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IDENTIFICATION AND ISOLATION OF FUNGI FROM *TRIGONELLA FOENUM-GRAECUM* L.

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ABSTRACT

In the present study, thirty three fungal species with 14 genera were isolated from 15 samples of *Trigonella foenum-graecum* L. (fenugreek) collected from different localities of Pakistan. Fungi include *Alternaria alternata* (Fr.) Keissler, *A. longipes* (Ellis & Everh.) E.W. Mason, *A. tenuissima* Wiltshire, *Aspergillus* spp. Micheli, *Botrytis cinera* Pers., *Chaetomium funicola* Cooke, *C. globosum* Kunze ex Fr., *C. indicum* Corda, *Cladosporium herbarum* (Pers.) Link, *Curvularia lunata* (Wakker) Boedijn, *Drechslera hawaiiensis* M.B. Ellis, *Fusarium oxysporum* Schlecht, *F. solani* (Mart.) Sacc, *Humicola fuscoatra* Traaen, *Mucor racemosus* Fresen., *Paecilomyces variotii* Bain, *Penicillium brevicompectum* Dierckx, *P. chrysogenum* Thom, *Phoma* sp. Sacc. and *Rhizopus* sp Ehrenb. Agar plate method was found to be the best for the detection of maximum number of fungi followed by blotter method and deep freezing methods. Reduced growth of saprophytic fungi was recorded due to surface sterilization by 1% sodium hypochlorite.

KEYWORDS: Fenugreek, ISTA technique, Mycoflora, Saprophytic and pathogenic fungi.

INTRODUCTION

Trigonella genus comes from latin which means 'little triangle' because of having triangular shaped flowers (Rosengarten, 1969). *Foenum-graecum* means 'Greek hay' as the plant was traditionally applied for flavouring poor quality hay in past (Acharaya *et al.,* 2006). According to Acharaya *et al.* (2006), Fenugreek is cultivated in Australia, Northern and Eastern Africa, Argentina, China, India, Africa and Mediterranean Europe. Leaves and seeds of fenugreek have tremendous medicinal value as they contains galactomannas, isoleucine and steroidal sapogenins (Acharaya *et al.,* 2006). Many researchers worked on medicinal properties of fenugreek and reported that it has antiparasitic, antifertility, anticancer, antimicrobial, antidiabetic and hypochloesterolemic activity (Al-Habori and Raman, 2002; Broca *et al.,* 2004; Devasena and Menon, 2003; Devi *et al.,* 2003; Hannan *et al.,* 2003; Suboh *et al.,* 2004; Tahiliani and Kar, 2003a, b; Thakaran *et al.,* 2003; Thompson and Ernst, 2003; Vats *et al.,* 2003; Venkatesan *et al.,* 2003).

Ecotophytic or endophytic seed borne pathogens act as primary source of disease and deteriorate seed quality. Many fungal and bacterial pathogens attacks on seed production of this crop like *Erysiphe polygoni* (caused powdery mildew), *Cercospora traversiana* (leaf spot), *Fusarium oxysporum, Rhizoctonia solani* (wilt diseases) respectively (Jongebloed, 2004; Prakash and Sharma, 2000). Jain and Jain (1995) reported 11 fungal species on fenugreek namely *Alternaria* sp, *Aspergillus flavus, A. niger, A. sydowii, Chaetomium* sp., *Cladosporium cladosporides, Drechslera spicifer, Emericella hidulans, Penicillium amantogriseum*, *Rhizopus stolonifer* and *Trichothecium roseum.* Besides this other researchers recorded data of fungi which causes soil borne, root rot, damping off, wilting, powdery mildew, downy mildew, rust diseases on fenugreek seeds (Hedawoo and Chakranarayan, 2011; El-Nagerabi, 2002; Uppal *et al.*,1934; Dwivedi *et al*.,1982; Hiremath *et al*., 1978; Acharya *et al.,* 2014) .

The present study was undertaken to investigate fungal pathogens associated with fenugreek collected from different localities of Pakistan.

MATERIALS AND METHODS

Fifteen Fenugreek samples were obtained from different localities of Pakistan including Karachi (8), Hyderabad (1), Azad Kashmir (1), Hub (1), Punjab (1), Sawabi (1), Sukkur (1) and Sajawal (1). Seed borne fungi were detected by the method of International Seed Testing Association (ISTA) in which 400 seeds were tested by using three methods namely Agar plate, Blotter and Deep freezing methods (Anonymous, 1993).

For standard blotter method, 200 untreated and 200 treated seeds (surface sterilization with 1 % sodium hypochlorite for 5 minutes) were placed on three layers of moistened blotter paper (20 seeds per petri plate). These petri plates were incubated for 8 days at 24±1^{o}C under 12 hours alternating cycles of light and darkness. In agar plate method, surface sterilized (with 1 % sodium hypochlorite) and non sterilized seeds were placed on Potato Dextrose Agar (PDA) supplemented with antibiotics (Penicillin @ 20,000 unit/L and streptomycin @ 200 mg/L) and incubated. In case of deep freezing method, sterilized and non sterilized seeds were placed on the moistened blotter paper and incubated for 24 h, of each 20^{o}C and -2^{o}C followed by 5 days of incubation at 24± 1^{o}C under 12 h, alternating cycle of artificial day light and darkness.

In all the three methods, fungi obtained on petri plates were identified on the basis of their color, mycelia texture, spores, pigmentation and by using different literature (Barnett and Hunter, 1998; Booth, 1971; Ellis, 1971; Gilman, 1950; MycoBank, 2013; Nelson *et al.,* 1983; Raper and Fennell, 1965). The Infection percentage of fungi was observed following the procedure given by Sokal and Rohlf (1995).

Table 1. Mycoflora recorded from Fenugreek seeds (Methi).

Name of fungi	Sterilized seed						Non-sterilized seed					
	Agar plate		Deep freezing		Blotter method		Agar plate		Blotter method		Deep freezing	
	NSI	MI ± SE	NSI	MI ± SE	NSI	MI ± SE	NSI	MI ± SE	NSI	MI ± SE	NSI	MI ± SE
Alternaria alternata (Fr.) Keissler	-	-	1	0.2 ± 0.774	-	-	2	0.93 ± 3.34	2	0.61 ± 1.599	-	-
Alternaria longipes Ellis & Everh. E.W. Mason	-	-	1	0.86 ± 3.35	-	-	-	-	1	1.66 ± 6.454	-	-
Alternaria tenuissima (Kunze) Wiltshire	1	0.06 ± 0.258	-	-	1	0.13 ± 0.5163	-	-	-	-	-	-
Aspergillus alutaceus Berk. & M.A. Curtis	1	0.13 ± 0.516	-	-	-	-	-	-	-	-	-	-
Aspergillus candidus Link ex Link	3	0.73 ± 1.869	2	0.53 ± 1.80	1	0.06 ± 0.258	1	0.313 ± 0.516	2	0.2 ± 0.560	-	-
Aspergillus carneus (Tiegh.). Blochwitz	3	0.26 ± 0.593	1	0.26 ± 1.03	-	-	3	0.6 ± 1.4505	2	0.6 ± 1.29	3	0.2 ± 0.4140
Aspergillus flavus Link	14	31.3 ± 52.07	15	24.2 ± 45.05	15	36.8 ± 45.81	15	44.3 ± 53.42	14	34.5 ± 5901	15	52.0 ± 48.0
Aspergillus fumigates Fresen.	1	0.33 ± 1.290	3	0.33 ± 0.72	4	1.2 ± 2.658	-	-	1	0.4 ± 1.549	5	1.53 ± 461
Aspergillus japonicas Saito	-	-	-	-	-	-	1	0.13 ± 0.516	-	-	1	0.06 ± 0.25
Aspergillus niger Van Tieghem	15	18.6 ± 27.34	14	7.6 ± 11.709	15	64.21 ± 574	15	32 ± 33.91	14	15 ± 11.08	15	74.73 ± 55.1
Aspergillus oryzae (Ahlburg) E. Cohn	3	0.2 ± 0.5936	1	0.2 ± 0.77	9	6.9 ± 16.35	5	1.6 ± 3.08	4	0.7 ± 1.38	10	5.2 ± 8.10
Aspergillus parasiticus Speare	1	0.2 ± 0.7745	2	0.2 ± 0.560	-	-	1	0.06 ± 0.25	1	0.26 ± 1.03	1	0.06 ± 0.25
Aspergillus sydowii (Bain. & Sart.) Thom & Church	1	0.6 ± 2.3237	2	0.33 ± 0.89	2	1.13 ± 4.120	1	0.2 ± 0.7745	2	0.4 ± 1.298	2	2.0 ± 5.29
Aspergillus terreus Thom	1	2.4 ± 0.385	5	3.2 ± 7.41	9	10.33 ± 161	6	44. ± 14.56	7	5±11.578	11	16.4 ± 22.06
Aspergillus ustus (Bain.) Thom & Church	-	-	-	-	-	-	1	0.46 ± 1.80	-	-	-	-
Aspergillus versicolor (Vuillemin) Tirabochi	-	-	1	0.06 ± 0.25	-	-	-	-	1	0.06 ± 0.25	1	0.2 ± 0.77
Aspergillus wentii Wehmer	3	0.33 ± 0.81	-	-	1	0.06 ± 0.25	3	0.8 ± 2.33	-	-	2	16.4 ± 22.0
Botrytis cinera Pers.	-	-	1	0.6 ± 0.258	2	0.53 ± 1.59	-	-	1	0.06 ± 0.258	-	-
Chaetomium funicola Cooke	1	1.2 ± 4.64	-	-	2	3.26 ± 12.1	1	0.66 ± 2.561	3	0.26 ± 0.617	1	0.06 ± 0.25
Chaetomium globosum Kunze ex Fr.	2	0.26 ± 0.798	-	-	-	-	1	0.13 ± 0.516	-	-	1	0.26 ± 1.032
Chaetomium indicum Corda	-	-	-	-	-	-	-	-	-	-	1	0.06 ± 0.258
Cladosporium herbarum (Pers.) Link	-	-	1	0.2 ± 0.77	1	0.13 ± 0.516	-	-	1	0.73 ± 2.84	-	-
Curvularia lunata (Wakker) Boedijn	-	-	-	-	1	0.06 ± 0.258	-	-	-	-	-	-
Drechslera hawaiiensis M.B. Ellis	-	-	-	-	-	-	-	-	-	-	1	0.06 ± 0.25
Fusarium oxysporum Schlecht	-	-	-	-	-	-	1	0.4 ± 1.549	-	-	-	-
Fusarium solani (Mart.) Sacc	-	-	-	-	-	-	1	0.26 ± 1.03	-	-	-	-
Humicola fuscoatra Traaen	2	0.46 ± 1.35	1	0.06 ± 0.25	1	0.26 ± 1.032	2	0.33 ± 0.899	1	0.2 ± 0.77	-	-
Mucor racemosus Fresen.	-	-	-	-	-	-	-	-	1	0.33 ± 1.29	-	-
Paecilomyces variotti Bain	-	-	-	-	-	-	-	-	1	0.13 ± 0.516	-	-
Penicillium bravicompectum Dierckx	2	5.4 ± 17.4	-	-	3	0.8 ± 2.33	2	1.2 ± 4.63	-	-	6	0.8 ± 1.78
Penicillium chrysogenum Thom.	2	0.4 ± 1.12	3	0.6 ± 1.40	7	1.6 ± 2.497	4	0.4 ± 0.736	6	1.06 ± 1.62	4	1.33 ± 2.5
Phoma sp. Sacc.	1	0.06 ± 0.25	-	-	-	-	-	-	-	-	-	-
Rhizopus sp. Ehrenb	2	2.0 ± 5.903	-	-	7	10.8 ± 14.82	3	7.0 ± 17.50	1	0.46 ± 1.807	8	24.4 ± 33.87

Where NSI = Number of samples infected; MI = Mean infection, ± SE = Standard error

RESULTS AND DISCUSSION

Thirty three fungal species belonging to 14 genera were found to be seed borne for this crop namely *Alternaria alternata* (Fr.) Keissler, *A. longipes* (Ellis & Everh.) E.W. Mason, *A. tenuissima* (Kunze) Wiltshire, *Aspergillus alutaceus* Berk. & M.A. Curtis, *A. candidus* Link ex Link, *A. carneus* (Tiegh.) Blochwitz, *A. flavus* Link, *A. fumigates* Fresen., *A. japonicas* Saito, *A. niger* Van Tieghem, *A. oryzae* (Ahlburg) E. Cohn, *A. parasiticus* Speare, *A. sydowii* (Bain. & Sart.) Thom & Church, *A. terreus* Thom, *A. ustus* (Bain.) Thom and Church, *A. versicolor* (Vuillemin) Tirabochi, *A. wentii* Wehmer, *Botytis cinera* Pers., *Chaetomium funicola* Cooke, *C. Indicum* Corda, *C. Globosum* Kunze ex Fr., *Cladosporium* herbarum (Pers.) Link, *Curvularia lunata* (Wakker) Boedijn, *Drechslera hawaiiensis* M.B. Ellis, *Fusarium oxysporum* Schlecht, *F. solani*, (Mart.) Sacc, *Humicola fuscoatra* Traaen, *Mucor racemosus* Fresen., *Paecilomyces variotii* Bain, *Penicillium brevicompactum* Dierckx., *P. chrysogenum* Thom., *Phoma* sp Sacc. and *Rhizopus* sp Ehrenb. Two species of *Aspergillus* like *A. flavus* and *A. niger* were isolated on most of the samples followed by *A. terreus* and *A. oryzae*. Of the 15 samples tested, only one seed sample was found to be infected with *Aspergillus alutaceus, A. ustus, Alternaria alternata, A. longipes, Chaetomium indicum, Curvularia lunata, Drechslera hawaiiensis, Fusarium oxysporum, F. solani, Mucor racemosus* and *P. variotii* (Table 1). Use of sodium hypochlorite (1%) significantly ($p<0.001$) reduced presence of saprophytic fungi on fenugreek. Main purpose of surface disinfection is to determining the presence of internal fungi on seeds. According to Sauer and Burroughs (1986), 1-5% solution of sodium hypochlorite killed the spores of *Aspergillus* spp present on seeds. Infection range of *A. flavus* was 24.2-36.8% on sterilized seeds and 34.5- 52.0% in non-sterilized seeds while *A. niger* has a range of 7.6- 64.2% in sterilized seeds and 15-74.73% in non-sterilized seeds.

Three methods used to determined the seed borne fungi of which, agar plate method showed presence of highest number of fungi compared to deep freezing and blotter method. Deep freezing method was suitable for the isolation of deep seated fungi like *Penicillium, Fusarium* and *Drechslera* sp. Similar results were recorded by Niaz and Dawar (2009) on maize seeds. Rahim *et al.* (2010) tested 21 samples of lentil seeds and observed the highest number of pathogenic fungi like *M. phaseolina, Fusarium aquaeductuum, F. oxysporum* from deep freezing method. Of the 15 sample used for the detection of seed borne mycoflora, 100% seeds samples were infected by *A. flavus* on surface sterilized seed while with 1% sodium hypochlorite significantly ($p<0.001$) minimize the incidence of *Aspergillus* spp (Table 1). Blotter and agar plate methods are simple and inexpensive methods for detection of seed borne fungi on basis of their sporulation. However, blotter method does not always suitable for mycelia growth, sporulation and presence of pathogenic symptoms. Agar plate method was best for these characteristics (Rao *et al.,* 2006; Nasir, 2003).

From the consumption point of view, saprophytic and pathogenic fungi present on seeds produced mycotoxins that cause the health hazards in human and animals. Fenugreek seeds are naturally contaminated with number of fungi which could be destructive under field and storage conditions.

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