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Short Communication

Management of Seed Borne Diseases of Cowpea [V*igna unguiculata* (l.) Walp] Using Bioagents

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Cowpea [*Vigna unguiculata* (L.) Walp] is an important vegetable in India popularly known as 'Chowli'. It probably originated in Asia, Africa and even South America. In India it is grown almost in all regions, most commonly in the central and peninsular regions. It is an important source of nutrients and provides high quality, affordable protein. It also plays an important role in agriculture for maintaining and increasing soil fertility through biological nitrogen fixation. It is consumed also as green pods, but mostly in the form of dried seeds, either as flour or split and provides a good source of carbohydrates and vitamins [16].

Fungi including *Curvularia*, *Fusarium*, *Rhizoctonia*, *Penicillium* and *Aspergillus* have been found associated with cowpea seeds [10]. Seed borne fungi are generally known to affect adversely the seed germination and vigour of the seedlings [9] besides seed decay, pre and post emergence mortality damping off and root rots [8].

The fungal bioagents *viz., Trichoderma* spp. and bacterial agent *Pseudomonas* have been reported to be effective against several plant pathogens [17]. The continuous use of chemicals for controlling these diseases may pose several problems like toxicity to non targeted organisms, development of resistance among the population of pathogen and environmental pollution. Therefore, the studies were undertaken to see the efficacy of bioagents *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas fluorescens* on the seed borne pathogens, seed germination, seedling vigour index and field emergence in cowpea.

The experiment on efficacy of talc based formulation of biocontrol agents along with recommended doses of fungicides were conducted on Research Farm and Seed Pathology Laboratory of Seed Technology Research Unit, MPKV, Rahuri during Kharif 2019-20 to 2021-22. The seeds of cowpea (Cv. Phule Pandhari) were collected from Seed Cell, MPKV, Rahuri (M.S.). The talc based formulations of *Trichoderma viride, Trichoderma harzianum* and *P. fluorescens* were received from the Project Director, Indian Institute of Seed Science, Mau (UP). The experiment included seed treatments with *P. fluorescens* @ 0.6 per cent, *Trichoderma viride* @ 0.6 per cent, *T. harzianum* @ 0.6%, *P. fluorescens* + *T. viride* @ 0.6% each, *P. fluorescens* + *T. harzianum* @ 0.6% each, Thiram + Carbendazim @ 0.2 per cent each and untreated control. The seeds of cowpea (Cv. Phule Pandhari) were treated with the biocontrol agents and recommended fungicides as per the required concentrations. For field experiment, the seeds were sown in the field having gross plot size 2.60 m x 2.50 m at 30cm x 10 cm spacing with four replications of 100 seeds each in randomized block design. The field emergence was recorded 21 days after sowing.

The seed borne fungi associated with the seeds of cowpea were detected by standard Blotter method as described by Neergaard, 1977 [11]. The blotters were soaked in distilled water and placed in three layers in transparent plastic plates after draining of excess moisture. Ten seeds were placed at equidistance from one another in each plate under aseptic condition. Total 400 seeds / treatment were plated. The plates were then incubated at 20+2 °C under near ultra violet/ fluorescent light with an alternate cycle of 12 hr light and 12 hr darkness in incubation room for 7 days. Thereafter seeds were examined under stereio-binocular microscope for the presence of seed borne pathogens. The fungi were identified on the basis of morphological characters of fungi / conidia / conidiophores and with the help of manual of Illustrated Genera of Imperfect fungi [3]. The per cent incidence of seed borne fungi was recorded and the reduction / inhibition of seed borne fungi by various treatments over untreated control was calculated.

The effect of bioagents and fungicides on seed germination and seedling vigour index of cowpea was

studied by towel paper method [6]. The experiment was laid out in CRD with four replications/ treatments in the laboratory. Fifty seeds were placed on each towel paper and rolled carefully to avoid disturbances of seeds from their places, for each treatment eight towels of 50 seeds (400 seeds) were used. The rolled towel paper were kept in slanting position and incubated at 24 ^{0}C temp. with relative humidity above 85 per cent in seed germinator [2]. A count of normal seedlings was recorded after 7 days. The mean seed germination was calculated. The seed with full growth of plumule and radical were considered as normal. The root and shoot length (cm) of randomly selected 10 normal seedlings from each towel paper were measured and seedling vigour index was calculated by the formula given [1].

Seedling vigour index (SVI) = [Mean root length (cm) + mean shoot length (cm)] × percentage germination (%)Seed mycoflora

The data obtained in respect of seed germination and field emergence were transformed into arc sin values and subjected to statistical analysis.

Effect of bioagents on seed borne fungi

Significant reduction in seed borne fungi, increased seed germination, seedling vigour index and field emergence of cowpea was recorded with various treatments over untreated control (Table 1 and 2). The seed borne fungi *viz*., *Macrophomina phaseolina*, *Alternaria alternata, Fusarium oxysporum, Curvularia lunata* and *Botrytis cineria* were found pre dominant on seeds of cowpea.

Table 1. Efficacy of bio-agents on seed mycoflora of cowpea

Treatment	Year	Cowpea				
		Mycoflora associated with seed (%)				
		M.p	*A.a*	*F.o*	*C.l*	*B.c*
P. fluorescens @0.6%	2019-20	2	2	1	1	1
	2020-21	4	3	2	2	2
	2021-22	3	4	3	2	1
	Mean	9	9	6	5	4
	% red^n	40.00	30.76	40.00	44.44	42.85
T. viride @ *0.6%*	2019-20	1	1	1	1	0
	2020-21	3	3	2	1	1
	2021-22	2	3	2	2	1
	Mean	6	7	6	4	2
	% red^n	60.00	46.15	40.00	55.55	71.42
T. harzianum @ *0.6%*	2019-20	1	1	1	0	0
	2020-21	2	2	1	1	1
	2021-22	2	3	2	0	1
	Mean	5	6	4	1	2
	% red^n	66.66	53.84	60.00	88.88	71.42
P. fluorescens + *T. viride* @0.6 % each	2019-20	2	1	1	0	0
	2020-21	3	2	1	1	1
	2021-22	2	2	1	1	1
	Mean	7	5	3	2	2
	% red^n	53.33	61.53	70.00	77.77	71.42
P. fluorescens + *T. harzianum* @0.6 % each	2019-20	1	1	1	0	0
	2020-21	2	2	1	0	1
	2021-22	1	1	1	1	0
	Mean	4	4	3	1	1
	% red^n	73.33	69.23	70.00	88.88	85.71
Thiram + Carbendazim @ 0.2 % each	2019-20	0	0	0	0	0
	2020-21	1	1	0	0	0
	2021-22	1	1	1	0	0
	Mean	2	2	1	0	0
	% red^n	86.66	84.61	90.00	100.00	100.00
Untreatedcontrol	2019-20	4	3	2	2	2
	2020-21	5	4	3	4	3
	2021-22	6	6	5	3	2
	Mean	15	13	10	9	7
	% red^n	—	—	—	—	

Where, *M. p* : *Macrophomina phaseolina* *A.a : Alternaria alternata* *F. o : Fusarium oxysporum*
C. l : Curvularia lunata *B.c : Botrytis cinerea*

The results (Table 1) indicated that the treatment to cowpea seeds with recommended fungicides and bioagents significantly reduced the occurrence of mycoflora on the seeds of cowpea. Thiram + Carbendazim @ 0.2 per cent each reduced the seed mycoflora by 84.61 to 100 per cent. The results are more or less in agreement with published data [10]. They reported significant reduction in seed mycoflora of cowpea with Carbendazim. Among the bio-control agents, seed treatment with *P. fluorescens* + *Trichoderma harzianum* @ 0.6 per cent each was found most effective in reducing the seed mycoflora, this was followed by treatment with *Pseudomonas fluorescens* + *Trichoderma viride* over untreated control. There was 77.33, 69.23, 70.00, 88.88 and 85.71 per cent reduction in *Macrophomina phaseolina*, *Alternaria alternata, Fusarium oxysporum, Curvularia lunata* and *Botrytis cineria* with the treatment of *P. fluorescens* + *Trichoderma harzianum* @ 0.6 per cent each respectively over control. Similar, reduction of many seed borne pathogens by *Trichoderma viride* and *P. fluorescens* were recorded earlier [7,17,5].

Treatment of cowpea seed with Thiram + Carbendazim @ 0.2 per cent each significantly increased seed germination (90.25%), seedling vigour index 2802.12 and field emergence (83.83%). The seed germination, seedling vigour index and field emergence in untreated control was 79.42 per cent, 2377.15 and 72.92 per cent, respectively. Thus seed germination, seedling vigour index and field emergence of cowpea was increased by 13.64, 17.88 and 14.97 per cent, respectively with the seed treatment of Thiram + Carbendazim over untreated control. Previous investigators [10] have reported that seed treatment of cowpea seeds with Carbendazim (0.2%) recorded minimum incidence of fungal diseases and highest seed germination. Similar results were also reported by other scientists [12,13,14 and 15] showing effectiveness of Thiram + Carbendazim seed treatment for inhibiting seed borne pathogens, increasing seed germination and seedling vigour index in safflower, soybean and pearl millet, respectively.

Among the bioagents, seed treatment with talc based formulation of *P. fluorescens* + *Trichoderma harzianum* was found effective in increasing the seed germination, seedling vigour index and field emergence by 11.86, 14.54 and 12.69 per cent, respectively over untreated control. The results are more or less in agreement with published data [4]. They reported the effectiveness of *P. fluorescens* + *Trichoderma viride* for increasing seed

Table 2. Efficacy of bioagents on seed germination, seedling vigour index and field emergence of cowpea

Treatment	Mean seed germination (%)					Mean seedling vigour index					Mean field emergence (%)				
	2019-20	2021-22	2021-22	Pooled mean	% Inc. over control	2019-20	2021-22	2021-22	Pooled mean	% Inc. over control	2019-20	2021-22	2021-22	Pooled mean	% Inc. over control
Pseudomonas fluorescens @ 0.6%	82.25 (65.11)	80.50 (63.86)	83.00 (65.68)	81.92 (64.84)	3.15	2537.57	2471.00	2620.16	2542.91	6.97	75.00 (60.04)	75.25 (60.26)	76.00 (60.74)	75.42 (60.28)	3.43
Trichoderma viride @ 0.6%	84.00 (66.47)	82.00 (64.93)	84.00 (66.43)	83.33 (65.91)	4.93	2570.90	2511.30	2604.60	2562.27	7.79	77.00 (61.45)	76.75 (61.22)	77.00 (61.40)	76.92 (61.29)	5.49
T. harzianum @ 0.6%	85.25 (67.48)	83.50 (66.12)	85.00 (67.24)	84.58 (66.89)	6.51	2623.37	2568.70	2692.50	2628.19	10.56	80.00 (63.48)	79.50 (63.12)	78.00 (62.16)	79.17 (62.85.)	8.57
P. fluorescens + *T. viride* @ 0.6% each	87.25 (69.11)	86.25 (68.37)	87.00 (68.98)	86.83 (68.73)	9.34	2714.52	2653.00	2714.60	2694.04	13.33	82.00 (64.95)	80.75 (64.03)	80.00 (63.51)	80.92 (64.10)	10.97
P. fluorescens + *T. harzianum*@ 0.6% each	89.50 (71.23)	88.00 (69.87)	89.00 (70.68)	88.83 (70.49)	11.86	2736.27.	2695.80	2736.30	2722.79	14.54	83.00 (66.16)	81.50 (64.58)	82.00 (65.28)	82.17 (65.02)	12.69
Thiram + Carbendazim @ 0.2% each	90.75 (72.35)	90.00 (71.67)	90.00 (71.62)	90.25 (71.81)	13.64	2777.97	2816.20	2812.20	2802.12	17.88	84.75 (67.13)	83.75 (66.38)	83.00 (65.74)	83.83 (66.30)	14.97
Untreated Control	80.25 (64.27)	78.00 (72.03)	80.00 (63.45)	79.42 (63.03)	—	2350.12	2299.20	2482.12	2377.15	—	72.00 (58.10)	73.75 (59.21)	73.00 (58.79)	72.92 (58.64)	—
SE+	1.76	1.25	1.20	0.42	—	75.82	37.95	49.20	32.97	—	1.80	1.34	2.17	0.31	—
CD at 5%	5.17	3.68	2.50	1.29	—	223.01	111.6	102.33	100.00	—	5.36	3.99	4.56	0.98	—

Figures in parenthesis indicates arc-sin transformed values

germination, seedling vigour index and field emergence in sunflower. The Scientist [10] evaluated the response of bioagents on seed mycoflora and seed quality parameters of cowpea and reported that the seeds treated with bioagents were found beneficial in reducing the pathogenic fungi and increasing the seed germination.

In recent years much attention has been given to non chemical systems for seed treatment to provide protection against seed borne pathogens. However, the present study has shown that the treatment of cowpea seeds with Thiram + Carbendezim was most effcetive for controlling seed borne pathogens and increasing seed germination. Among bioagents, treatment of seeds of cowpea with *P. fluorescens* + *Trichoderma harzianum* @ 0.6 per cent each was found effective in reducing seed borne fungi, increasing seed germination, seedling vigour index and field emergence. Thus, if necessary, as in case of organic production of cowpea, these bioagents can sucessfully be used.

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