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Management of seed mycoflora of fenugreek (*Trigonella foenum-graecum* Linn.*)* by fungicides, antibiotics and sulphadrugs *in vitro*.

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ABSTRACT: *Seed borne fungi of fenugreek (Trigonella foenum-graecum Linn.) act as basic source for pathogenic diseases of this crop, thus affect the cultivation of crop and causing considerable damage of the leafy vegetable and seeds. Five predominant fungi (Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata and Fusarium moniliforme) were encountered during study on fenugreek seeds as the major contaminants due to lack of proper post harvest operations which may cause substantial yield losses. To overcome the contaminations of fenugreek seeds during storage and transport, Five fungicides viz. Bavistin, Blitox, Dithane M-45, Thiram and Vitavax, two antibiotics viz. Griseofulvin and Streptocycline and one sulphadrug viz. Sulphamethoxazole were tested in vitro for their inhibitory effect on the radial growth of above five dominant seed mycoflora of fenugreek. It was observed that the radial growth of seed mycoflora was highly affected by Vitavax, Bavistin and Thiram while other tested fungicides, antibiotics and sulphadrugs were moderately inhibited the radial growth of selected seed mycoflora. Based on the findings, seed treatment of Vitavax, Bavistin and Thiram may be recommended for management of seed mycoflora of fenugreek before storage and sowing.*

Key words*: Fenugreek, seed mycoflora, in vitro inhibition, fungicides, antibiotics and sulphadrugs.*

I. INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* Linn. belongs to family Fabaceae. The crop is also known as Methi. The seeds of fenugreek are widely used as spices, condiments and medicines. It is one of the most geographically widespread spices having excellent medicinal properties. The seeds of fenugreek are colonized by a number of fungi during post harvest and under storage conditions. It depletes the marketing value of seeds by changing their physical characteristics and reduces their edible quality by altering the biochemical nutrients of stored seeds. Moreover, the seed mycoflora acts as potential source for a number of seed-borne diseases of this crop (Singh and Singh, 2022). Several fungi colonizing on seeds have been reported to cause as internal and external seed borne diseases (Trigo et al., 1996, Giridhar and Reddy,1999; EI-Nagerabi,2002; Mohamed and Ghoneem, 2002; Gowdar et al., 2007 and Kulshrestha et al., 2014). Therefore, the present study was conducted in vitro to find out the efficacy of fungicides, antibiotics and sulphadrugs in inhibition of radial growth of the pathogenic fungi in order to control the seed borne diseases in field.

II. MATERIAL AND METHODS

Seeds samples of Fenugreek (*Trigonella foenum-graecum* Linn.) were collected from local market of Bareilly and harvested seeds were sampled at the time of harvesting from experimental fields in the month of March and experimental work was conducted in Phytopathology lab, Botany department, Bareilly College, Bareilly, to find out appropriate management and control procedure of seed mycoflora of fenugreek. Five selected fungicides, viz. Thiram, Dithane-M-45, Bavistin, Blitox and Vitavax, two antibiotics viz. Griseofulvin and Streptocycline and one sulphadrugs i.e. Sulphamethoxazole were assayed *in vitro* for their inhibitory effect on radial growth of selected fungi. Five concentrations (50, 100, 250, 500 and 1000 ppm) were employed. The radial growth of pathogen was measured on potato dextrose agar medium amended with said concentrations of test compounds (Nene, 1971). Only 20 ml of the medium was poured in each Petri-dish. Inoculation was done with 5mm disc cut from 6 day old culture of test pathogen. Proper replicates and control were prepared for each pathogen and each treatment, separately *in vitro*. The Petri-dishes were incubated at 25 ± 2^0C. Percentage inhibition was calculated after 6 days using the formula of Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where

I = Percent inhibition

C = Radial growth in control set (mm)

T = Radial growth in treated set (mm)

The efficacy of tested therapeutants was compared by calculating the amount of material required for the 50% inhibition in radial growth of fungi (ED-50).

III. RESULTS AND DISCUSSION

Five pre-dominant fungi (*Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata and Fusarium moniliforme*) of fenugreek isolated from seeds were selected to evaluate the inhibitory effect of fungicides, antibiotics and sulphadrug on these fungi. Radial growths of the tested seed borne fungi were significantly decreases in different concentrations of therapeutants. This study can be helped for minimizing seed borne disease in fields growing fenugreek crops. Among seed borne pathogenic fungi were studied due to lack of proper management of post harvested seeds preservation techniques. A large percentage of annual yields get damaged by fungal action. Fenugreek seeds could be subjected for contamination with fungi mainly during storage and transport. Improving the conditions of fenugreek under processing, storage and transport and continuous mycological. However, it shows the fungal efficiency in developing association with broad spectrum of fenugreek seeds.

The detailed results are shown in Table-1-3. All the test therapeutants exhibited positive inhibitory effect on selected mycoflora on seeds of fenugreek. *Aspergillus niger, A. flavus* and *A.fumigatus* have been recorded as most dominant fungi. During the predominant contaminants seeds are harvested of fenugreek. Then storage of fenugreek seeds with mycoflora are probably regarded as commensally residents on the plant that survived may be responsible for spoilage and degradation of spice and vegetable quality by producing enzymes, mycotoxins and other secondary metabolites. The range of inhibition markedly varied with different therapeutants and concentrations. Maximum inhibition (100%) in radial growth of *Alternaria alternata* and *Curvularia lunata* was exhibited by Vitavax, (250ppm and 500ppm), followed by *Aspergillus niger,and A. flavus* caused by Bavistin (250ppm and 500ppm) and *Fusarium moniliforme* by Thiram (500pmm).Therapeutic agents like fungicides, antibiotics and sulphadrugs are heterogeneous group of organic compounds. They prevent or delay spore germination of pathogen when present on seed surface resulting in failure of infection. They act directly on the pathogen and control the fungal diseases by the inhibition of protein synthesis, nucleic acid metabolism and cell wall formation in pathogens (Dimand and Horsfall,1959).Biochemically the fungicides directly inhibit DNA, RNA and protein synthesis of pathogens (Singh, 1983). Several researchers have reported the positive efficacy of fungicides in controlling the fungal pathogens of various crops (Sagar and Hegde, 2006; Purshothaman, 2007; and Idries *et al.,* 2008). Out of two tested antibiotics i.e. Griseofulvin and Streptocycline, Griseofulvin was most effective showing maximum antimycostatic properties. It inhibited the radial growth of all the test pathogens. Antibiotics generally exert adverse effect on the metabolism related to development process of fungi. Most of the antibiotics affect DNA synthesis and thereby hamper the replication system of fungal cells. Effective control of pathogenic fungi by antibiotics has been reported by Singh *et al*., 2005 and Karande *et al*., 2007. Sulphadrugs are comparatively less effective against the test pathogens. All the five concentrations of Sulphamethoxazole had little effect on the radial growth of test pathogen *in vitro* in present investigation. Similar findings are reported by Grover and Joshi, (1962) and Kumar, (1989).

Table 1: Inhibitory effect of fungicides, antibiotics and sulphadrugs on radial growth of seed mycoflora of fenugreek (Trigonella foenum-graecum Linn.).

Treatments	Inhibition in radial growth (mm) (%)											
	Alternaria alternate						*Aspergillus flavus*					
	50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	ED 50 level	50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	ED 50 level

Thiram	83.3	93.3	96.6	98.6	100.0	>50	43.3	51.6	60.0	96.6	100.0	57.73
Dithane M-45	51.1	66.2	69.8	73.3	88.8	>50	50.0	66.6	71.4	79.0	89.8	>50.00
Bavistin	62.5	78.5	85.0	100.0	100.0	>50	62.9	78.4	87.7	97.7	100.0	>50.00
Blitox	32.2	41.3	44.0	69.0	78.0	284.08	56.6	66.6	76.0	85.5	95.5	>50.00
Vitavax	87.0	96.6	100.0	100.0	100.0	>50	47.9	56.8	65.3	75.0	93.2	52.19
Griseofulvin	41.3	58.3	85.00	88.0	95.0	60.53	42.3	54.5	69.0	88.8	100.0	59.10
Sulphamethoxazole	41.6	50.0	69.0	83.3	90.0	60.09	55.8	63.6	77.3	81.1	86.1	>50.00
Streptocycline	36.5	48.8	54.5	63.6	69.0	102.45	10.0	15.5	22.2	33.3	53.8	750.7
	Treatment		Concentration		Treatment × Concentration		Treatment		Concentration		Treatment × Concentration	
S.Em.±	0.55		0.44		1.24		0.56		0.44		1.26	
CD at 5%	1.56		1.24		3.50		1.59		1.25		3.56	
F-value	*		*		*		*		*		*	

Table 2: Inhibitory effect of fungicides, antibiotics and sulpha drugs on radial growth of seed mycoflora of fenugreek (Trigonella foenum graecum Linn.).

Treatments	**Inhibition in radial growth (mm) (%)**											
	Aspergillus niger						***Curvularia lunata***					
	50 ppm	**100 ppm**	**250 ppm**	**500 ppm**	**1000 ppm**	**ED 50 level**	**50 ppm**	**100 ppm**	**250 ppm**	**500 ppm**	**1000 ppm**	**ED 50 level**
Thiram	62.2	76.9	92.3	100.0	100.0	>50.00	41.1	55.8	83.3	91.6	100.0	60.82
Dithane M-45	54.0	68.9	77.3	92.0	100.0	>50.00	25.0	32.1	55.8	69.1	85.2	115.76
Bavistin	85.7	93.0	100.0	100.0	100.0	>50.00	63.6	76.7	83.3	95.5	100.0	>50
Blitox	40.0	60.5	76.7	88.3	100.0	62.50	37.5	43.1	63.6	72.7	90.9	116.0
Vitavax	52.3	68.2	88.3	100.0	100.0	>50.00	72.7	80.3	92.2	100.0	100.0	>50.00
Griseofulvin	12.6	26.3	32.1	35.7	41.0	<1000	38.4	52.2	69.2	85.5	97.7	65.10
Sulphamethoxazole	10.5	16.2	22.8	28.1	35.7	<1000	16.5	21.3	28.5	35.0	44.5	<1000
Streptocycline	33.3	64.0	80.0	91.6	100.0	75.07	52.8	71.4	89.5	100.0	100.0	>50.00
	Treatment		Concentration		Treatment × Concentration		Treatment		Concentration		Treatment × Concentration	
S.Em.±	0.53		0.42		1.19		0.53		0.42		1.20	
CD at 5%	1.50		1.18		3.36		1.51		1.19		3.39	
F-value	*		*		*		*		*		*	

Table 3: Inhibitory effect of fungicides, antibiotics and sulpha drugs on radial growth of seed mycoflora of fenugreek (*Trigonella foenum graecum* Linn.).

Treatments	**Inhibition in radial growth (mm) (%)**					
	Fusarium moniliforme					
	50 ppm	**100 ppm**	**250 ppm**	**500 ppm**	**1000 ppm**	**ED 50 level**

Thiram	60.0	71.0	88.0	100.0	100.0	>50.00
Dithane M-45	31.8	55.4	63.6	72.66	79.5	78.61
Bavistin	58.0	73.3	84.1	91.8	100.0	>50.00
Blitox	48.3	60.0	86.0	91.5	100.0	51.75
Vitavax	56.0	65.5	82.4	100.0	100.0	>50.00
Griseofulvin	40.6	78.1	82.0	90.0	100.0	60.32
Sulphamethoxazole	14.4	16.3	28.1	33.3	48.8	<1000
Streptocycline	45.5	67.7	74.1	83.8	100.0	54.94
	Treatment		Concentration		Treatment × Concentration	
S.Em.±	0.59		0.46		1.32	
CD at 5%	1.66		1.31		3.72	
F-value	*		*		*	

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