Common seed borne fungi of fenugreek (*Trigonella foenum graecum* L.)

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ABSTRACT

Fungi are most destructive pathogen for the crop plants as compared to other pathogenic microorganisms. Fungi infects the crop plants during the growth, developmental condition and after harvesting crops. Many microorganisms growing on the stored seed material and quality of seed material decreases. Seeds loses their viability. Fungal pathogen spores are attached externally as well as internally to fenugreek seeds. Infected damaged seeds could not germinates and yield decreases significantly.

In the present study fenugreek vegetable seeds have been selected in this study. Fenugreek seeds used for the detection of seed mycoflora, sporulation, dry mycelial weight and sporulation (ISTA, 1966). Many fungi were isolated from the fenugreek seeds. Out of these very common seed borne fungi were selected for further study. All these selected fungi were brought into pure culture and used for further investigation. *Aspergillus niger*, *Fusarium oxysporum*, *Curvularia lunata* and *Drechslera longirostrata* were selected for the further study. The leaf extract of selected medicinal plants were analyzed against the selected parameters of seed borne fungi of fenugreek. Parameters selected like seed mycoflora, growth in the form of dry mycelial weight, sporulation, spore germination have been carried out. It was found that *Semecarpus anacardium, Solanum xanthocarpum and Abrus precatorius* was found more inhibitory effect on seed mycoflora, spore germination, dry mycelial weight (DMW) and sporulation as compared to other selected medicinal plants (Mashooda Begam and Lokesh 2008). Similar study was carried out by Pandey (1982) and Muzumdar et al (2004).

Key Words: Seed mycoflora, Spore germination, Dry mycelial weight, fenugreek, leaf extract etc.

INTRODUCTION:

Agriculture is a main business in India. Economy of India is directly related with agriculture and its related businesses. India is having the highest population of human in the whole world. Natural resources and growing demand increase day by day. So we need to find out some alternative pathway to fulfill increasing demand of food materials and others. Plants are used for the purpose of food, clothes, shelter, medicinal, fertilizer, antimicrobial agent and many other purposes. India is a one of mega biodiversity center of world having more than 45000 plant species. These plant resources may be utilized for agriculture purposes because most of plant having specific antimicrobial activity.

India have a rich biodiversity center of world. If wild medicinal plants biomass utilized for the welfare in agriculture, it improves crop yield eco-friendly without any type of pollution. Every year large amount of plant biomass produced by plants which may be used in the agriculture as a pesticides, insecticides, Biofertilizers, antimicrobial activity (Musyimi, 2008). It may be utilized for agricultural purpose because most of plants are easily available,

cheaper in cost and having anti-microbial biochemicals. By using chemical biofertilizer, biopesticides causes imbalance in biodiversity. If we used plant based product as fertilizer, pesticide and seed dressing material which do not causes any hazardous effect on plants and soil micro flora. By considering above importance this study is undertaken. Fenugreek is important and the nutritious vegetable commonly grown in our area throughout the year.

In the present study seeds of fenugreek have been selected for this study. Fenugreek seeds were collected from different market places and all the seeds samples were mixed together. Combined seeds were used for detection of seed mycoflora and germination of seeds. Isolation of many fungi have been carried out by the paper blotter method (ISTA, 1966). Many fungi were isolated from fenugreek seeds, out of which four very common fungi have been chosen and these were brought into pure cultures (Vyas, 1988). Out of these fungi Aspergillus *niger* and *Fusarium oxysporum* were very common than the *Curvularia lunata* and Drechslera *longirostrata.* Effect of very common ten medicinal plants leaf extract have been tested against seed mycoflora, spore germination percentage, dry mycelia weight and sporulation have been studied.. It is clear from the study that *Solanum xanthocarpum, Semecarpus anacardium, Dioscorea bulbifera and Aegle marmelos* were found more inhibitory for spore germination, growth and sporulation of seed borne fungi of fenugreek. Similar study in Okra carried out by Mashooda Begam and Lokesh (2008).

MATERIALS AND METHOD:

1. Collection of plant materials:

Wild selected ten medicinal plants leaves were collected, surface sterilized and dried in oven. After drying ten medicinal plants leaves were ground with the help of blender. Dry leaf powder preserved separately in the water proof containers. 5% aq. leaf extract was found more effective than the other concentrations for control of seed mycoflora of vegetable seeds fenugreek. Hence 5% leaf extract used for further study.

2. Effect of leaf extract on incidence of seed mycoflora:

Aqueous extract of different percentage of the selected leaf biomass was prepared by dissolving 1, 2, 3..... gms. of leaf powder in 100ml sterile distilled water. The leaf extract of selected medicinal plants were tested against seed mycoflora. The leaf extract of effective percentage was determined. Five percent (5%) leaf extract was found more effective than the others. Therefore five percent extract of medicinal plant leaf extract were used for further studied.

3) Detection of seed mycoflora:

a) Moist blotter plate method:

Seed borne fungi of fenugreek was determined with the help of seed blotter test method Agarwal and Sorbhoy (1978) and Amer Habib et al (2007). Blotting paper of Petridish size was cut down and soaked in the sterile distilled water. Petridish were presterilized in autoclave. 10 Seeds of Fenugreek were placed equal distance on moist blotter paper. More than 400 seeds were tested for each treatment. Blotter seeds plates were incubated at room temperature for seven days. After incubation preliminary Identification and confirmation of different fungi on seeds were carried out (Nisha Moshahary et al, 2020). Slides of seed borne fungi were prepared and observed under the microscope (Mukadam, D.S., 1997). More than twelve fungi were isolated, out of these four very common fungi were brought in to the pure culture and further used.

2. Identification of seed borne fungi:

Identification of fungi were carried out with help of reproductive characters. Vegetative body of fungus is similar in all fungi but asexual and sexual structures are unique, hence identification on the basis of asexual spores or sexual spores or fruiting body of fungi. Detail structure of fungi were carried out with the help of microscope, recent manuals, internet and expert person (Subramanian, 1971; Jha, 1993 and Mukadam, 1997). Identified seed borne fungi were brought in the pure culture and preserved for further use.

3. Preparation of spore suspension:

Pure cultures of selected seed borne fungi were incubated for one week on the PDA medium in the culture room and after complete incubation period add 10 ml of sterile distilled water in the sporulated test tube. The slants were shaken and filtered with the help of muslin cloth. Filtrates were conserved and used as a spore suspension.

4. Study of spore germination:

In 100 ml of sterilized borosil conical flasks were taken and in this added with 25 ml of GN medium and 2 ml of 5% leaf extracts poured separately in each flask. In these flasks were added separately with 2ml spore suspension of selected seed borne fungi. Pure cultures were maintained on PDA slants for seven days. Inoculated flasks were incubated in the culture rooms. After the incubation selected seed borne fungi spore germination tested and effect of leaf extract on spore germination noted down in the table no. 2. The flask with 25 ml of GN medium and no supplements of plant extract were considered as a control.

5. Study of sporulation and dry mycelial weight of seed borne fungi:

During the present studies some common and dominant seed borne fungi of vegetable fenugreek like *Aspergillus niger*, *Curvularia lunata*, *Drechslera longirostrata and Fusarium oxysporum* were grown separately in GN medium. Each flask added separately with 2 ml of 5% leaf extract of selected medicinal plants. After incubation of seven days each flask were separately filtered through pre-weighed Whatman filter paper No.1. The filter papers with mycelial mat were put into the oven for drying for twenty four hours at sixty degree centigrade. After incubation reweighed the filter paper with mycelial mat. Growth of selected seed borne fungi were calculated with the help of subtracting initial weight from final weight of filter paper.

Table no. 1: Effect of leaf biomass on seed mycoflora and seed germination of fenugreek.

Sr. No.	**Extract of leaf biomass**	**Incidence of seed mycoflora (%)**	**Seed germination**		
			%	RL (mm)	SL (mm)
01.	*Abrus precatorius* L.	46	82	32	23
02.	*Aegle marmelos* (L.) Corr.	53	83	35	26
03.	*Balanites aegyptica* Delile.	52	82	31	24
04.	*Datura metel* L.	58	72	30	18
05.	*Dioscorea bulbifera* L.	62	68	23	20
06.	*Helicteres isora* L.	48	62	20	24
07.	*Sapindus laurifolius* Vahl.	45	83	35	25
08.	*Semecarpus anacardium* L.	38	88	38	28
09.	*Solanum xanthocarpum* Schra.	40	84	22	23
10.	*Vitex negundo* L.	70	60	20	18
	Control	90	70	36	22

RL= Root length, **SL**= Shoot length

It is clear from the results presented in table – 1 that the leaf extracts of all the test medicinal plants were found to be more or less inhibitory for the incidence of seed mycoflora. The leaf extracts of *Semecarpus anacardium* was found to be more inhibitory and leaf extract of *Vitex negundo* was found to be very less inhibitory for the incidence of seed mycoflora of fenugreek as compared to the leaf extracts of other test medicinal plants. Highest seed germination was found in the leaf extract of *Semecarpus anacardium* and very low seed germination in *Helicteres isora.* Root and shoot length of fenugreek was found more in the leaf extract of *Semecarpus anacardium* as compared to other selected medicinal plant leaf extract. Similar study was carried out by Nisha Moshahary et al, 2020 and Singh et al, 2009.

Chart no. 1: **Showing seed mycoflora %, seed germination % of fenugreek seeds**

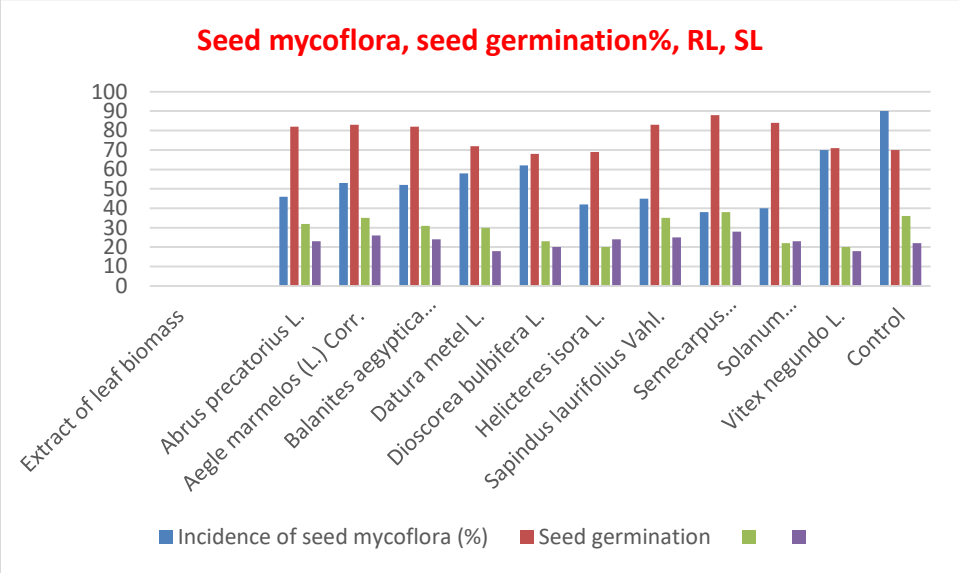
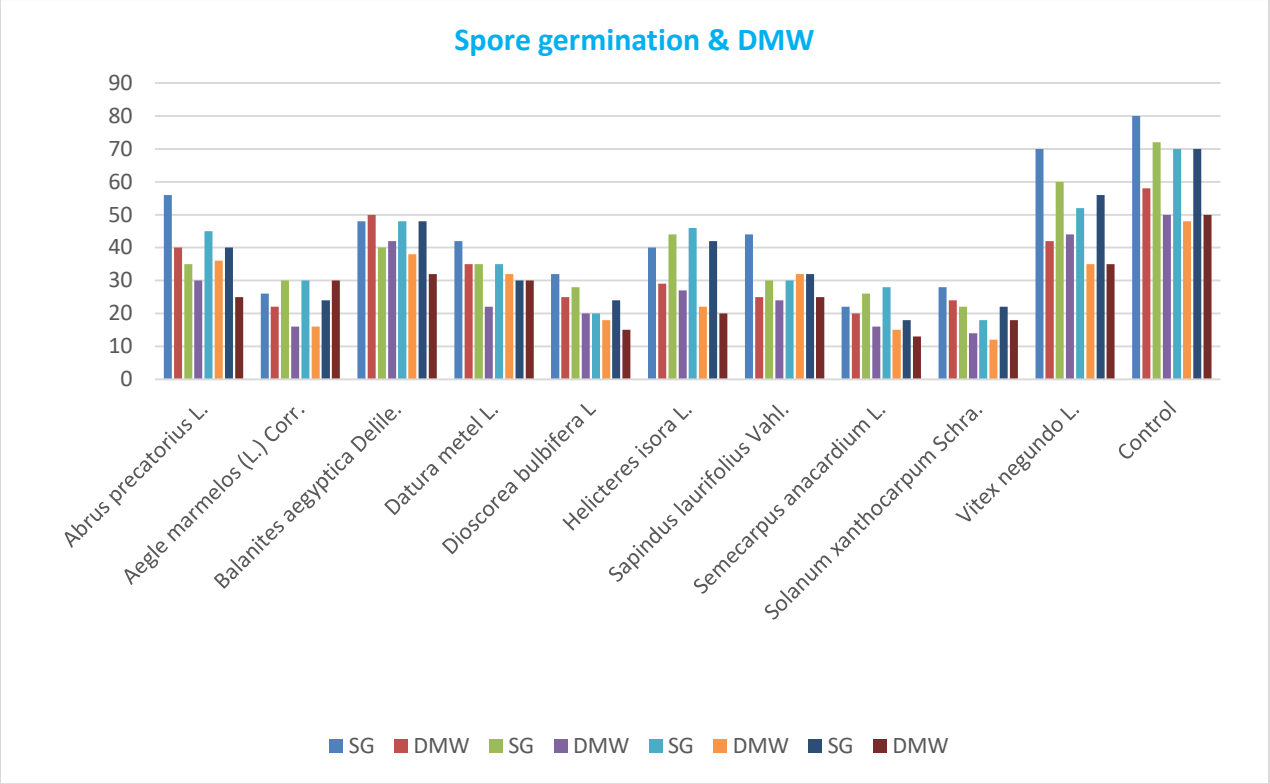


Table-2: Effect of leaf biomass of selected medicinal plants on spore germination, growth and sporulation of *Aspergillus niger*, *Curvularia lunata*, *Drechslera longirostrata* and *Fusarium oxysporum*.

Sr No	Medicinal plants	*Aspergillus niger*			*Curvularia lunata*			*Drechslera longirostrata*			*Fusarium oxysporum*		
		SG	DMW	SPN	SG	DMW	SPN	SG	DMW	SPN	SG	DMW	SPN
1	***Abrus precatorius* L.**	56	40	++	35	30	++	45	36	++	40	25	++
2	***Aegle marmelos* (L.) Corr.**	26	22	++	30	16	+	30	16	+	24	30	++
3	***Balanites aegyptica* Delile.**	48	50	+++	40	42	+++	48	38	+++	48	32	+++
4	***Datura metel* L.**	42	35	++	35	22	++	35	32	++	30	30	++
5	***Dioscorea bulbifera* L**	32	25	++	28	20	++	20	18	++	24	15	++
6	***Helicteres isora* L.**	40	29	+++	44	27	+++	46	22	+++	42	20	++
7	***Sapindus laurifolius* Vahl.**	44	25	+++	30	24	+++	30	32	++	32	25	+++
8	***Semecarpus anacardium* L.**	22	20	+	26	16	+	28	15	+	18	13	+
9	***Solanum xanthocarpum* Schra.**	28	24	++	22	14	++	18	12	+	22	18	+
10	***Vitex negundo* L.**	70	42	+++	60	44	+++	52	35	+++	56	35	+++
	Control	80	58	+++	72	50	+++	70	48	+++	70	50	+++

SG: spore germination %; **DMW:** dry mycelial weight (mgs); **SPN:** sporulation; **+= Low, ++=Medium, +++= High**

Chart No. 2: Showing spore germination % and DMW of seed borne fungi



RESULT AND DISCUSSION:

All plants showing antimicrobial activity against specific pathogen. Plants having secondary metabolites for defense mechanism like alkaloids, glycosides, tannins, phenolic compounds, latex etc. In the present study ten wild medicinal plants leaf extract treated against seed seed mycoflora, fungal growth and sporulation of selected seed borne fungi of fenugreek. All the selected medicinal plants showing antimicrobial activity (Perumal, 2005)

It is clear from the results presented in table – 1 that the leaf extracts of all the test medicinal plants were found to be inhibitory for the incidence of seed mycoflora. The leaf extracts of *Semecarpus anacardium* was found to be more inhibitory and leaf extract of *Vitex negundo* was found to be very less inhibitory for the incidence of seed mycoflora of fenugreek as compared to the leaf extracts of other test medicinal plants. Highest seed germination percentage was found in the leaf extract of *Semecarpus anacardium* and very low seed germination percentage in *Vitex negundo.* Root and shoot length found of fenugreek was found more in the leaf extract of *Semecarpus anacardium* as compared to other selected medicinal plant leaf extract. Similar study was carried out by Nisha Moshahary et al, 2020 and Singh et al, 2009. Highest shoot and root of seedlings were found in *Semecarpus anacardium* and low in the *Vitex negundo* (Mughal, 2000).

It is clear from the result presented in Table 2 that the 5% leaf extract of all the wild selected medicinal plants were found more or less inhibitory for spore germination, growth in the form of dry mycelial weight and sporulation of all selected seed borne fungi.

It was reported from Table No.2 that leaf extract of *Semecarpus anacardium* found more inhibitory for spore germination percentage, dry mycelial weight and sporulation of *Aspergillus niger* and *Vitex negundo* showed less inhibitory spore germination, growth in the form of dry mycelial weight and sporulation of *Aspergillus niger* as compared to other test medicinal plants. Leaf extract of *Solanum xanthocarpum* was found more inhibitory and *Vitex negundo* showed less inhibitory spore germination, growth in the form of dry mycelial weight and sporulation of *Curvularia lunata* as compared to other test medicinal plants.

Leaf extract of *Solanum xanthocarpum* was found more inhibitory and Leaf extract of *Vitex negundo* showed less inhibitory spore germination, growth in the form of dry mycelial weight and sporulation of *Drechslera longirostrata* as compared to other test medicinal plants.

Leaf extract of *Semecarpus anacardium* was found more inhibitory and Leaf extract of *Vitex negundo* showed less inhibitory spore germination, growth in the form of dry mycelial weight and sporulation of *Fusarium oxysporum* as compared to other test medicinal plants.

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REFERENCES:

Agarwal D. K. and A. K. Sorbhoy (1978): Physiological studies of four species of *Fusarium* pathogenic to soybean. Indian Phytopathology, 31:24-31.

Amer Habib, S.T. Sahi, M.U. Ghazanfar and S. Ali (2007): Location of seed borne mycoflora of eggplant (*Solanum melongena* L.) in different seed component and impact on seed germinability, Int. Journ. Of Agriculture and Biology, 1560-8530/2007/09-3-514-516

I.S.T.A. (1966): International rules of seed testing, 1966. Inter. seed test. Ass. 31:1-152.

Jha, D.K. (1993): A text book on seed pathology. Vikas publishing house pvt. Ltd. New Delhi, 132 pp. (reprint 1995).

Mashooda Begum and S. Lokesh (2008): Synergistic effect of fungicides on the Incidence of Seed mycoflora of Okra. International Journal of Botany 4(1) : 24-32, 2008.

Mazumdar, A.; B.P. Saha; S.P. Basu and R. Mazumdar (2004): Antibacterial activity of methanolic extracts of leaves of *Lagestroemia parviflora*. Indian journal of Natural products 2003; V/ 19(3): p-20-23.

Mughal, A.H. (2000): Allelopathic effect of leaf extract of *Morus alba* L. on germination and seedling growth of some pulses. Range management and Agro forestry 2000, 21(2):164-169.

Mukadam, D.S. (1997): The illustrated kingdom of fungi (some selected genera). Published by Aksar Ganga Prkashan, Aurangabad, India.

Musyimi, D.M.; J.M. Ogur and P.M. Muema (2008): Phytochemical compounds and antimicrobial activity of extracts of *Aspilia* plant *(Aspilia mossambicensis)*(Oliv) Wild. International Journal of Botany 4(1): 56-61.

Nisha Moshahary, Riya Paul and Phatik Tamuli (2020): Seed Mycoflora of Fenugreek (*Trigonella foenum- graecum* L.) and its Management

Pandey, K.N. **(1982):** Antifungal activity of some medicinal plants on stored seeds of Eleucine cornacana. Indian phytopath 35:499-501

Perumal, G.; C. Subramanyam; D, Natrajan; K. Srinivasan; C. Mahanasundari and K. Prabhakar (2005): Antifungal activities of traditional medicinal plant extracts- A preliminary survey. Journal of physiological Research, 2004, V. 17(10) : p.81-83.

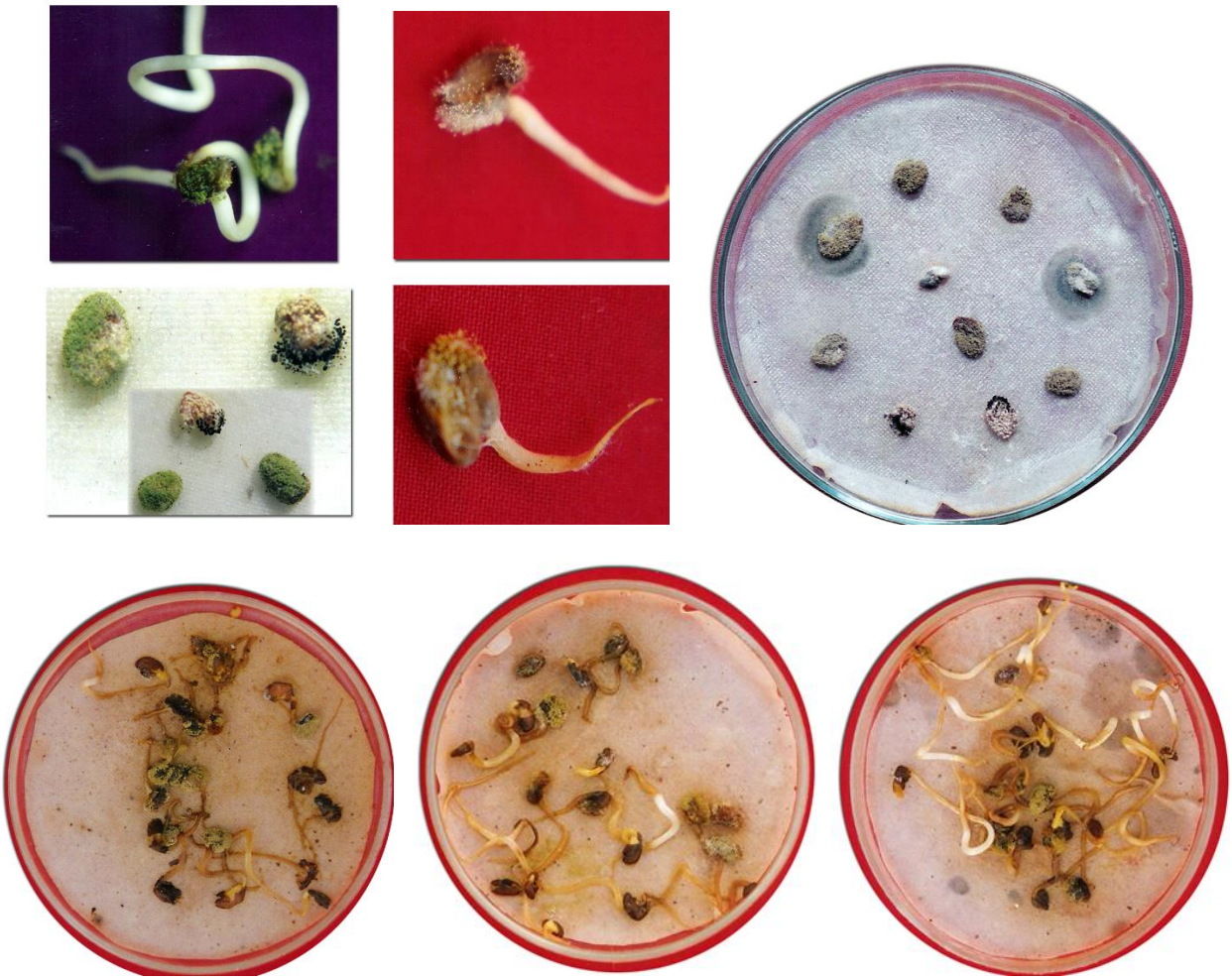
Singh, Mishra C.P. and U.S. Mishra, Nishant (2009): Dynamics of seed mycoflora on Fenugreek seeds during storage, Semantic Scholar, 22(1): 47-53

Subramanian, C.V. (1971): Hyopomycetes. An account of Indian species except *Cercospora*. ICAR, New Delhi: 930 pp.

Vyas, N.L. (1988): Observation on some cucurbitaceous seed mycoflora in Mizoram. Indian J. Mycol. And plant pathology, 2, 5, (1, 2): 44pp.

Photographs

Seed mycoflora of Fenugreek



Untreated and Treated seeds of fenugreek

Untreated seeds of Methi

Treated seeds of Methi