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Efficacy of Fungicides and Bioagents on Seed Mycoflora of Mung Bean (*Vigna radiata* L.)

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ABSTRACT

The effect of different fungicides and bioagents on seed mycoflora of mung bean was evaluated. The seed treatment improved the seed germination and reduced seed borne mycoflora of mung bean seeds. In total, twelve fungi belonging to ten genera were isolated from mung bean seeds collected from different districts of Karnataka. Isolated fungi were identified as Aspergillus niger, Aspergillus flavus, Aspergillus candidus, Alternaria alternata, Penicillium notatum, Rhizopus stolonifer, Cladosporium sp., Fusarium oxysporum, Mucor sp., Curvularia lunata, Chaetomium globosum and Macrophomina phaseolina. Out of seven fungicides tested, seed treatment with captan at the rate 4 g kg^{-1} of seed significantly reduced seed mycoflora (78.68 %). Seed treatment with Trichoderma harzianum at the rate of 8 g kg^{-1} of seed reduced the seed mycoflora up to (69.63 %) followed by Pseudomonas fluorescences (66.49 %) and Trichoderma viride (64.39 %).

Key words: *Mung bean, Seed treatment, Mycoflora, Fungicides, Bioagents.*

INTRODUCTION

Seed health is an important factor in the control of diseases, since an infected seed is less viable, has low germination, reduced vigour and yield[1]. Mung bean [*Vigna radiata* L. Wilczek] is the third most important pulse crop among the thirteen food legumes grown in India. It is also known as green gram, which is an ancient and well known leguminous crop of Asia. It is an important wide-spreading, herbaceous, self-pollinating crop and an excellent source of protein and minerals.

Seed is the focal point in agriculture development, without which an agriculture system is meaningless[2] and high quality seed is an important pre-requisite for sustainable and profitable crop production. Recently reported seed borne mycoflora associated with Mung bean includes *Macrophomina phaseolina, Aspergillus flavus, Colletotrichum globosum Alternaria alternata, Penicillium notatum, Rhizopus stolonifer, Cladosporium* sp. and *Fusarium oxysporum*[3].

The seed mycoflora are carried over from year to year and from one place to another with the seeds and which serves as primary source of infection for subsequent crop.

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Management of seed borne diseases has been judiciously achieved through fungicides and biological agents. Seed treatment is one of the key method in the integrated management of many seed borne diseases and has provided excellent results in reducing losses caused by diseases with increase in quality and quantity of seed. Seeds are the efficient carriers for survival, large scale and long distance spread of pathogens. Infected or contaminated seeds serve as major source of inoculum for large number of plant pathogens which may infect the seeds and survive as spore or resting structures on or with in the seeds[4].

Currently, the information on the management of mung bean seed mycoflora is meagre. Hence, there is a prerequisite to generate information on the management of seed mycoflora. Keeping this in view, present investigation was envisaged and this investigation opens the new avenues in studying and managing the seed mycoflora to get better management strategies.

MATERIALS AND METHODS

The present investigation on seed mycoflora of mung bean (*Vigna radiata* L.) includes, identifying the suitable fungicides and Bioagents for the seed treatment of mung bean to reduce the seed mycoflora. The investigation was carried out at the College of Agriculture, University of Agricultural Sciences, GKVK, Bengaluru during 2014-2016.

Fungicides used for seed treatment

Mung bean seeds were treated with seven fungicides *viz*., captan, thiram, mancozeb, chlorothalonil, carbendazim, vitavax and thiophanate methyl at the rate of 2, 3 and 4 g kg^{-1} of seed at each treatment level mentioned in the table.1. Treated seeds were assayed for seed mycoflora by employing standard blotter method as per the International Rules for Seed Testing. According to ISTA, four hundred seeds of each sample were placed equidistantly, aseptically on three layers of moist blotters moistened with sterile distilled water in sterile petriplates of 90 mm diameter at the rate of twenty five seeds per plate and the plates were incubated for seven days under diurnal cycles of 12 h light and 12 h darkness at room temperature of 22±2 °C [5]. Seeds without treatment served as control. Observations on seed mycoflora was recorded on eighth day of incubation by observing fungal growth on seeds under stereobinocular microscope and expressed in percentage. The results were statistically analyzed through arc sin transformation and two-way ANOVA.

Table 1: List of fungicides used for seed treatment against mung bean seed mycoflora

Sl. No.	Common name	Chemical name	Trade name
1.	Captan	N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide	Captan 50 % WP
2.	Thiram	Dimethylcarbamothioylsulfanyl N,N-dimethylcarbamodithonate	Thiram 75 % WP
3.	Mancozeb	Zinc and Manganese Ethylene bis dithiocarbamate	Indofil M-45 75 % WP
4.	Chlorothalonil	Tetrachloroethylene bis dithiocarbamate	Kavach 75 % WP
5.	Carbendazim	Methyl 1-2 benzimidazole carbamate	Bavistin 50 % WP
6	Vitavax	5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide	Vitavax 75 %WP
7	Thiophanate methyl	Dimethyle 1,2-phenylene-bisiminocarbonothioyl bis carbamate	Topsin-M 70% WP

Bioagents used for seed treatment

In this experiment, mung bean seeds were treated with commercial formulations of *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* at the rate of 2, 4, 6 and 8 g kg^{-1} of seed. Treated seeds are plated on moist blotter by using standard blotter method as per the

International Rules for Seed Testing. Seeds were incubated for seven days and observations on seed mycoflora was recorded on eighth day of incubation by observing fungal growth on seeds under stereo binocular microscope and expressed in percentage. The results were statistically analyzed through arc sin transformation and two way ANOVA.

RESULTS AND DISCUSSION

Efficacy of fungicide treatment:

Among different fungicides tested, the maximum reduction of seed mycoflora (49.18 %, 65.57 % and 78.68 %) was recorded when the seeds were treated with captan at the rate of 2, 3 and 4 g kg^{-1} of seed respectively. Similar trend was observed when the seeds were treated with carbendazim, thiram, mancozeb, cholorothalonil, vitavax and the minimum reduction was counted with thiophanate methyl. Minimum seed association of 12 per cent by *Fusarium oxysporum* was recorded when the seeds were treated with captan at 4 g kg^{-1} of seed followed by *Macrophomina phaseolina* (9 %), *Cladosporium* sp. (7 %), *Aspergillus flavus* (5 %), *Rhizopus stolonifer* (5 %), *A. niger* (3 %). *A. candidus* (3 %) *Penicillium notatum* (2 %) and *C. lunata* (1%).

Maximum seed germination of 92 per cent was observed when the seeds were treated with captan at the rate of 2g kg^{-1} of seed and carbendazim at the rate of 2 and 3 g kg^{-1} of seed respectively. Minimum seed germination of 81 per cent was recorded when the seeds were treated with vitavax and thiophanate methyl at the rate of 4 g kg^{-1} of seed respectively.

The results of the chemical seed treatment revealed that seed treatment with captan inhibited 78.68 per cent of seed mycoflora followed by carbendazim (68.30 %), thiram (65.57 %), chlorotholonil (62.29 %) vitavax (62.84 %), mancozeb (60.10 %) and thiophanate methyl (57.92 %) at the rate of 4 g kg^{-1} of seed as shown in Table. 2 and fig.1.

The maximum reduction of seed mycoflora 78.68 per cent was observed when the seeds are treated with captan at the rate 4 g kg^{-1} of seed. This may be because captan is a non-specific thiol reactant with protective and curative action that works by inhibiting respiration of numerous species of fungi and bacteria, the mechanism of action may involve the degradation of captan into the short-lived thiophos gene, which is a highly reactive with thiols and other functional groups which appears to be the optimum rate and best treatment among the chemical fungicides that reduces seed mycoflora and enhanced seed germination.

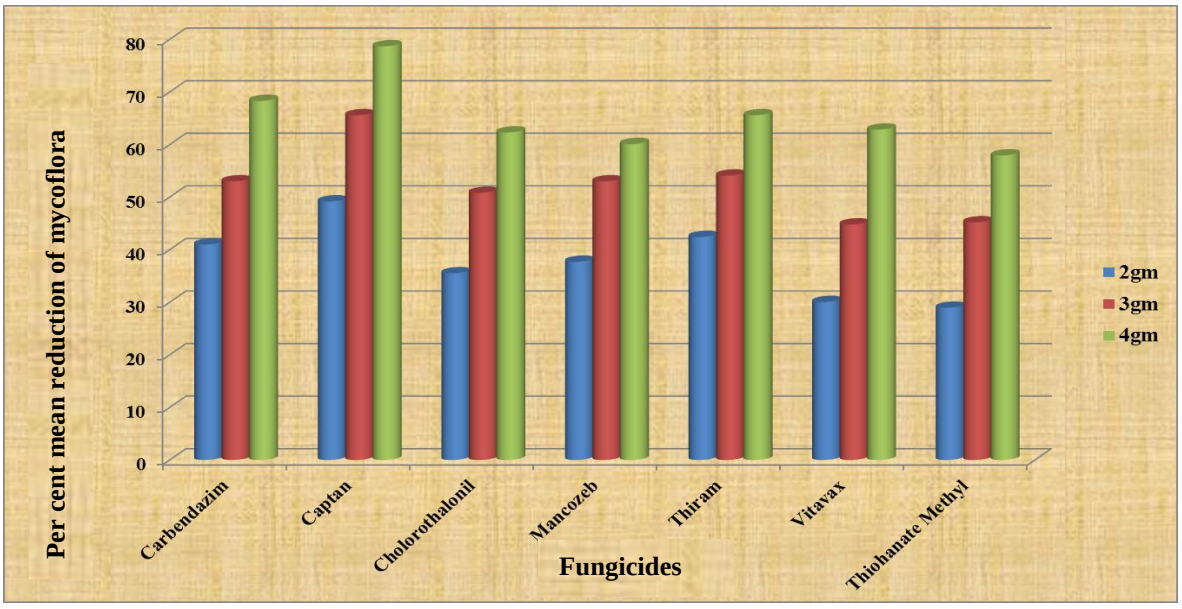

Fig. 1: Effect of chemical seed treatment on mung bean seed mycoflora

Singh *et al*.[6] also made the similar observations in the *in vitro* study of some fungicides *viz.*, Captan, Dithane M-45, Vitavax and Bavistin as seed dressing fungicides for their efficacy in controlling *Fusarium* species on mung bean seeds. He found that fungicidal treatment induced metabolic changes leading to development of toxic factors, resulting in the internal environment unfavourable for pathogens growth and activity, ultimately inducing the resistance and protection against the infection. These results are also in agreement with the observations made by Sandrou *et al.*[7] and Kumar and Dubey[8] in cowpea, black gram, brinjal and sunflower in controlling seed borne pathogens.

Seed treatment with Bioagents:

Seeds of the mung bean were treated with commercial formulation of bioagents *viz*., *Tricoderma viride, T. harzianum, Bacillus subtilis,* and *Pseudomonas fluorescens* at rate of 2, 4, 6 and 8 g kg^{-1} of seed. The treatment details and results are presented in Table. 3. Among the bioagents, the maximum inhibition (69.63 per cent reduction over control) of seed mycoflora was recorded when seeds were treated with commercial formulations of *T. harzianum* at the rate of 8 g kg^{-1} of seed followed by treatment with *Pseudomonas fluorescens* (66.49 %) at the rate 8 g kg^{-1} of seed, *Tricoderma viride* (64.39 %) at the rate 8 g kg^{-1} of seed and 63.79 % when seed treated with *B. subtilis* @ 8 g kg^{-1} of seed.

Minimum association of 22 per cent was observed with *Penicillium notatum* followed by *Fusarrium oxysporum* (14 %), *Microphomina phaseolina* (14 %), *Aspergillus niger* (7 %) *A. flavus* (5 %) *Cladosporium* sp. (5 %), and *R. stolonifer* (3 %) was recorded when seeds were treated with *T. harzianum* at the rate 8 g kg^{-1} of seed. Maximum association of *P. notatum* (34 %), followed by *M. phaseolina* (28 %), *F. oxysporum* (26 %), *A. flavus* (21 %), *A. niger* (21 %), *Cladosporium* sp.(20 %), *A.candidus* (14 %) *Rhizopus stolonifer* (12 %), *A. alternata* (4 %), *C. globosum* (2 %), *C. lunata* (1 %), and *Mucor* sp. (1 %) was observed when the seeds were treated with *T. viride* at the rate 2 g kg^{-1} of seed.

The germination percentage was ranged from 86 to 92 per cent when seeds were treated with different bioagents at different concentrations. There is no effect on seed germination when the seeds are treated at the rate of 8 g kg^{-1} of seed. Hence, this appears to be the optimum rate and best treatment among the bioagents that reduced seed mycoflora and enhances seed germination.

As in the present study, Kumar *et al*.[9] also observed that complete elimination of *A. alternata, Phyllosticta cajani, R. solani, Curvularia lunata, R. bataticola, Alternaria* spp., *C. dematicum* and *Trichothecium roseum* and reduce the colonies of *A. niger, A. flavus, B. cineria, F. moniliforem* and *F. semitectum* from pigeon pea seeds when treated with *T. viride*. Manjunatha and Rao[10] also reported that seed treatment with *T. viride, T. harzianum* and *P.fluorescences* effectively reduced *A. alternata, F. moniliforme, A. flavus, A. niger,R. stolonifer, C. lunata* and *C. globosum* infection from wheat seeds.

In the present investigations, all the fungicides and bio-agents significantly reduced seed mycoflora growth as compared to control. At higher concentration, all fungicides were found effective in controlling seed borne mycoflora. These results are in agreement with the findings of Pankaj[11] where the seed borne mycoflora was effectively controlled by fungicide treatment. The metabolic activities of fungi completely destroyed at higher concentration of chemical treatment[12].

Table 2: Effect of chemical seed treatment on mung bean seed mycoflora

Sl. No.	Fungicides	Dosage (g kg^{-1})	Seed germination (%)	Per cent seed mycoflora													Per cent reduction over control
				Aspergillus niger	*Alternaria alternata*	*Curvularia lunata*	*Aspergillus flavus*	*Aspergillus candidus*	*Cladosporium* spp.	*Fusarium oxysporum*	*Penicillium notatum*	*Macrophomina phaseolina*	*Rhizopus stolonifer*	*Mucor* spp.	*Chaetomium globosum*	Mean	
1.	Carbendazim	2	92	10	2	4	16	10	17	25	8	20	11	4	2	10.8(19.1)*	40.98
		3	91	8	0	2	14	10	14	20	5	17	10	3	0	8.6 (17.0)	53.00
		4	88	6	1	0	10	6	9	14	4	12	7	0	0	5.8 (13.9)	68.30
2.	Captan	2	92	8	3	2	12	8	19	23	6	17	9	4	1	9.3 (17.7)	49.18
		3	90	5	0	1	8	5	14	17	5	15	6	0	0	6.3 (14.5)	65.57
		4	90	3	0	1	5	3	7	12	2	9	5	0	0	3.9 (11.3)	78.68
3.	Cholorothalonil	2	90	11	4	8	15	8	24	30	10	20	12	0	0	11.8(20.0)	35.51
		3	88	8	2	6	10	7	20	24	7	14	9	1	0	9.0 (17.4)	50.81
		4	87	6	0	5	8	5	15	20	5	10	9	0	0	6.9 (15.2)	62.29
4.	Mancozeb	2	91	9	4	6	12	10	25	30	9	16	11	3	2	11.4(19.7)	37.70
		3	90	8	3	5	8	8	23	26	6	9	7	0	0	8.6 (17.0)	53.00
		4	88	6	1	2	6	7	18	25	5	10	6	1	1	7.3 (15.6)	60.10
5	Thiram	2	90	11	3	7	12	13	18	29	10	15	7	0	1	10.5(18.9)	42.42
		3	89	9	0	5	10	10	16	28	6	12	4	0	1	8.4 (16.8)	54.09
		4	90	7	0	4	9	8	11	21	4	8	4	0	0	6.3 (15.5)	65.57
6	Vitavax	2	89	13	1	4	17	18	26	31	14	17	7	3	2	12.8(20.9)	30.05
		3	89	8	0	3	15	13	24	26	11	12	7	1	1	10.1(18.5)	44.80
		4	81	7	0	1	10	9	20	18	7	5	4	1	0	6.8 (15.1)	62.84
7	Thiophanate Methyl	2	87	12	6	6	18	19	26	26	9	18	13	2	1	13.0(21.1)	28.96
		3	85	11	4	2	17	16	18	17	7	17	10	1	0	10.0(18.4)	45.20
		4	81	10	2	2	9	12	14	15	6	13	8	1	0	7.7 (16.1)	57.92
Control			92	22	8	12	26	20	22	36	25	21	16	7	4	18.3(25.3)	-

	Mycoflora	Fungicide	Dosage
SEm±	0.85	0.69	0.62
CD (0.01)	2.97	2.25	2.07

*****The values in the parenthesis are arc sin transformed values

Table 3: Effect of seed treatment with bioagents on mung bean seed mycoflora

Sl. No.	Bioagents	Dosage (g kg^{-1})	Seed germination (%)	Per cent seed mycoflora													Per cent reduction over control
				Aspergillus niger	*Alternaria alternata*	*Curvularia lunata*	*Aspergillus flavus*	*Aspergillus candidus*	*Cladosporium* spp.	*Fusarium oxysporum*	*Penicillium notatum*	*Macrophomina phaseolina*	*Rhizopus stolonifer*	*Mucor* spp.	*Chaetomium globosum*	Mean	
1.	*Trichoderma viride*	2	92	21	4	1	21	14	20	26	34	28	12	1	2	14.8(22.6)*	22.51
		4	90	19	3	0	18	10	18	22	28	27	8	0	1	12.8 (20.9)	49.21
		6	89	16	1	0	14	6	10	19	22	19	6	0	0	9.4 (17.8)	50.78
		8	88	10	1	0	9	2	6	14	21	13	6	0	0	6.8 (15.1)	64.39
2.	*Trichoderma harzianum*	2	92	17	5	2	15	11	18	28	38	25	13	2	1	14.6 (22.4)	23.56
		4	90	12	2	1	12	5	12	18	31	22	7	0	0	10.2 (18.6)	46.59
		6	89	11	2	0	8	1	8	20	28	19	5	1	0	8.6 (17.0)	54.97
		8	89	7	0	0	5	0	5	14	22	14	3	0	0	5.8 (13.9)	69.63
3.	*Bacillus subtilis*	2	89	19	5	1	24	16	17	20	20	31	15	1	2	14.3(22.2)	25.13
		4	88	15	4	1	22	10	16	16	14	27	13	1	0	11.6 (19.9)	39.26
		6	86	11	2	1	18	8	12	12	11	24	10	0	0	9.1 (17.5)	52.35
		8	85	8	2	3	11	7	10	8	9	20	7	0	0	7.0 (15.3)	63.79
4.	*Pseudomonas fluorescences*	2	90	14	2	1	11	13	21	24	37	28	15	3	1	14.2(22.1)	26.10
		4	88	9	0	1	8	9	16	20	33	24	11	2	0	11.1 (19.4)	41.88
		6	87	7	1	0	6	6	12	17	28	19	8	0	0	8.7 (17.1)	53.89
		8	86	7	0	0	4	4	7	14	21	15	5	0	0	6.4 (14.6)	66.49
Control			92	24	6	3	26	21	23	36	35	27	22	4	2	19.1(25.9)	-

	Mycoflora	Bioagents	Dosage
SEm±	1.07	0.76	0.74
CD (0.01)	3.64	2.57	2.48

Note: The values in the parenthesis are arc sin transformed values

CONCLUSION

Out of seven fungicides tested, seed treatment with captan at the rate 4 g kg^{-1} of seed significantly reduced seed mycoflora (78.68 %). Among four bioagents used, seed treatment with *Trichoderma harzianum* at the rate of 8 g kg^{-1} of seed reduced the seed mycoflora up to (69.63 %) followed by *Pseudomonas fluorescences* (66.49 %) and *Trichoderma viride* (64.39 %).

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