Screening and Management of fungi from Trigonella foenum- graecum seeds

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Abstract: Trigonella foenum-gracum L. belongs to family Fabaceae commonly termed as Methi in Marathi. Seeds of the plant are bitter, mucilaginous, aromatic, carminative, tonic, thermo genic, galactagogue, astringent and emollient. Powder of seeds use in veterinary medicine, an aqueous extract of the seeds possesses antibacterial property.

Seeds of fenugreek (Trigonella foenum-graecum L.) are infected during storage condition, which affect the germination percentage. Seeds were evaluated using blotter method to determine the fungal association. Fungal species were isolated from the internal and external seed surfaces of fenugreek, viz., Alternaria alternate, Aspergillus flavus, Aspergillus niger, Cladosporium, Colletotrichum dematum, Mucor, Fusarium cassiae, and Rhizopus stolonifer. Fungi toxicants Antracol and Bavistin are used among them Antracol is more effective than Bavistin to control seed mycoflora of Trigonella foenum-graecum seeds. Treated seeds showed better germination percentage as well as root and shot length than control.

Keywords: Seeds mycoflora, Trigonella foenum-graecum, fungicide.

1 Introduction:

1.1 Description: *Trigonella foenum-graecum L*. an aromatic, erect annual, 30-60 cm in height; leaves pinnate, 3-foliate, leaflets toothed; flowers white or yellowish white, axillary; fruits pods, 5-7.5 cm long with long persistent beak; seeds 10-20 per pod, greenish brown along with a deep groove across one corner, leaves and seeds are used for various purpose.

1.2 Chemical Constituents: The endosperm of the seed is rich in galactomannan, young seeds mainly contain carbohydrates and sugar; mature seeds content amino acid, fatty acid, vitamins, saponins etc., it also content diosgenin, gitogenin, homoorientin, and tigogenin.

1.3 Uses: The leaves are refrigerant and aperient and are given internally for vitiated conditions of pitta. A poultice of the leaves is applied for swellings and burns. Seeds are bitter, mucilaginous, aromatic, carminative, tonic, thermogenic and galactagogue. They are good for fever, vomiting, anorexia, cough and bronchitis. Externally in the form of poultice they are used for boils, abscess and ulcers. An infusion of the seeds is good cool drink for smallpox patients. Powdered seeds find application in veterinary medicine. An aqueous extract of the seeds possesses antibacterial and anti- diabetic property [1, 7, 10, 11].

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, seed mycoflora also affect germination [27,37,38,39,42].

2 Materials and Methods

2.1 Collection of Seeds:

Seeds of Fenugreek were collected from different local part of Jintur taluka of Parbhani district and their after separated as healthy and infected on the basis of visual observations and characteristic feature of seeds, for the detection of seed mycoflora blotter paper method are used. For isolation of external and internal seed mycoflora associate with the healthy and unhealthy seed sample of *Trigonella foenum-graecum L.*, the seed sample were store in cloth bag at room temperature in the laboratory. Seed germination, percent seed mycoflora, seed ling vigour was calculated by using blotter paper method. The seeds were divided in to three lots and one lot used for isolation, purification and pathogenicity. Second lot used for effect of fungicides on seed mycoflora, germination and vigour index and third used as control. [2, 25-29]

2.2 Blotter paper method: -

Healthy and infected seeds were selected for detection and isolation of seed borne fungi. Five seeds were placed in circle in each petri plate. The seeds were surface sterilized by 0.1% $Hgcl^2$ solution for 1-2 minutes and then washed three times by sterile distilled water. 5 healthy and unhealthy seeds were placed separately in petri plate containing two layered blotter paper. Sterilised distilled water was added to moisten the blotter paper. All plates were exposed to 12 hr. light and 12 hr. dark and incubated at 28 + 10°c. After 10 days, the seeds and seedlings were examined by using research microscope and seed mycoflora and germination have been recorded [2, 25,26,29,33,40,43].

2.3 Isolation Purification and Pathogenicity:

The seed borne fungi were isolated and cultured on potato dextrose agar media. The single culture of fungi was isolated and purified on potato dextrose agar. After 10 days these cultures were transferred on fresh potato dextrose agar for three times subsequently. These pathogens were preserved on slants in test tubes and stored at room temperature (23+3°c). These seed borne fungi were identified by using the standard literature. The pure cultures were transferred on potato dextrose agar media in the petri plates for mass multiplication to test the pathogenicity [3,6,17-19].

Spore suspension was prepared separately in sterile distilled water from 10 days old cultures grown on potato dextrose agar (Om Parkash and Babusingh Siradhana, 1978). A 4 mm disc of culture was taken out by with the help of sterilized cork borer and spore suspension was made in test tube up to 10 ml. Seeds were surface sterilized by using 0.1 % $Hgcl^2$ solution. Seeds were dipped in spore suspension for 3-5 minutes and were placed on moist blotter papers in a circle in the petri plate [33,40,43]. The sterile distilled water was used time to times for moistening blotter paper. For pathogenicity test, seedlings were rubbed by

brush at radical and plumules for development of wound. Same brush was dipped in spore suspension and inoculated on wounds of seedlings. The inoculated seedlings were placed in petri plate containing moist blotter papers. The petri plates were incubated for 10 days at 28° ± 3°c temperature [4,5,13].

The observations were made of the fungi and development of seedling. The diseases were observed and pathogens were isolated and cultured on potato dextrose agar media. The seedlings also shows growth of fungus viz also cultured on potato dextrose agar media. The fungi and disease of seedlings were found to be identical to that viz earlier identified, on the basis of spores and other characters [30,32,44].

2.4 Effect of Fungicides: -

For determination of effect of different fungicides viz Antracol and Bavistin were used to study seed mycoflora, germination and vigour index of which lethal does was from 0.1 to 5%. The different concentration of each fungicide used was from 0.5 to 5 %. International rules for seed testing (ISTA) were followed i.e. seeds were surface sterilized by 0.1% $Hgcl^2$ solution and drying in sunlight. The seeds were divided into 10 fractions for treatment of different concentration of fungicides and one set was kept as untreated control.

The seeds were dipped in different concentrations of fungicides for 3 minutes and plated on moist blotter paper in the petri plates. Sterile distilled water was added time to time for moistening. The petri plates were incubated at 28 ± 1°c and exposed for 10 days dark/light cycle. Observations were recorded on 10 days for seed mycoflora, germination and root length shoot length to calculate

vigour index [8,15,28,31] as shown in table no. 01, 02 & 03.

Vigour index = germination (%) x Seedling length

(Seed ling length = Root length + Shoot length)

3 Result and Discussion:

The seeds of Fenugreek (*Trigonella foenum-graecum L.*) were collected from different parts of Jintur, all collected seeds were separated as in three lots i.e., infected and healthy. Infected seeds separated on the basis of discoloration, rotting, size by visual observation.

3.1 Screening for germination and mycoflora:-

For screening the seeds for germination and seed mycoflora of Fenugreek (*Trigonella foenum-graecum L.*), the blotter paper was used, as shown in Plate a,b,c. The experimental observations clearly indicate that unsterilized seed of Fenugreek (*Trigonella foenum-graecum L.*) shows least germination and more seed mycoflora as compared to the sterilized seeds. The Blotter paper methods gives the maximum seed germination and found most suitable, therefore, used for the detailed further investigation. The percent seed mycoflora was observed in infected seeds of Fenugreek (*Trigonella foenum-graecum L.*) the seeds were surface sterilized by 0.1% $Hgcl^2$ solution.

The different seed mycoflora detected from sterilized and unsterilized seed was *Alternaria alternata, Alternaria cassia, Aspergillus flavous, Aspergillus niger, Cercospora cassia, Cepholosporium sp, Cladosporium sp, Colletototrichum dematium, Colletotrichum fragariae, Colletotrichum truncata, Curvularia cymbopogoni, Fussarium cassiae, Fusarium moinliformae, Fusarium oxysporium, Macrophomina phaseolina, Nigrospora sparica,Penicillium sp, Phoma medicagini, Psudocercospora angustifolia, Rhizopus nigricans, Rhizopus stolonifer,* as shown in Table No. 01& Plate: a, b, c and d.

Table 01: Percent mycoflora isolated from sterilized and unsterilized Fenugreek (*Trigonella foenum-graecum L.*) seeds by Blotter paper method

Fungi isolate	S.S.	S. U. S	Fungi isolate	S. S	S. U. S.
Alternaria alternata	6.9	***8.5***	***Fusarium cassiae***	7.0	8.9
Alternaria cassia	***6.0***	***7.0***	***Fusarum monoliformae***	8.0	9.9
Aspergillus flavus	***7.8***	***8.9***	***Fusarium oxysporium***	9.9	10.5
Aspergillus niger	***9.7***	***10.4***	***Macrophomina phaseolina***	4.4	4.5
Cercospora cassia	***6.0***	***8.5***	***Nigrospora spharica***	7.1	8.2
Cephalospoarium sp.	***6.5***	***8.4***	***Penicillum sp.***	4.7	5.8
Cladosporium sp.	***6.3***	***7.4***	***Phoamea medicagini***	2.9	3.1
Colletotrichum dematium	***6.2***	***7.9***	***Psudeocercospora angustifolia***	6.1	7.2
Colletotrichum fragariae	***8.2***	***9.4***	***Rhizopus nigricans***	10.2	12.1
Colletotrichum trumcatum	***8.5***	***9.7***	***Rhizopus stolanifer***	9.9	10.2
Curvularia cymbopogani	***8.0***	***9.0***	***Rhizoctonia solani***	9.7	10.1
			Sclerotium rolfsii	8.1	9.4
			S. E. ±	0.39	0.42
			C.D. P.= 0.05	1.33	1.42

S.S. = Surface sterilized seeds. S. U. S. = Surface Unsterilized Seeds.

3.2 Effect of Fungicides on seed mycofiora, germination and vigour index:-

The Fungicide like Antracol and Bavistin were tested and the percent seed mycoflora, germination and vigour index was recorded. The results indicated that as the concentration increases, the percent seed mycoflora decreases and germination percentage increases up to certain concentration, above that there was total inhibition of fungal pathogen.

3.2.1 Antracol: - The fungicide namely Antracol was used at different concentration for different incubation period. The results were recorded on 10^{th} day incubation period. At 0.0, 0.5, 1.0, 2.0, 2.5, 3.0 and 3.5 % concentration the percent seed germination was 30, 28, 24, 20, 15, 09, 6, and 00, 35, 37,40, 45, 60, 70, 75 and 80 and vigour index was 2100, 2405, 2800, 3375, 4800, 5750, 6450, 6940. The vigour index was 2100 at 0.5 percent concentration while 6960 at 3.5 percent concentration and complete

inhibition of seed mycoflora and higher germination was noted at 3.5 percent. It was observed that as a concentration increases the seed mycoflora decreases and percent germination increases, vigour index also increases as shown TableNo.02, Fig No.01.

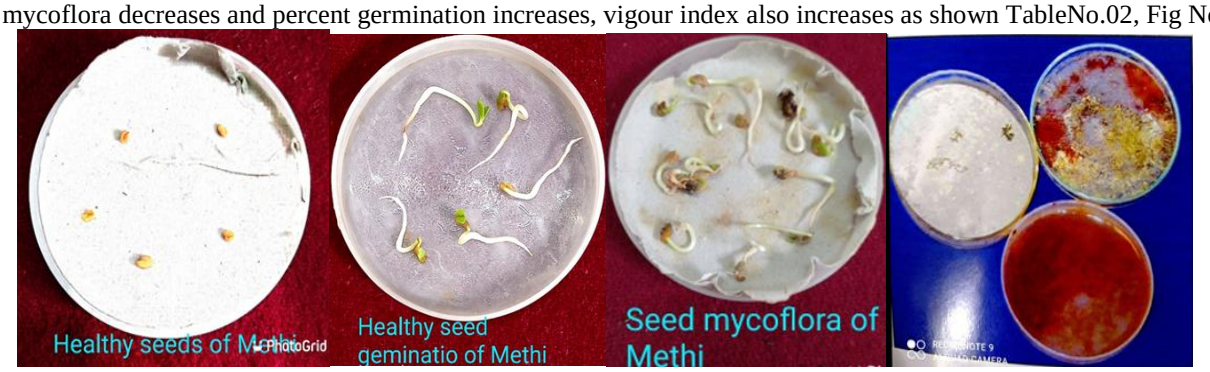

Plate – a, b, c, d Fungi detected

Table 02: Effect of Antracol on percent seed mycoflora, germination and vigour index

Concentration (%)	Seed Mycoflora	Seed germination	Vigour index
Control	30	35	2100
0.5	28	37	2405
1.0		40	2800
1.5	24	45	3375
2.0	20	60	4800
2.5	15	70	5750
3.0	09	75	6450
3.5	06	80	6960
S. E. +	0.0	6.46	4.31
C.D.P = 0.05	3.14	21.65	14.46
	10.53		

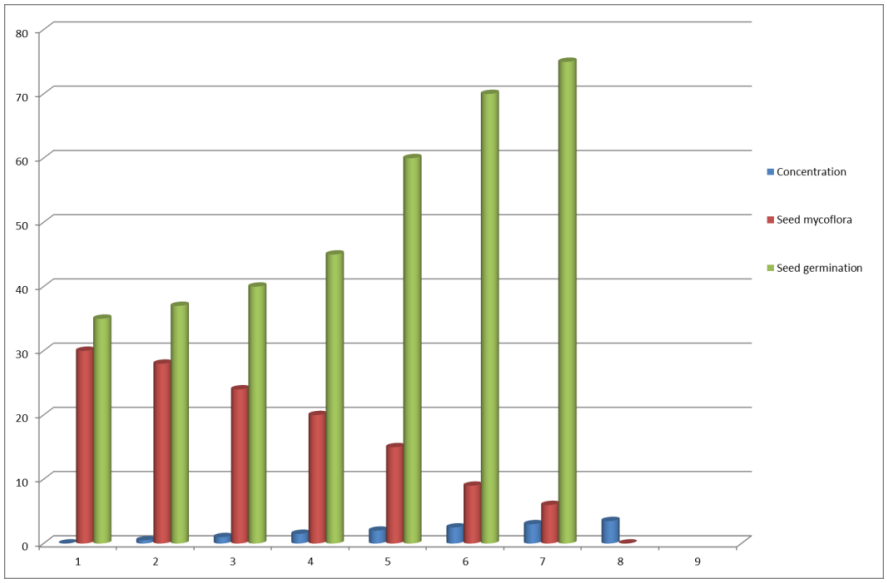

Fig: 01: Effect of Antracol on percent seed mycoflora, germination and vigour index

3.2.2 Bavistin (Carbendazim): - It is the most common fungicide, it was also tested at different concentration i.e., 0.0-4.5 % and observing were recorded on 10 th days of incubation period. The control (0.0 %) showed 30 % seed mycoflora, 35 % seed germination and 2100 vigour index calculated. 4.5 % concentration observed 0.0 % seed mycoflora, 85 % germination and 6899 vigour index as compared to control. At 4.0% concentration 4%, 70% and 5460 seed mycoflora, germination and vigour index. 5768 vigour index, 68% germination and 6% mycoflora at 3.5 % germination. At 3.0 % concentration 8 %, 61 and 4514 seed mycoflora, germination and vigour index observed. At 2.5% concentration 10 % seed mycoflora, 55 % germination and 3960 vigour index. At 2.0 % concentration 12 % seed mycoflora, 50 % germination and 3550 vigour index. At 1.5% concentration 14%, 45% and 3105 seed mycoflora, germination and vigour index, at 1.0 % concentration 2814, 42 % and vigour index, germination and seed mycoflora. At 0.5% concentration 18 %, 37 % and 2331 vigour index. Which shown in Bavistin was also tested against seed mycoflora and to increase germination percent of seed. Vigour index calculated and found to be increase with increases in percent concentration and the seed mycoflora decreased and percent germination increased. 4.5 % percent shows complete inhibition of seed mycoflora, higher germination and 6800 vigour

index is given in Table No. 03 Fig No. 02

Table 03: Effect of Bavistin on percent seed mycoflora, germination and vigour index.

Concentration (%)	Seed Mycoflora	Seed germination	Vigour index
Control	30	35	2100
0.5	18	37	2331
1.0	16	42	2814
1.5	14	45	3105
2.0	12	50	3550
2.5	10	55	3960
3.0	08	61	4514
3.5	06	68	5168
4.0	04	70	5460
4.5	00	85	6800
S. E. +	2.37	5.10	15.21
C.D.P = 0.05	7.94	17.09	50.97

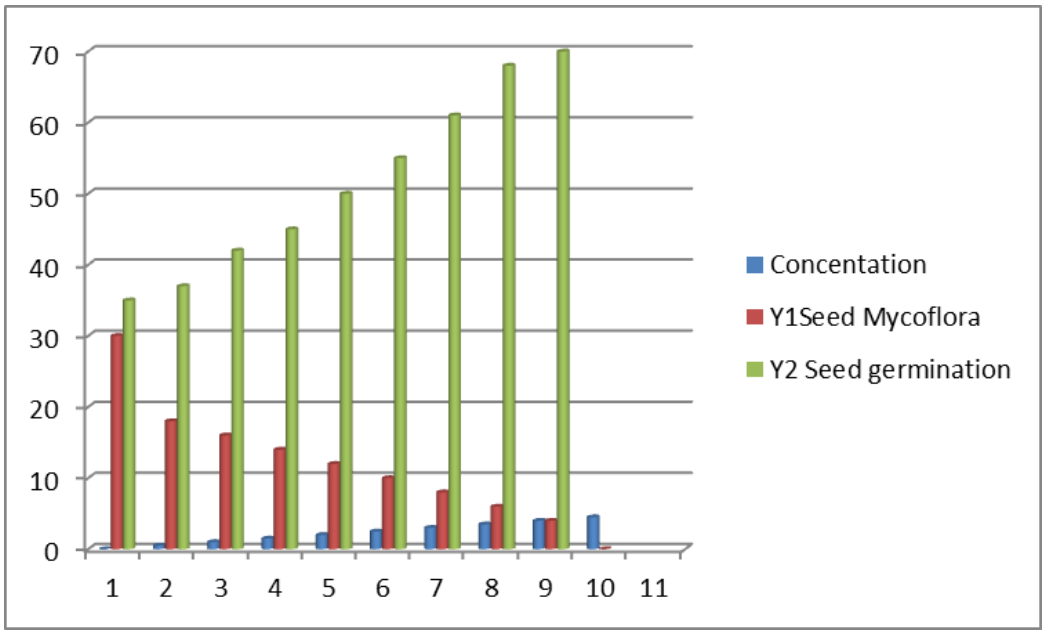

Fig 02: Effect of Bavistin on percent seed mycoflora, germination and vigour index.

Trigonella foenum-gracum L. belongs to family Fabaceae commonly termed as methi in Marathi.

The leaves, pods and seeds of this species used as medicineand other purpose. It is mostly cultivated in all part of India including Maharashtra. The plant contain galactomannan, young seeds mainly contain carbohydrates and sugar; mature seeds content amino acid. Fatty acid, vitamins, saponins etc., it also content diosgenin, gitogenin, homoorientin, and tigogenin.

The plants are of great importance as a medicine, the leaves are refrigerant and aperient and are given internally for vitiated conditions of pitta. A poultice of the leaves is applied for swellings and burns. Seeds are bitter, mucilaginous, aromatic, carminative, tonic, thermogenic, galactagogue, astringent and emollient. They are good for fever, vomiting, anorexia, cough, diabetic and bronchitis [9,12,14,16].

This economically and medicinaly impartant crop get affected by various pest and diseases. Out of these diseases, fungi play significant role in distruction. The fungi causes foliar diseases, seed rot, pod rot, seedling blight, coller rot, wilt, damping off. Among these, seed rot and seedling blight disease is serious problem in Fenugreek as it affects germination and seedling growth. Seed rot and seedling blight are caused by *Alternaria alternata (Fr) keissler, and Colletotrium dematium* van Arx.

Seed mycoflora has been deteted by Blotter paper method[20-24, 34-36, 41].

Janardhanan and Ganguly, (1963), observed that the seed samples of some medicinal plant collected soon after harvest, were divided in two lots. One lot treated with 1.5 percent murcury acetate and another untreated seed. Treated and untreated (100) were plated in petriplates containing 2.0 percent malt extract agar, at the rate 20 seeds per plate, and incubated at 32°C. It was observed that certain variations in the fungal flora of seeds predaminantly consisted of species of Alternaria, Aspergillus, Penicillium, Cladosporium, Mucor, Chaetomium and Rhizoctonia. Alternaria sp. seemed to be comparatively less affected by surface sterilization.

Patel and Patel, (1984) stated that senna (Cassia angustifolia vahl) an additional host of Macrophomina phaseolina in Gujarat. Pathak et. al., (2001) deteded nine fungi namely Aspergillus aculeatus, A. flavus A. fumigatus, A. niger, A. ustus, A. verisicolar offinis, pacelomyces variotil and penicillium aurarntigoniseum were detected from the surface sterilized seeds of the Mimosa leaves Jacaranda by agar plate and blotter method. Fungi isolated by blotter method were Aspergillus flavus, A. versicolar,

Curvularia affinis and Penicillium aurantiogriseum. The fungi detected by agar plate method were Aspergillus aculeatus, A. flavus, A.. fumigatus, A. niger, A. ustus pacilomycel variotii and Penicillium aurantigoriseum.

Gadauskas et al., (1977) also proved the pathogenicity test by aqueous suspension of conidia prepared from old culture of Colletotrichum dematium f. sp. truncata. in V & Juice agar. He also stated this is the best sutible method to test pathogenicity. The effect of different fungicides was tested for control the seed mycoflora of Fenugreek viz. Antracol and Bavistin. The different concentration of each fungicides used was from 0.5 to 5 %. Antracol showed least seed mycoflora and more seed germination up to 80 % and vigour index 6960 at 3.5 % percent concentration.

Solanke (1997) proved that the germination percentage was significantly superior in thiram treated seed than ABC dust and untreated control. Seed mycoflora was also less in thiram treated seeds i.e. 83% germination and 7 % mycoflora and control showed 78% seed mycoflora and 8% seed germination. Pensalwar et al., (1997) detected seed mycoflora of ground by agar plate, blotter paper, rolled towel and moist sand method, detected mycoflora were Fusarium moniliforme, Alternaria alternata, Aspergillus flavus, A. niger, Macrophomina phaseolina and Verticillium sp. All the method yield the same set of fungs however, frequency of seed mycoflora was more in agar plate than other method. It was also observed that the fungi were more in unsterilized seed than sterilized seed in all the method.

Das et al., (1997) reported that the Tilt, Opus, Calixin, Indofil, Blitox, Bavistin control the stem rot and coller rot of groundnut caused by Sclerotium rolfsii. Tilt (propiconazole) was more effective to control diseases than other fungicides. Kareppa (1998) recommended that for control of seed borne pathogen of groundnut, the thiram was more effective than carbendazim. Pandey and Upadhya (1999) confermed that wilt of pegion pea can be reduced by seed treatment or soil drenching with fungicides. Gupta and Garg (2000) tested ten systemic and non-systemic fungicide i.e. for seed treatment with bavistin, indofil M-45 topsin-m and aureofungin which gives more yield.

Peshney and Mahant (1994) reported that the soybean seed borne fungi were controlled by fungicides. Thiram 0.3 % was more effective against Aspergillus and Penicillium, where as captain 0.3 % and carbendazim 0.1 % were highly effective against Fusarium semilectum and Rhizoctonia bataticola respectively.

Kareppa and Kalburge (2000) also stated that the Groundnut infected by Aspergillus flavus, A. niger, Alternaria alternata, Fusarium moniliformae, Microphamina phaseolina, Rhizoctonia bataticola and Sclerotium rolfsii. The infection percentage was reduced by seed treatment with carbendazim and thiram. Treatment of seed of soybean with carbendazim and thiarm increase percent germination as evaluted by Lakade and Kareppa (2003) for the manangment of seed borne diseases.

The findings of present investigation were found similar with earlier workers regarding seed mycoflora, seed germination percentage. It is, therefore, suggested that fenugreek seeds should be treated with Antracol and Bavistin before showing to achieve better germination and vigorous growth.

References

1. Agrawal R.L. Seed technology, Oxford and IBH publishing Co, Pvt. Ltd, New Delhi, (1993).
2. Allen, S. J; Brown J. F. and J. K. Kochman. (1983). Effect of temperature, Dew period and light on the growth and development of Alternaria helianthi. Phytopath. 73 (6): 893-896.
3. Alexopolous, C. J; Mims C. W. and M. Blackwell. (1996). Introductory mycology, John willey and sons. Inc, New yourk, Winchester, Brisbane, Toronto, Singapur.
4. Amsellem, Z, Sharon, A; Gressel, J and Quimby, P. C. (1990). Complete abolition of high inoculum threshold of two mycoherbicide (A. cassiae and A. crassa), when applied in vert emulsion. Phytopath. 80 (10): 925-929.
5. Armstrong, G. M. and J. K. Armstrong. (1965). Further studies on pathogenicity of three forms of Fusarium oxysporium causing wilt of Alfalfa. Pl. Dis. Report. 49 (5): 412-416.
6. Barnett, H. L. (1970). Illustrated genera of fungi imperfecti, Burgess Publ. Minn. U.S.A.
7. Bhattacharjee S.K. Hand book of medicinal Plants, Pointer publishers, Jaipur, India, (2000).
8. Chowdhury, A. K. (2000). Interaction of resistance in chickpea plant against Fusarium wilt infection by seed treatment with non- conventional chemical J.Mycol.Pl. Pathol. 30 (1): 53-56.
9. Das, S.R; Pani, B.K. and Panda, S. N. (1997). Control of stem Rot diseases of Tuberose with Fungicides. Pl. Dis. Res. 12 (2) 143- 144.
10. Deshmukh, Laxman P. (2003). Medicinal plants of India. Oxford Book Company Jodhpur.
11. Deshpande, D. J (2008). Hand Book of Herbal remedies Agrobios (India) Jodhpur.
12. Dhavle, S. D. and B. M. Kareppa. (2000). Effect of carbendazim on *Colletotrichum capsici* causing leaf spot of turmeric. *Proc. National conference on frontiers in biotechnological aspects of plant sciences, held at Dr. BAM University,* Aurangabad from 30 to 31 Janauary: 58.
13. Duhan J. C. and L. S. Sugha. (1989). Studies on the Alternaria leaf and pod blight of couliflower. Pathogenicity and distribution in Haryana. Indian Phytopath. 42 (1): 87-94.
14. Gudouskas R.T. Teem D.H., Morgan-Jones G., Anthracnose of *Cassia accidentalis* caused by *Colletotrichum dematum f. truncate*. Pl. Dis. Reptr. 61 (1977) 468-470.
15. Gupta, Meenu and R. C. Garg. (2000). Chemical controlay ripe fruit Rot of Chilli caused by Colletotrichum capsici J. Mycol. Pl.Pathol. 30 (2): 253.
16. Janardhanan, K. K. and Ganguly. (1963). Fungul flora of seed of some important medicinal plants. Indian Phytopath. XVI. 379-381.
17. Jha, D. K. (1993). Seed pathology, vikas publishing house, pvt., Ltd,
18. Kamat D. V. (1961). Management of crop diseases. Oxford Book Company, Jodhpur.
19. Kamat D. V. (2007). Management of crop diseases. Oxford Book Company, Jodhpur.

20. Kareppa, B. M. and L. V. Gangawane. (1992). Methods for evaluation of fungicides against tuber fungal diseases of potato. *J. Phytopathol. Suppl.* 45.
21. Kareppa, B. M. (1998). Studies on variation in seed mycoflora of Groundnut. J. Ind. Bot. Soc. (Suppl): 17-18.
22. Kareepa, B. M. and D. V. Kalburge. (2000). Effect of fungicides on A. flavus, a major seed borne pathogen of groundnut. Proc. Seminar on plant resource development held on 9th Dec. at Dr. B.A.M. University Aurabgabad.
23. Kaurow, L.P. (1986). Effect of fungicides on the germination, root/shoot growth and incidence of seed-borne pathogens in rice. Indian Phytopathology, 39: 609-610.
24. Lakade, H. M. and B. M. Kareppa. (2003). Effect of carbendazim on *Alternaria alternata* and *Fusarium oxysporum* causing seed rot and seedling blight of soybean. *National conference on biotechnological approaches in conservation, utilization and improvement of plant health held at Yeshwant College, Nanded* from 14 to 15 Feb, 2004: 137.
25. Mukadam, D. S. and L. V. Gangwane. (1982). Method in experimental plant pathology. A publication of college science improvement program university leadership project in biology sponsored by UGC.
26. Mukadam, D. S. (1997). The illustrated kingdom of fungi, Aksharganga prakashan, 24, Pannalal Nager, Aurangabad.
27. Naik, V. N. (1998). Flora of Marathwada Region, Vol. I & II, Amrat Prakashan Aurangabad.
28. Nene, Y. L. (1971). Fungicides in plant diseases control, oxford and IBH, publishing company New Delhi.
29. Neergard, Poul. (1986). Seed pathology, Abridged Edition, S. Chand and company Ltd. Rama Nagar, New Delhi.
30. Om Prakash and Babu Singh Siradhana. (1978). Ear rot of maize caused by four Aspergillus species. J. Indian Phytopath 31: 115-116.
31. Pandey, K. K. and J. P. Upadhyay. (1999). Comparative study of Chemical, Biological and Intergrated approach for management of Fusarium wilt of pigeon pea. J. Mycol. Pl.Pathol. 29 (2): 214- 216.
32. Patel, B. K. and A. J. Patel. (1976). Fungi causing damping off Tomato seedlings. *Indian Phytopath.* 6 (12): 87-88.
33. Patel, D. J and S. J. Patel. (1984). Seena an additional host of Macrophomina phaseolina in Gujarat. Indian J. Mycol. Pl. Pathol. 14 (3): 293.
34. Pathak, A. K; Shotles. Godikas; J. P. Jain and Suresh Muralia. (2001). Effect of antogonistic fungi and seed dressing fungicides on the incidence of seed rot of mustard. J. Mycol. Pl. Pathol. 31 (20): 327-329.
35. Pensalwar, S. N; R. B. Solanke and S. S. Kore. (1997). Association of fungi with groundnut seeds and effect of various seed dresser on Fussarium moniliformae inoculation. J. Mycol.Pl. Pathol. 27 (3): 299-302.
36. Peshney, N. L. and S. F. Mahant. (1994). Detection and chemical control of seed borne fungul pathogen of soybeen. J. Indian Phytopath. 47 (3): 350.
37. Prajapati, Purohit, Sharma and Kumar (2007). A Hand Book of Medicinal Plants a complete Source Book. Agrobios (India).
38. Rangaswami G., Mahadevan A. (1999). Diseases of crop plant in India. Prentice Hall India.
39. Rasheeduz, Zafar. (1994). Medicinal plant of India. CBS publishers & Distributors. Jain Bhavan, Bhola Nath Nagar Shahadara Delhi.
40. Solanke, R. B; S. S. Kore and S. N. Pensalwar. (1997). Effect of fungicidal seed treatment on seed viability and mycoflora during storage of sorghum. J. Mycol. Pl. Pathol. 27 (3): 297-298.
41. 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.
42. Srinivasan (2006). Fenugreek (Trigonella foenum-graecum): A review of health beneficial physiological effects. Food Rev. Int., 22(2): 203-224.
43. Srivastava, R. N. and J. S. Gupta (1981). Histopathological studies of yellow cosmos seed. Indian J. Mycol. Pl. Pathol. 12 (3): 267-270.
44. Walker, H. L. (1982). Seedling blight of sickle pod caused by *Alternaria cassiae.* Plant disease. 66 (5): 426-428.