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SEED MYCOFLORA OF FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) AND ITS MANAGEMENT

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Abstract

Seeds of fenugreek (*Trigonella foenum-graecum* L.) are infected during storage condition, which affect the germination percentage. Seeds were evaluated using blotter test and agar plate method to determine the fungal association. Seven fungal species were isolated from the internal and external seed surfaces of fenugreek, viz., *Aspergillus flavus, Aspergillus niger, Cladosporium, Penicillium italicum, Mucor, Fusarium solani and Rhizopus stolonifer*. Out of five fungitoxicants used, Carbendazim and Indofil M-45 were found effective to control seed mycoflora of fenugreek. Treated seeds showed better germination percentage as well as root and shot length than control.

Key words : Management, mycoflora, *Trigonella foenum-graecum.*

Introduction

Fenugreek (*Trigonella foenum-graecum* L., Family: Fabaceae) is an annual plant and is cultivated worldwide as a semiarid crop. Fenugreek is used as a herb, spice, and vegetable. Its seeds and leaves are common ingredients in dishes from the Indian subcontinent. Fenugreek seeds are a rich source of protein, dietary fiber, B vitamins, and dietary minerals, particularly manganese and iron. Fenugreek dietary supplements are manufactured from powdered seeds into capsules, loose powders, teas, and liquid extracts in many countries. Although fenugreek is used in traditional medicine to promote digestion, induce labour, increase breast milk supply in nursing mothers, and reduce blood sugar levels in diabetics, there is insufficient scientific evidence that fenugreek has any of these properties (Bazzano *et al.,* 2016). Seeds of fenugreek spice have medicinal properties such as hypocholesterolemic, lactation aid, antibacterial, gastric stimulant, for anorexia, antidiabetic agent, galactogogue, hepatoprotective effect and anticancer. These beneficial physiological effects including the antidiabetic and hypocholesterolemic effects of fenugreek are mainly attributable to the intrinsic dietary fiber constituent which have promising nutraceutical value (Srinivasan, 2006).

Seeds play a vital role for the healthy production of crop, which are carriers of some important seed-borne diseases which cause considerable losses in yield. Studies on seed mycoflora have greatly increased in recent times in view of their importance in toxin production, seed deteriorating agents and disease carriers (Tamuli *et al.,* 2007). Seed mycoflora also affect germination (Tamuli and Boruah, 2001). Since this valuable crop is propagated by seed, an investigation was carried out to study the seed mycoflora and its management.

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Materials and Methods

Collection of Seeds

Seeds of Fenugreek were collected from different local markets of Tezpur. They were mixed and then preserved in a room temperature during the studies.

Seed Viability Test

Seed Viability Test was done by moisten a sheet of paper towel and uniformly damp it. Then placing ten seeds in a row among the damp soil we insert it inside a plastic

bag and seal it. Then place the plastic in room temperature. After two days we can observe the sprouting. Check seed packet for average germination times for particular seed, but generally 3-7days should be enough time for the test. After 7 days we can count how many seeds are sprouted.

Preparation of Germination

Five fungicides namely- Captan, Carbendazim, Indofil M-45, Ridomil Gold and Valxtra were collected from local market. Those five fungicides are treated with distilled water at a concentration of 0.03% in five beakers and one beaker is left with untreated distilled water only. Total 200 seeds were treated with fungicide solutions and 40 seeds treated with sterilized distilled water served as a control group. Then, petridishes were assembled by placing four in each required group which altogether made 24 petridishes. Finally, the treated and untreated seeds were plated on sterilised moist blotting papers. The rate of germination was recorded and studied after 5 days of plating the germination percentage was recorded following ISTA rules.

$$\text{Germination}\,\% = \frac{\text{Number of seeds germinated}}{\text{Number of seeds tested}} \times 100$$

Isolation and Identification of Seed Borne Fungi

The surface mycoflora of selected seeds were isolated by blotter paper test as well as agar plate method as recommended by International Seed Testing Association ISTA.

Agar Plate Method

In Northern Ireland, Musket first used this method for seed health management 28. In this method, pre sterilized petriplates were poured with 15 mL of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Incubation and other details of the study were same as described for blotter test method. The fungi occurring on seeds plated on moist blotter paper and agar plate were preliminary identified on the basis of sporulation characters. Detail examination of fungal characters was done by using compound microscope and identification was confirmed. Total percentage of fungal incidence of fungi was calculated by using the following formula:

$$\text{Frequency} = \frac{\text{Total no.of seeds in which particular fungus appeared}}{\text{Total no.of seeds studied}} \times 100$$

Application of Fungicides in Controlling the Seed Mycoflora

Five fungicides are taken viz. Captan, Carbendazim, Indofil M-45, Ridomil Gold and Valxtra in a concentration of 0.03% mixed with distilled water in five individual beaker. Ten untreated seeds were soaked in distilled water and ten treated seeds are soaked in each five beaker treated with different fungicides and treated them for 30 minutes.

Detection of Seed Mycoflora

The treated ten seeds were equidistantly placed aseptically on each six petriplates. The petridish were incubated at 25+ 1°C. On the fourth day of incubation, observations were made for determining various fungal growths. The identification is done with the help of microscopes and slide is being prepared for the further confirmation of seed borne fungi. Fungicides are used at the very beginning of the germination process.

Identification of Seed Borne Fungi

Incubated seeds were observed under light microscope at 10x and 40x magnification. The incidence of seed borne fungi was detected by observing their growth characters on the incubated seeds. Temporary slides were prepared from the fungal colony and observed under compound microscope.

Results and Discussion

The Fenugreek seeds showed a variation in germination percentage with treated and control seeds. Control seeds germinate only 70%. With respect to the treated seeds (5 fungicides), one fungicide namely Carbendazim showed the highest germination percentage of fenugreek seed as it showed 98% of germination. Similarly, Ridomil Gold showed 97.5%, Valxtra showed 96%, Indofil M-45 showed 95%, Captan showed 90% of germination percentage respectively.

The root and shoot length is mostly based on the quality of germination percentage. After fungicidal treatment, the growth of root and shoot length of seeds have increased as compared with the normal untreated seeds. Carbendazim holds the highest average root and shoot length whereas Control holds the least average root and shoot length. Nene and Thapliyal (1971) reported besides disease control, beneficial side effects of Carbendazim like stimulation of growth, flowering and yield of plants as well as reduction of mite population on the treated host plants. Carbendazim shows a broad spectrum of fungitoxic activity being effective against actinomycetes, deuteromycetes and various basidiomycetes. Tamuli (2000) found carbendazim as the most effective fungicide to control seed mycoflora of mustard. Kaurow (1986) also found better germination percentage of rice by treating with carbendazim. Studies

Table 1: Effects of fungicides on seed germination percentage and the root/shoot length of Fenugreek.

Treatment (0.03%)	Germination (%)	Root Length (Avg.) (cm)	Shoot Length (Avg.) (cm)
Captan	90	1.6	4.77
Carbendazim	98	1.79	6
Indofil M-45	95	0.44	4.38
Ridomil Gold	97.5	1.39	4.66
Valxtra	96	0.49	2.48
Control	70	0.34	1

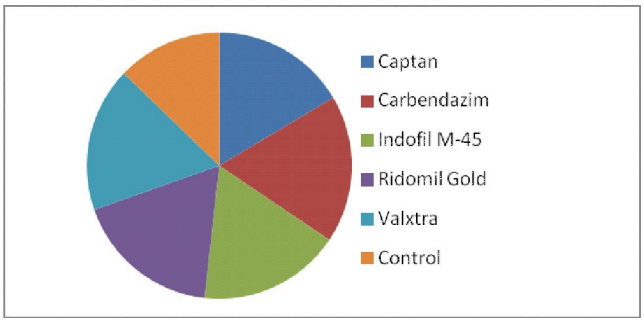

Fig. 1: Effects of fungicide on seed germination.

Table 2: Efficacy of different fungicides in controlling the seed mycoflora of Fenugreek (Methi).

Fungicides (0.03)	Percentage of seed showing incidence after the treatment with fungicides						
	Af	An	Cl	Pi	Ms	Fs	Rs
Captan	3	4	-	-	8	11	8
Carbendazim	-	3	-	-	-	-	-
Indofil M-45	-	-	-	-	-	10	6
Ridomil Gold	-	12	-	10	15	-	-
Valxtra	15	20	-	-	-	-	-
Control	42	44	38	48	50	55	51

Key: Af= *A. flavus* , An= *A. niger*, Cl= *Cladosporium*, Pi= *Penicillium italicum*, Ms= *Mucor*, Fs= *F. solani* and Rs= *R. stolonifer*.

Fig. 3: Germination study.

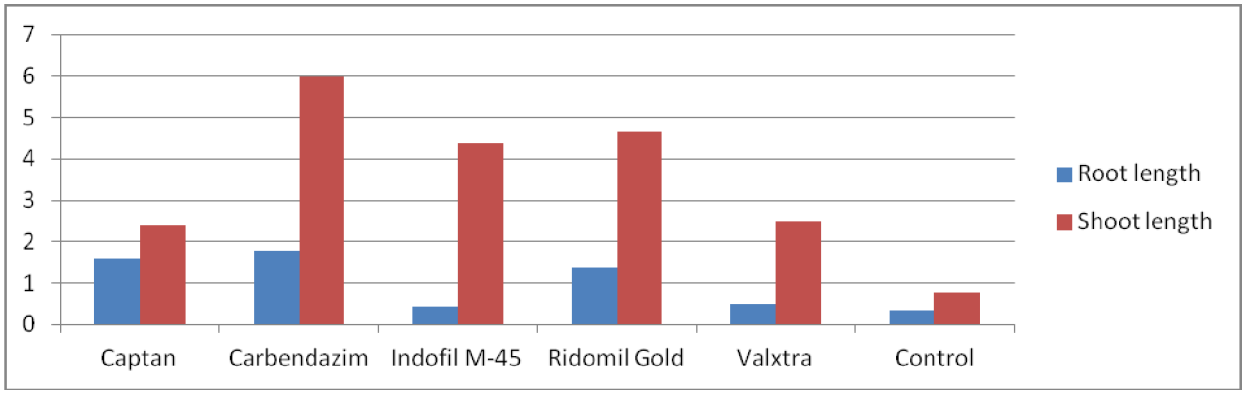

Fig. 2: Effects of fungicides on root/shoot length of fenugreek.

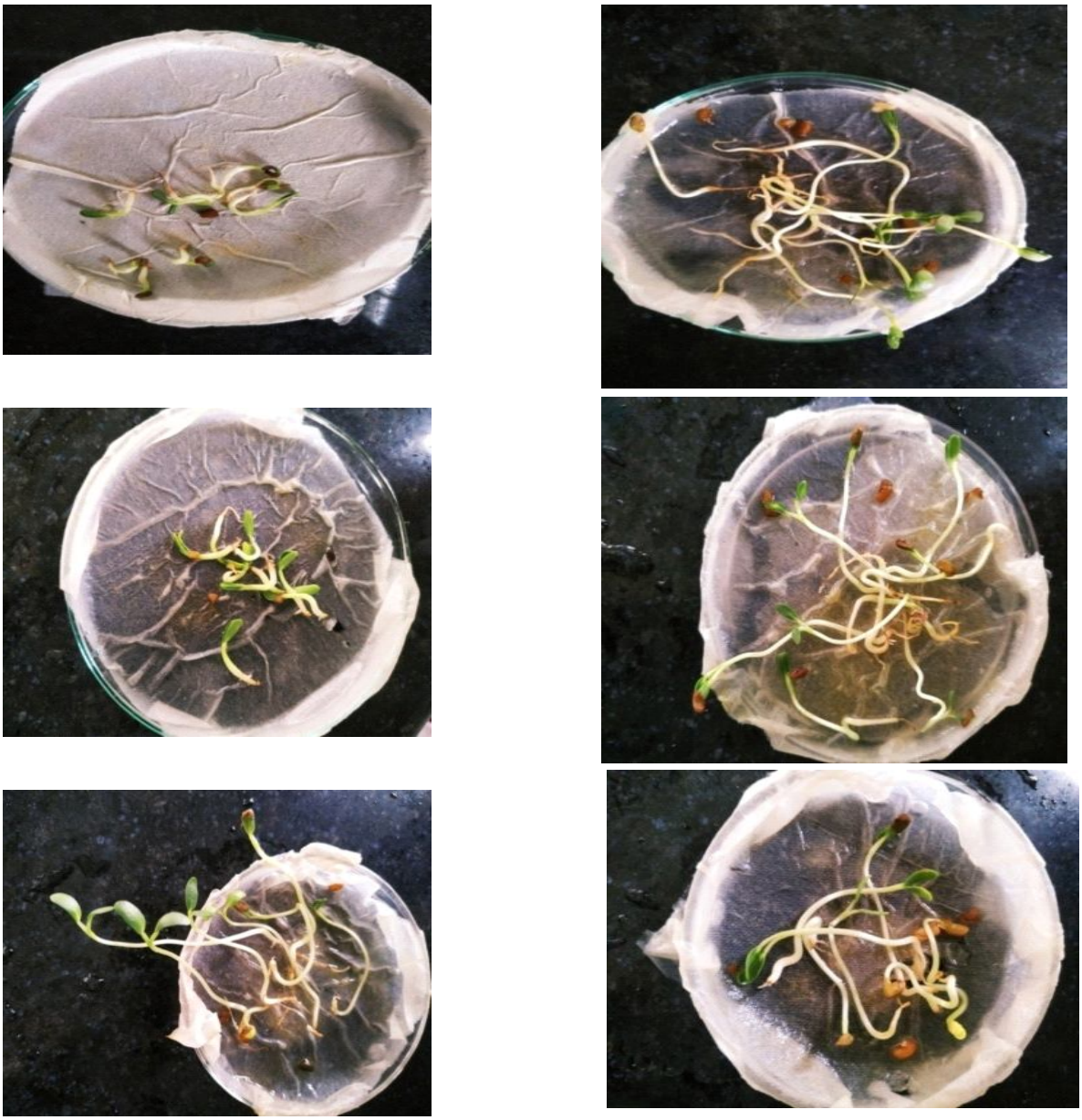

Fig. 4: Root/shoot length.

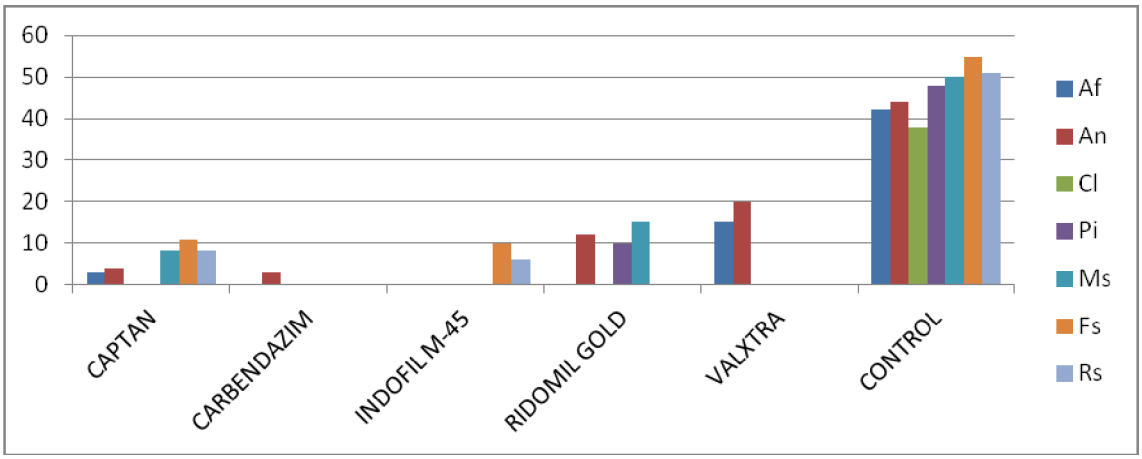

Fig. 5: Efficacy of different fungicides in controlling the seed mycoflora of Fenugreek.

showed that the maximum affected seeds in ninth day were Control. Out of 10 seeds, 5 seeds get affected. The comparative study shows that amongst those five fungicides the maximum treated seed is affected by Captan- 4 out of 10, Valxtra – 3 out of 10, Carbendazim, indofil m 45 and ridomil gold is affected with only 1 seed out of 10. Results show that fungicides not only help in decreasing the growth of the pathogen but simultaneously it also helps in growth regulation. Total 7 fungi have been identified. They are *Aspergillus flavus, Aspergillus niger, Cladosporium, Penicillium italicum, Mucor, Fusarium solani* and *Rhizopus stolonifer*. Singh C.P, Mishra U.S, Nishant Mishra (2009) also reported *Aspergillus flavus*, *A. niger* on Fenugreek seeds. The findings of our observation were found similar with earlier workers regarding seed mycoflora, germination percentage as well as root/shoot length. It is, therefore, suggested that fenugreek seeds should be treated with carbendazim before showing to achieve better germination and vigorous growth.

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