Pak. J. Bot., 20(2): 233–237, 1988.

SEED-BORNE MYCOFLORA OF *TRIGONELLA FOENUM-GRAECUM* L.

M.H. HASHMI

Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Seed samples of *Trigonella foenum-graecum* (fenugreek) obtained from 7 countries were analyzed for seed-borne mycoflora. Of the 23 samples examined, the samples from Pakistan showed maximum infection of seed-borne fungi alongwith unidentified yeasts and bacteria. Fungi isolated were: *Alternaria alternata, Botrytis cinerea, Curvularia inaequalis, C. lunata, Drechslera tetramera, Epicoccum purpurascens, Fusarium moniliforme, F. oxysporum, F. semitectum, F. solani, Phoma* sp., *Stemphylium botryosum, Ulocladium* sp., and *Verticillium albo-atrum.* Of these *A. alternata* and *F. moniliforme* were predominant. Storage fungi like *Aspergillus, Penicillium* and *Rhizopus* were rarely found except in a few heavily infected samples. In infection experiments *F. moniliforme, F. oxysporum* and *F. solani* caused seed rot and wilting of the seedlings of fenugreek. These species appear to be new records not hitherto reported on fenugreek.

Introduction

Trigonella foenum-graecum, commonly called fenugreek is used as a condiment in several Asian and African countries. It is well known for its alkaloids trigonelline, cholesterin, lecithin and choline. Fenugreek is also applied as a green manure to control *Verticillium dahliae* wilt of cotton (Askarova & Golovchenko, 1962). In the present study the seed-borne mycoflora of fenugreek was examined and compared with that given in the Annotated List of Seed-borne Diseases (Richardson, 1979, 1981, 1983). The pathogenic potential of *Fusarium* spp., on fenugreek were also investigated.

Materials and Methods

Twenty three seed samples of fenugreek obtained from 7 tropical countries through the courtesy of the Danish Government Institute of Seed Pathology, Copenhagen, Denmark were used in this study.

The standard blotter method for seed testing was used (Anonymous, 1966). The seeds transferred on filter paper in Petri dishes were incubated for 7 days at 22°C and examined under a stereobinocular microscope (6-50x magnification). Pure cultures of fungi were maintained on potato dextrose agar (pH 5.6) and identified according to Nelson *et al.,* (1983), Ellis (1971) and Barron (1968).

Table 1. Fungi isolated from seeds of *Trigonella foenum-graecum* obtained from different countries of the world.

Countries	Number of samples tested	*Alternaria alternata* A	*Alternaria alternata* B	*Botrytis cinerea* A	*Botrytis cinerea* B	*Fusarium moniliforme* A	*Fusarium moniliforme* B	*Fusarium oxysporum* A	*Fusarium oxysporum* B	*Fusarium semitectum* A	*Fusarium semitectum* B	*Fusarium solani* A	*Fusarium solani* B	*Phoma* sp. A	*Phoma* sp. B	*Verticillium albo-atrum* A	*Verticillium albo-atrum* B
Egypt	1	1	6.00 ± 0.00 (6.00)			1	1.00 ± 0.00 (1.00)										
India	2	1	7.00 ± 0.00 (7.00)	1	2.50 ± 0.00 (2.50)	1	10.00 ± 0.00 (10.00)	1	7.00 ± 0.00 (7.00)	1	5.00 ± 0.00 (5.00)	1	5.50 ± 0.00 (5.50)				
Nepal	1					1	6.00 ± 0.00 (6.00)					1	3.50 ± 0.00 (3.50)			1	1.00 ± 0.00 (1.00)
Pakistan	16	11	3.94 ± 1.79 (1.00-11.00)	1	1.00 ± 0.00 (1.00)					2	2.00 ± 0.70 (1.00-3.00)	2	1.00 ± 0.00 (1.00)			1	2.00 ± 0.00 (2.00)
Sri Lanka	1	1	3.50 ± 5.00 (2.50)					1	1.00 ± 0.00 (1.00)					1	1.00 ± 0.00 (1.00)		
Sudan	1	1	2.00 ± 0.00 (2.00)			1	1.00 ± 0.00 (1.00)					1	1.00 ± 0.00 (1.00)	1	1.00 ± 0.00 (1.00)		
Syria	1					1	4.00 ± 0.00 (4.00)										
$\overline{X}$			4.48		1.75		4.40		4.00		3.50		2.75		1.00		1.50
Standard Error			1.00		0.26		0.63		0.53		0.21		0.18		0.00		0.01

A: No. of infected samples. B: Infection percentage.
Numbers in parentheses indicate infection range.

Four hundred naturally infected seeds were sown in pots containing peat soil. In another set water agar slants were used. The pots were kept for 2 weeks at 20-25°C under illumination of fluorescent day light tubes for 12 h each day. Seedlings were classified according to severity of infection based on visual inspection. Each seedling was treated with 1% sodium hypochlorite for 5 min and cut into four components viz., cotyledonary leaves, upper half of hypocotyl, lower half of hypocotyl and root. These components were incubated on wet blotters and fungi developing on different components were examined under stereobinocular microscope and identified.

Results and Discussion

The seed-borne fungi isolated from fenugreek showed that 65.2% of the samples were infected by *A. alternata*, 21.7% by *F. moniliforme* and 21.7% by *F. solani* (Table 1). This was followed by *F. semitectum* (Av. 3.50 ± 0.21) in 13.0%, *B. cinerea* in 8.8%, *F. oxysporum* in 8.6%, *V. albo-atrum* in 8.0% and *Phoma* sp., in 8.0% of the samples. While *A. alternata* was predominant in seed samples of Pakistan, *F. moniliforme* and *F. solani* were present as trace infections.

One of the characteristics of the mycoflora of fenugreek seed was the ubiquity of *A. alternata* and *F. moniliforme*. In contrast, fungi of the genera *Aspergillus*, *Penicillium* and *Rhizopus* were comparatively rare except in a few heavily infected samples, hence not recorded here. Colonies of bacteria were frequently found arising from seed coat.The colony colour of bacteria was usually white or yellow, and no attempt was made to identify the species.

When naturally infected seeds of fenugreek were sown in peat soil, *F. moniliforme*, *F. oxysporum* and *F. solani* showed symptoms of seed rot, root rot and seedling stem blight (Fig. 1A, 1B). Rotting normally began as whitish pink to brownish discoloration when seedlings were young as was evident when seeds were sown on agar slants. The symptoms of the disease appeared more severe as the plants were left to mature. Leaves of affected plants became chlorotic, withered and eventually dropped. Roots of such plants occasionally developed beadlike swellings (arrows in Fig. 1C). The most characteristic symptom of blight was browning or blackening of the vascular system in stems which was evident when stems were split open. Infection by *F. moniliforme* (2.0 to 7.5%) was comparatively more than *F. oxysporum* (1.5 to 3.0%) or *F. solani* (1.0 to 5.9%) both in water agar slants and in peat soil (Table 2). Association of *F. moniliforme* together with *F. solani* caused greater incidence of seed rot. Seed rot and death of seedlings by different species of *Fusarium* was more pronounced in test tubes where comparatively more moisture was available than in pots (Table 2). In recovery experiments, *F. moniliforme* and *F. solani* were isolated from 6.1% of the healthy looking seedlings whereas *F. oxysporum* was detected from 4.0% seedlings. There does not appear to be any previous report of seed rot and pathogenic activity of *Fusarium* spp., in fenugreek.

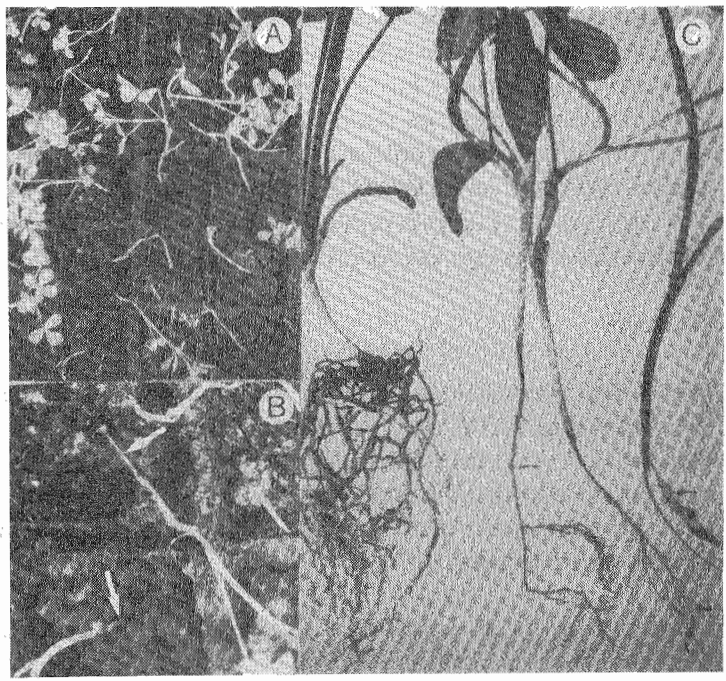

Fig. 1. Root rot and stem blight caused by *Fusarium* spp., in fenugreek plants grown from naturally infected seeds. Note the rotting (arrows in B) and beadlike swellings (arrows in C).

Table 2. Seed rot and death of seedlings of fenugreek due to *Fusarium* spp. (400 naurally infected seeds were used in each experiment).

Pathogen isolated	Expt. 1 % seed rot		Expt. 2 % dead seedlings		% recovery of *Fusarium* spp. from healthy looking seedlings* from pot experiments
	in pots	in water agar slants	in pots	in water agar slants	
Fusarium moniliforme	2.0	5.0	4.0	7.5	6.1
Fusarium oxysporum	1.5	2.0	2.5	3.0	4.0
Fusarium solani	1.0	3.5	3.5	5.9	6.1
Fusarium moniliforme + *Fusarium solani*	4.0	7.0	—	1.0	—
Other fungi**	8.8	13.0	1.2	2.0	—

*Number of healthy looking seedlings: 297.

***Aspergillus* sp., *Cladosporium herbarum*, *Cladobotryum variospermum*, *Curvularia lunata* and *Rhizopus stolonifer*.

The development of wilting in fenugreek has several features in common with the comparable symptoms observed in coriander and probably has a common biochemical basis. In coriander, *F. solani* caused annellations on deformed stems (Hashmi, 1988) whereas the cumulative pathogenicity of *F. solani*, *F. moniliforme* and *F. oxysporum* caused beadlike swelling on the roots of fenugreek seedlings. To account for the variation in the syndromes across the range of diseases, presumably one pathogen or one host may lack a component which prevents that symptom from developing (Dimond, 1955). Yellowing of leaves in fenugreek is most probably caused by ethylene formation by the pathogen and possibly to some extent by the host. The formation of ethylene, its liberation into the transpirational stream and subsequent systemic distribution has been demonstrated in *Fusarium*-wilt of tomato (Dimond, 1955).

References

Anonymous. 1966. International Rules for Seed Testing, 1966. *Proc. Int. Seed Test. Assoc.*, 31: 1-152.

Askarova, S.A. and S.G. Golovchenko. 1962. On the question of biological methods for the control of cotton wilt. *Uzbek, Biol. Zh.*, 6: 8-12 (RAM 42, p. 386).

Barron, G.L. 1968. *The Genera of Hyphomycetes from Soil.* Baltimore, Williams and Wilkins Co. 364 pp.

Dimond, A.E. 1955. Pathogenesis in the wilt diseases. *Ann. Rev. Pl. Physiol.*, 6: 329-350.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.* Commonw. Mycol. Inst., Kew, Surrey, England, 608 pp.

Hashmi, M.H. 1988. *Seed-borne mycoflora of some spices, detection techniques and pathogenicity.* Ph.D. Thesis, Univ. of Karachi, Karachi, Pakistan. 164 pp.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium species.* An Illustrated Manual of Identification. The Pennsylvania State Univ. Press, University Park, Pennsylvania. 203 pp.

Richardson, M.J. 1979. *An Annotated List of Seed-borne Diseases.* Int. Seed Test. Assoc., Zurich, Switzerland. 320 pp.

Richardson, M.J. 1981. *An Annotated List of Seed-borne Diseases.* Supplement 1. Int. Seed Test. Assoc., Zurich, Switzerland. 78 pp.

Richardson, M.J. 1983. *An Annotated List of Seed-borne Diseases.* Supplement 2. Int. Seed Test. Assoc., Zurich. Switzerland, 108 pp.

(Received for publication 16 August 1988)