Symptomatology, *Fusarium oxysporum*, Pathogenicity

Introduction
Fenugreek (*Trigonella foenum graecum* L.) is an important seed spice, originated in South-Eastern Europe belonging to the family Fabaceae. It is an annual herb, commonly known as Methi. It is a native of India. India is the leading fenugreek producing country in the world (Edison, 1995) [1]. It is the third largest seed spice in India after coriander and cumin. In India, it is grown in about 66,000 ha with an annual production of about 90,000 tonnes (Anon., 2014) [2]. Rajasthan is the fenugreek bowl of country, contributing 90 per cent to the country's production (Kumawat and Sharma, 2014) [3]. Fenugreek suffers from many of fungal diseases *viz.*, Cercospora leaf spot caused by *Cercospora traversiana*, root rot (*Rhizoctonia solani)*, leaf spot (*Ascochyta* sp.), powdery mildew (*Erysiphe polygoni)*, downy mildew (*Peronospora trigonellae)* and Fusarium wilt (*Fusarium oxysporum*) (Prasad *et al.*, 2014) [4]. Fenugreek is mainly grown as leafy vegetable throughout Karnataka and there is ample scope for its cultivation as seed spice. But there is lack of systematic work on diseases of fenugreek in Karnataka. Many diseases are reported in fenugreek, wilt is becoming more severe in recent years (Rani *et al.*, 2014) [5]. The present study is concerned with symptomatology, proving pathogenicity and standardization of inoculum density one of the major disease of fenugreek called wilt caused by the fungus *Fusarium oxysporum.*

Material and methods
Symptomatology of root rot/wilt disease of fenugreek
Fenugreek plant samples showing typical wilting symptoms were recorded during survey and were subjected for the isolation of the pathogen.

Isolation of pathogens
The infected plants showing typical symptoms of the disease were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogens. The infected parts were surface sterilized with 1% sodium hypochlorite solution for 30 seconds and washed serially in sterilized distilled water to remove the traces of the chemical if any and then transferred to sterilized Petriplates containing potato dextrose agar (PDA). The Petriplates were incubated at room temperature (27±1°C) and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants and incubated at 27±1°C for 10 days. Then such slants were used to study the pathogen characters. Similar method was followed for other pathogens.

Identification of pathogen
The identification of *Fusarium oxysporum* was done based on the spore morphology and colony characters of the fungi as described by Ainsworth, (1971) [6], Barnett and Hunter, (1972) [7] and Leslie and Summerell (2006) [8]. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions. Such culture tubes were preserved in a refrigerator at 5ºC and used for further studies.

Preparation of giant culture of *Fusarium* oxysporum for inoculum density assesment
Sand and corn meal (90:10) was used as substrate for gaint culture preparation in conical flask and sterilized alternatively for two consecutive days. Fresh culture of seven days old *Fusarium oxysporum* of 5 mm disc was inoculated to flask and incubated for 20 days for full growth of the fungi, during incubation the culture was mixed thoroughly to get uniform growth of *Fusarium oxysporum*. After full growth, it was used for further studies.

Proving the pathogenicity
Sterilized soil was taken in earthen pots. Giant culture was mixed thoroughly with soil at the rate of eight per cent to get sick soil. Then five healthy fenugreek seeds were sown in pots filled with sick soil. Seeds sown in pots without inoculum served as control. Observations were taken at regular interval for symptoms development. Reisolation was made from such infected plant and compared with that of original culture. Same procedure was followed for *Rhizoctonia solani* and *Sclerotium rolfsii* for proving pathogenicity.

Standardization of inoculum density
The gaint culture was inoculated to each pot with 300 g soil holding capacity. The inoculum was added at the rate of 2, 4, 6, 8, 10, 12 and 14 per cent inoculum density. Five seeds were sown in each pot. Healthy plant without adding inoculum was maintained as control. Three replications were maintained for each treatment. Observation on per cent disease incidence was recorded.

Results and Discussion
Symptomatology of root rot/wilt of fenugreek
The various types of symptoms of fenugreek root rot/wilt disease are presented in Plate 2.

Fusarium wilt
Wilt of fenugreek caused by *Fusarium oxysporum*, exhibited various types of symptoms at any stage of the crop. Infection at seedling stage resulted in complete drying. Infected plants exhibited various types of symptoms like stunting of the plants, gradual yellowing of lower leaves, drooping, epinasty, drying of leaves and in some cases partial wilting of the plant was noticed. Dried leaves were retained on the wilted plants. Wilted plants when splited longitudinally, showed brown vascular discoloration. The wilted plants ultimately showed the loss in vigour leading to death of entire plant (Plate 2).

Root rot
The root rot symptoms of the infected plants by *Rhizoctonia solani* are many and varied. The infected plants exhibited symptoms of yellowing and drying of the leaves. Affected plants showed discolouration of roots and complete decaying of tap and lateral root system. The root/ bark of infected plants can be easily peeled off, with extensive sloughing and shredding of affected bark. Finally infected plants showed the death of entire plants. Similar symptoms were recorded by Dwivedi *et al.* (1982) [9] and Bose *et al.* (2003) [10]
(Plate 2).

Sclerotium wilt
The sclerotium wilt symptoms appeared generally during late season of crop growth. The first symptoms appeared as yellowing of lower leaves or drying of the leaves. White cottony mycelial growth of the fungus was observed at collar region of the plan. Infected plant lacks lateral root system. Stunted growth of the plant and fungus spread more rapidly and destroyed the root system leading to death of plant was observed. In severe cases, pathogen produced the sclerotia on the infected tissues. This is the new report of the Sclerotium wilt of fenugreek. Similar symptoms were observed by Agarwal and Kotasthane (1971) [11] and Dange (2006) [12] in *Sclerotium* wilt of soybean and chilli respectively
(Plate 2).

Isolation, identification and proving pathogenicity
During survey, characteristic symptoms of fenugreek wilt samples were collected. The pathogen *Fusarium oxysporum* was isolated from the infected root portion of fenugreek plant by following standard tissue isolation method. Similarly, Chaudhary (2013) [13] isolated *Fusarium oxysporum* in wilt of fenugreek caused by *Fusarium oxysporum*.
The major pathogens isolated were *Fusarium oxysporum* causing Fusarium wilt, *R. solani* causing root rot, and *S. rolfsii* causing Sclerotium wilt. The individual pathogen alone cause wilt or in combination can lead to root rot/wilt complex.

Fusarium oxysporum
Standard tissue isolation technique was used to get pure culture of this organism from diseased root samples. Colonies were cottony white to dark pink. Mycelium was septate and hyaline and produced both macro and microconidia. Macroconidia were moderately curved, stout, sickle shaped, usually 1-3 septate, with size ranged from 3.78-45.16 × 1.00-6.28 µm. Microconidia were oval to oblong shaped, with size of 1.70- 13.76 × 1.05-5.36 µm. Chlamydospore were single celled, dark coloured, thick walled and occurred either singly or in chain, intercalary or at terminal end of hyphae. The identification of *Fusarium oxysporum* was done based on the spore morphology and colony characters of the fungus by referring to "The Genus *Fusarium*" (Booth, 1971) [14], "Illustrated genera of imperfect fungi" (Barnett and Hunter, 1972) [7]. Chlamydospores were coloured, usually vacuolated and spherical (Plate 3).

Rhizoctonia solani Kuhn.

The tissue from infected root samples showing symptoms like easy peeling of the bark was used to get pure culture of *R. Solani*. The fungus produced black colour colony, dense, radiating luxurious mycelial growth on potato dextrose agar medium and branching of the mycelium was at right angle. After 6 days of inoculation, the pathogen produced the microsclerotial bodies in the culture. The pathogen was identified using characters described by Ainsworth (1971) [6]. *R. solani* (Telomorph: *Thanatephorus cucumeris* (Frank)) Donk is a cosmopolitan in soils. It is a destructive plant pathogen with an almost unlimited host range (Plate 3).

Sclerotium rolfsii Sacc

The infected root samples showing growth of white cottony mycelium or presence of sclerotial bodies, were used to get pure culture of *S. rolfsii*. The fungus produced silky white, dense, radiating mycelial growth on potato dextrose agar medium. In early stages, the fungus produced silky white mycelium and gradually lost its lustre and became dull in appearance. Aerial hyphae were not uniformly distributed, Initiation of sclerotial bodies were obtained from sixth day after inoculation. In the beginning, the sclerotial bodies were white but gradually turned to buff brown colour and then to chocolate brown at maturity, as descbribed by Barnett and Hunter (1972) [7] (Plate 3).

The fungus isolated from the infected plant tissue was compared with the type species originally described and was found resembling with *Sclerotium rolfsii* in all the morphological character (Saccardo, 1911) [18].

Pathogenicity test

Pathogenicity test was done for all three pathogens was carried out and observed for symptom expression. The pathogen was reisolated from infected roots and identified the causal organism and was confirmed by comparing with the original culture by standard procedures.

Fusarium wilt (*Fusarium oxysporum*)

Pathogenicity test of *F. oxysporum* was observed for symptom expression. Infection in the beginning was typically in the form of yellowing of lower leaves was observed on 14 days after sowing. Roots of the diseased plants showed brownish to blackish lesions. The affected plants wilted and dried up and death of the plant was observed at 28 days after sowing. Isolation from artificially inoculated plant yielded *Fusarium oxysporum* which was identical to original one. These studies were supported by the earlier report of Chaudhary (2013) [13] (Plate 4).

Root rot (*Rhizoctonia solani* Kuhn)

In *Rhizoctonia solani* inoculated plants exhibited foliar yellowing and stunting of the plants at 12 days after sowing. Death of plants was noticed at 14 days after sowing. The bark of such infected plants came out very easily. Re-isolation from infected plant yielded the *Rhizoctonia solani* which was identical to original culture (Plate 4).

Sclerotium wilt (*Sclerotium rolfsii* Sacc.)

In *S. rolfsii* pathogenicity study, yellowing of lower leaves was noticed at 14 days after sowing and finally death of the plants was noticed at 18 days after sowing. The fungus was reisolated from infected plant tissue and compared with the original culture, thus proving the pathogenicity following Koch's postulates (Plate 4).

Assessment of minimum inoculum level of *Fusarium oxysporum*

Inoculum density is bound to influence the disease incidence. Infection takes place only when minimum inoculum potential of the pathogen is present in the soil. The knowledge of the relationship between pathogen inoculum density and disease severity is useful, but it does not necessarily provide predictive data for determining the level of disease that might be observed in a particular field. The reason for this is the extent of saprobic competition for nutrients in the soil can influence the subsequent level of disease (Martin and Loper, 1999) [20]. Since the soil microflora at any given location will be different, the data on the effect of inoculum density on disease severity in one soil will not always be correlated with another soil. The presence of other soil borne pathogens can also influence disease severity caused by a given amount of inoculum.

It was conducted by inoculating the giant culture of *Fusarium oxysporum* to the sterilized soil at various concentrations on weight by weight basis as explained in 'Material and Methods' and planted the susceptible DFC-26 cultivar. The symptoms were observed as mentioned above. In the present investigation, a minimum of eight per cent inoculum was found to be optimum to cause the disaese. As there was increase in per cent inoculum level there was significant increase in the disease incidence (Fig. 2). Per cent disease incidence increased as the inoculum concentration increased. At 8, 10, 12 and 14 per cent of inoculum, there was cent per cent disease incidence and death of the plants was noticed. Seed germination was inhibited at more than 12 per cent. These findings are in agreement with the reports of Rao and Rao (1963) [21] in case of *F. oxysporum* f. sp. *vasinfectum*, Shalini (2008) [22] in rhizome rot of ginger and Raghu (2014) [23] in chilli wilt caused by *F. solani*. The results are presented in Table 2 and Plate 5.

Table 1: Assessment of minimum inoculum level of *Fusarium oxysporum*

Per cent inoculum level	Incidence of wilt (%)
0	0.00 (0.00)*
2	20.00 (26.55)
4	40.00 (39.22)
6	66.67(54.97)
8	100.00 (89.96)
10	100.00 (89.96)
12	100.00 (89.96)
14	100.00 (89.96)
S.Em.±	1.49
C.D. at 1%	6.16
CV %	4.30

*Arc sine transformed values

Plate 1: Symptoms of fenugreek wilt

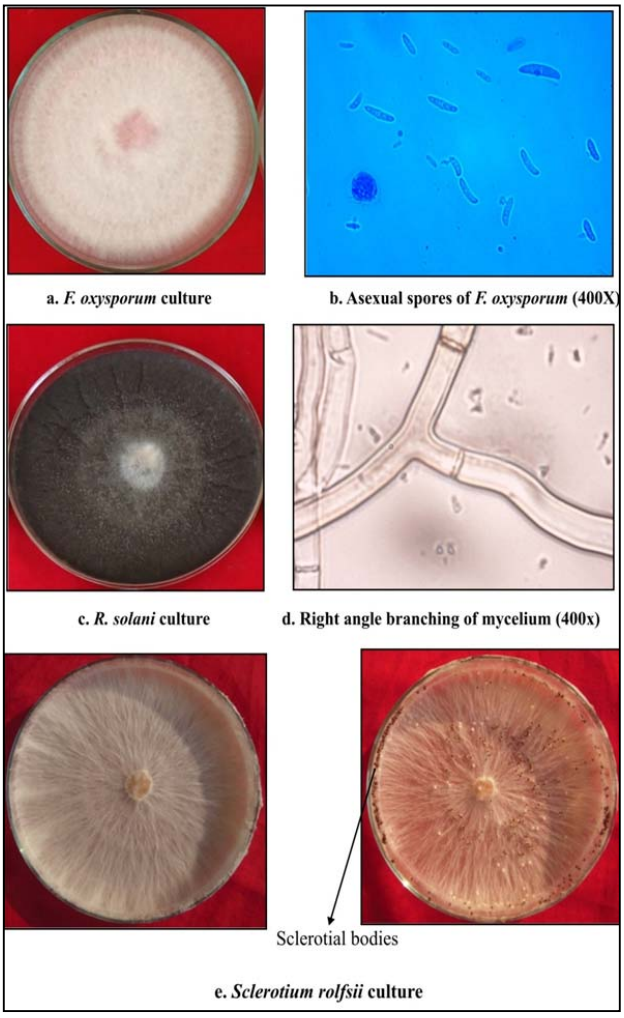

Plate 2: Cultural characterstic of wilt pathogens

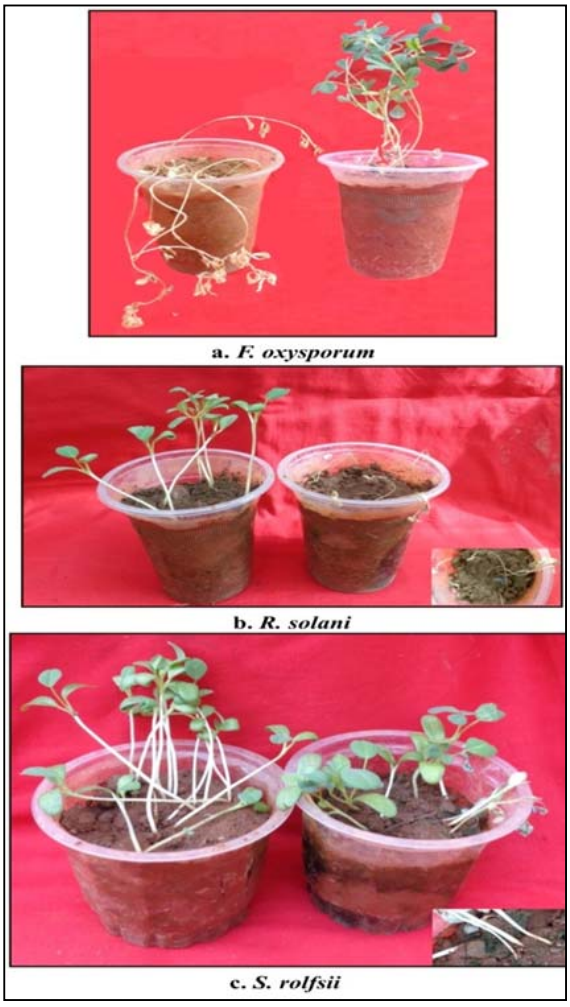

Plate 3: Proving pathogenicity of wilt complex pathogens

Plate 4: Assessment of minimum inoculums level of *Fusarium oxysporum*

References

1. Edison S. Spices-research support to productivity. In: The Hindu Survey of Indian Agriculture. (Ed. Ravi, N.), Kasturi and Sons Ltd., National Press, Madras, 1995, 101-105.
2. Anonymous,http://www.indiastat.com/agriculture/2/spices/262/fenugreek/20663. 2014.
3. Kumawat SK, Sharma R. A review on management of fungal diseases associated with *Trigonella foenum graecum* L. (fenugreek). Int. J. Pha. Biol. Sci. 2014; 2(6):14-25.
4. Prasad R, Acharya S, Erickson S, Thomas J. Identification of cercospora leaf spot resistance among fenugreek accessions and characterization of the pathogen. Australian J. Crop. Sci. 2014; 8(6):822-830.
5. Rani N, Hegde YR, Nargund VB, Lingaraju S, Nagangowda SB. Scenario of wilt complex of fenugreek and its management. Nation. Symp. Plant diseases: New perspectives and innovative management strategies. 5. Epidemiology in relation to climate change, UAS, Dharwad (India), 2014, 33.
6. Ainsworth GC. Ainsworth Bisby's dictionary of the fungi. VI Edn. Common wealth Mycological Institute, Kew, Surrey, England, 1971, 663.
7. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. Burgess Publication Ltd. St. Paul, Minnesota, USA, 1972, 241.
8. Dwivedi DK, Shukla DN, Bhargava SN. Two new root rot disease of spices. Curr. Sci. 1982; 51(5):243-244.
9. Leslie JF, Summerell BA. The fusarium laboratory manual. Black well publishing, Ames. IA, 2006.
10. Bose TK, Kabir J, Maity TK, Parthasarathy VA, Som MG. Vegetable crops, 2003, 315-334.
11. Agarwal SC, Kotasthane KE. Resistance in some of soybean varieties against Sclerotium rolfsii Sacc. Indian Phytopath. 1971; 24(2):401-403.
12. Dange V. Studies on root rot of chilli caused by Sclerotium rolfsii Sacc. M. Sc. (Agri) Thesis. Univ. Agric. Sci., Dharwad, Karnataka (India), 2006.
13. Chaudhary S. Detection and management of wilt of fenugreek (*Trigonella foenum graecum*. L.) incited by *Fusarium oxysporum. M. Sc. Thesis*, Univ. Agric. Sci., Bikaner, Rajasthan (India), 2013.
14. Booth C. The genus Fusarium. Common wealth Mycological Institute, Kew Surrey, England, 1971, 132.
15. Saccardo PA. Notae mycologicae. Ann. Mycol. 1911; 9:249-257.
16. Martin FN, Loper JE. Soil borne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. Critical Rev. Pl. Sci. 1999; 18:111-181.
17. Rao AS, Rao MV. Inoculum potential and fusarium wilt of cotton. Nature. 1963; 200:598-599.
18. Shalini DS, Kulkarni S. Survey, surveillance and etiology of rhizome rot of ginger in Karnataka. J. Pl. Dis. Sci. 2008; 3(1):37-39.
19. Raghu S. Studies on chilli wilt complex disease. Ph. D. *(Agri)*, Thesis, Uni. Agric. Sci., Dharwad (India), 2014.