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Biocontrol Strategies for Managing Seed Borne Pathogens in Mungbean (*Vigna radiata* L.): Efficacy and Potential

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ABSTRACT: During present investigation it is observed that Biopriming a new technique that integrates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects of disease control used as an alternative method for controlling many seed and soil borne pathogens. It is an ecological approach in which selected fungal antagonists used against soil and seed borne pathogens. Biological seed treatments provide an alternative to chemical control with additional benefits of induced diseases resistance, ecofriendly nature and sustainable disease management. Mungbean (*Vigna radiata*) is susceptible to seed-borne pathogens, causing significant yield losses. Biopriming, a non-chemical seed treatment, was evaluated for its efficacy in managing seed-borne pathogens. *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* are different bio control agents frequently used for biopriming treatment in present investigation. Also it shows reduction in disease incidence, increase seed germination and seedling vigour.

Keywords: Biopriming, Mungbean, Seed-borne pathogens, *Trichoderma harzianum, Pseudomonas fluorescens, Bacillus subtilis.*

INTRODUCTION

Green gram (*Vigna radiata* L.) is predominantly self-fertilizing and its chromosome number is 2n=22 in family Fabaceae. It is most important pulse crop native of India. Mungbean (*Vigna radiata* L.) is predominantly self-fertilizing and its chromosome number is 2n-22 in the family Fabaceae. It is important pulse crop native of India. Mungbean is one of the most ancient crops among cultivated plants. It contains 26% protein, 51% carbohydrate, 10% moisture, 4% minerals, and 3% vitamins. It is also rich in Ca, Fe, K and is an excellent source of vitamins such as thiamine, niacin, and vitamin A. Although systematic efforts have been made to upgrade pulses production in order to meet the minimum dietary requirements such efforts are quite inadequate due to various constraints like poor cultivation practices, availability of quality seeds of improved cultivar is considered crucial for realizing productivity and adoption of cultivars in different agro-climatic conditions.

Poor germination and poor seedling vigour are due to contamination of seeds which results in an unhealthy plant. Field pathogens that are associated with seeds cause depletion in seed quality, affect the viability and decrease germination percentage Kantha and Vaidehi (1980). A large number of fungi were found to be associated with the mung bean seeds. *Alternaria* sp., *Fusarium* sp, *Aspergillus flavus, A. niger and Macrophomina* sp. were found in germinating seed and seedling of mung bean Pradhan (2017). Mungbean is produced primarily for its protein content.

Seed borne mycoflora associated with mung bean reported recently include *Aspergillus* sp, *Alternaria* sp, *Fusarium* sp, *Macrophomina* sp., *Penicillium* sp., *Rhizopus* sp. These fungi reduce the germination percentage, viability and vigour of seeds. Because of seed borne infections, there is a reduction in production and incapable to satisfy the requirements for mungbean seeds. Some control measures may be useful for increasing the supply to meet the demand. Seed borne mycoflora are more easily managed as compared to air-borne and soil-borne mycoflora. Seed priming is a quality enhancement technique for rapid uniform germination of seeds and optimum plant stand in the field. This technique is often used as a seed invigoration treatment for improving germination and vigour in low vigour lots. Hence, it appears to reverse the detrimental effects of seed deterioration (Srinivasan *et al.*, 2009).

Biopriming is a new technique of that integrates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects of disease control. It is recently used as an alternative method for controlling many seed and soil borne pathogens. It is an ecological approach using selected fungal antagonists against soil and seed borne pathogens. Biological seed treatments provide an alternative to chemical control with additional benefits of induced diseases resistance,

ecofriendly nature and sustainable disease management. *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens and Bacillis subtilis* are different bio control agents frequently used for biopriming treatment. Several researchers have investigated the use of beneficial micro-organisms in the priming medium to control disease proliferation during priming itself (Warren and Bennet 2000). The main causes for poor yield in mungbean are fungal, viral and bacterial diseases. The amount of yield loss depends on the intensity of the disease and environmental conditions. Several researchers have investigated the use of beneficial micro-organism in the priming medium to control diseases proliferation during priming itself (Waren and Bennet 2000). *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens and Bacillis subtilis.*

MATERIALS AND METHODS

Present investigation were carried out to study the effect of biopriming on mungbean in the PGI glasshouse M.P.K.V., Rahuri. The experimental seed crop was raised during kharif 2022-2023 in glasshouse conditions. Seeds of mungbean variety phule chetak was selected. The seeds were inoculated by dipping the seed in concentrated suspension of spores/active hyphae of important seed mycoflora of mungbean for 12 hours and then seeds were dried under shade for 12 hours. The biopriming of bioagents to the artificially inoculated seeds of mungbean was given in plastic vessels. Talc based formulations of the bioagents were weighted on weighing balance as per the dose and mixed with water. Then seeds were soaked in the volume of respective concentration in each of the biopriming agents for 8 hours. After soaking the seeds were removed from the solutions and dried in shade up to original moisture content (8-10%) The inoculated and untreated seeds were served as control (Meena *et al.*, 2016). A portion of seeds of each bio-priming treatments was used for pot culture.

The study of percent disease incidence was recorded applying 0-9 grade disease rating scale (Mayee and Datar 1986). The Incidence was calculated by using following formula.

$$\text{Per cent disease intensity} = \frac{\text{Sum of total rating} \times 100}{\text{Total number of observation} \times \text{Highest grade in the scale}}$$

$$\text{Per cent disease intensity} = \frac{\text{PDI in control - PDI in treatment} \times 100}{\text{PDI in control}}$$

The root and shoot length (cm) of randomly selected seedlings from each treatment were measured with the help of measuring scale and seedling vigour index was computed by using formula given by Abdul Baki and Anderson (1973)

Biopriming agents and their Duration of soaking:

Sr. No.	Treatment	Hour of soaking
1.	*Trichoderma viride* @10g/kg seed	08
2.	*Trichoderma harzianum* @10g/kg seed	08
3.	*Pseudomonas fluorescens* @10g/kg seed	08
4.	*T. viride* + *P. fluorescens* @ 5 g each/kg seed	08
5.	*T. harzianum* + *P. fluorescens* @ 5 g each/kg seed	08
6.	*Bacillus subtilis* @ 10 g each/kg seed	08
7.	Control	-

RESULT AND DISCUSSION

The result in respect of efficacy of seed biopriming with bioagents on incidence of seed borne *Fusarium oxysporum*, seed germination and seedling vigour index of artificially infected seeds of mungbean (phule chetak) are presented in Table 1. All treatments were statistically significant over control in respect of incidence of seed borne *Fusarium oxysporum*, seed germination and seedling vigour index. The bioprimed seeds with *Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5 g each/kg seed was found most effective followed by *T. viride*+*P. fluorescens.* The results of present are in agreement with Mukhopadhyaay (1989); Kumar and Dube (1992); Mahamune and Kakde (2011).

The result in respect of efficacy of seed biopriming with bioagents on incidence of seed borne *Aspergillus niger*, seed germination and seedling vigour index of artificially infected seeds of mungbean (phule chetak) are presented in Table 2. All treatments were statistically significant over control in respect of incidence of seed borne *Aspergillus niger*, seed germination and seedling vigour index. The seed biopriming with bioagent *Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5 g each/kg seed was found most effective followed by *T. viride* + *P. fluorescens.* The results of present study are in agreement with Pradeep *et al.* (2000); Koche *et al.* (2009); Mahamune and Kakde (2011); Gawade *et al.* (2016).

The result in respect of efficacy of seed biopriming with bioagents on incidence of seed borne *Alternaria alternata*, seed germination and seedling vigour index of artificially infected seeds of mungbean (phule chetak) are presented in Table 3. All treatments were statistically significant over control in respect of incidence of seed borne *Alternaria alternata*, seed germination and seedling vigour index. The seed biopriming with bioagent *Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5 g each/kg seed was found most effective, followed by *T. viride* + *P. fluorescens*@ 5 g. The results of present study, on effect of various bioagents on the incidence of seed borne fungi, seed germination and seedling vigour index are in agreement with Koche *et al.* (2009).

The result in respect of efficacy of seed biopriming with bioagents on incidence of seed borne *Penicillium* spp. seed germination and seedling vigour index of artificially infected seeds of mungbean (phule chetak) are presented in Table 4. All treatments were statistically significant over control in respect of incidence of seed borne *Penicillium* spp., seed germination and seedling vigour index. The seed biopriming with bioagent *Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5 g each/kg seed was

found most effective followed by *T. viride* + *P. fluorescens*@ 5 g each/kg seed The results of present study, on effect of various bioagents on the incidence of seed borne fungi, seed germination and seedling vigour index are in agreement with Bodhe (2019).

The result in respect of efficacy of seed biopriming with bioagents on incidence of seed borne *Aspergillus flavus*, seed germination and seedling vigour index of artificially infected seeds of mungbean (phule chetak) are presented in Table 5. All treatments were statistically significant over control in respect of incidence of seed borne *Aspergillus flavus*, seed germination and seedling vigour index. The seed bioprimng with bioagent *Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5 g each/kg seed was found most effective followed by *T. viride* + *P. fluorescens*@ 5 g each/kg seed The results of present study, on effect of various bioagents on the incidence of seed borne fungi, seed germination and seedling vigour index are in agreement with Pradeep *et al.* (2000), Koche *et al.* (2009); Gawade *et al.* (2016); Bodhe (2019).

The result in respect of efficacy of seed biopriming with bioagents on incidence of seed borne *Colletotrichum* spp, seed germination and seedling vigour index of artificially infected seeds of mungbean (Phule chetak) are presented in Table 6**.** All treatments were statistically significant over control in respect of incidence of seed borne *Colletotrichum* spp, seed germination and seedling vigour index. Among tretments, biopriming with *Trichoderma harzianum* +*Pseudomonas fluorescens* @ 5 g each/kg seed was found most effective, followed by *T. viride*+*P. fluorescens* @ 5 g each/kg seed. The present results on effect of various bioagents on seed borne mycoflora, seed germination and seedling vigour index in mungbean are in agreement with Shivanna and Shetty (1989).

Table 1: Efficacy of seed biopriming with bioagents on incidence of seed borne *Fusarium oxysporum* (Artificially inoculated to seed) and their effect on seed germination and seedling vigour index of mungbean.

Sr. No.	Treatment	Incidence of *F. oxysporum* (%)	Reduction in incidence over Control (%)	Seed germination (%)	Increase in seed germination over Control (%)	Seedling Vigour Index (SVI)	Increase in SVI over Control (%)
1.	*Trichoderma viride*@ 10g/kg seed	21.03 (27.26)	65.75	70.12 (70.00)	16.66	2105.83	23.61
2.	*Trichoderma harzianum*@ 10g/kg seed	18.03 (25.08)	70.49	71.12 (71.25)	18.33	2216.35	30.10
3.	*Pseudomonas fluorescens* @10g/kg seed	22.04 (27.95)	63.93	69.12 (67.45)	15.00	2074.78	21.79
4.	*Trichoderma viride* + *Pseudomonas fluorescens* @5g/kg seed	17.02 (24.33)	72.13	73.13 (71.86)	21.66	2313.18	35.79
5.	*Trichoderma harzianum* + *Pseudomonas fluorescens* @5g/kg seed	15.05 (22.77)	75.40	74.11 (75.82)	23.33	2412.01	41.59
6.	*Bacillus subtilis*	52.08 (46.12)	14.75	67.10 (53.71)	11.66	1908.18	12.03
7.	Control	61.10 (51.33)		60.11 (49.58)		1703.82	
	S.E.±	0.579		1.172		35.75	
	CD at 1%	2.435		4.933		150.5	

(Figures in parentheses indicates arc sin transformed values)

Table 2: Efficacy of seed biopriming with bioagents on incidence of seed borne *Aspergillus niger* (Artificially inoculated to seed) and their effect on seed germination and seedling vigour index of mungbean.

Sr. No.	Treatment	Incidence of A. niger (%)	Reduction in incidence over Control (%)	Seed germination (%)	Increase in seed germination over Control (%)	Seedling Vigour Index (SVI)	Increase in SVI over Control (%)
1.	Trichoderma viride@ 10 g/kg seed	20.03(26.55)	70.14	78.13(66.01)	20.00	2137.22	32.80
2.	Trichoderma harzianum @ 10 g/kg seed	18.03(25.08)	73.13	79.13(62.71)	21.53	2152.58	33.75
3.	Pseudomonas fluorescens@ 10 g/kg seed	21.04(18.73)	68.66	76.12(60.64)	16.92	1991.31	23.73
4.	Trichoderma viride +Pseudomonas fluorescens @ 5 g each/kg seed	16.04(27.26)	76.11	80.12(63.41)	23.07	2272.11	41.18
5.	Trichoderma harzianum + Pseudomonas fluorescens @ 5 g each/kg seed	15.02(22.74)	77.61	81.14(64.15)	24.61	2429.34	50.95
6.	Bacillus subtilis	55.09(47.85)	17.91	72.10(58.03)	10.76	1800.00	12.03
7.	Control	67.11(54.91)		65.11(53.70)		1602.67	
	S.E.±	0.612		1.286		34.988	
	CD at 1%	2.576		5.415		147.29	

Figures in parentheses indicates arc sin transformed values)

Table 3: Efficacy of seed biopriming with bioagents on incidence of seed borne *Alternaria alternata* (Artificially inoculated to seed) and their effect on seed germination and seedling vigour index of mungbean.

Sr. No.	Treatment	Incidence of *A. alternata* (%)	Reduction in incidence over Control (%)	Seed germination (%)	Increase in seed germination over Control (%)	Seedling Vigour Index (SVI)	Increase in SVI over Control (%)
1.	*Trichoderma viride*@ 10 g/kg seed	21.04 (27.26)	63.15	72.12 (58.02)	18.03	2265.43	30.58
2.	*Trichoderm harzianum*@ 10 g/kg seed	19.03 (25.83)	66.66	73.11 (58.67)	19.67	2293.82	32.21
3.	*Pseudomonas fluoescens*@ 10 g/kg seed	22.04 (27.95)	61.40	71.10 (57.39)	16.39	2227.04	28.36
4.	*Trichoderma viride +Pseudomonas fluorescens* @ 5g/kg seed	17.03 (24.33)	70.17	74.12 (59.32)	21.31	2453.08	41.39
5.	*Trichoderma harzianum + Pseudomonas fluorescens* @ 5g/kg seed	16.03(23.56)	71.92	76.13 (60.64)	24.59	2576.95	48.53
6.	*Bacillus subtilis*	49.08 (44.40)	14.03	68.11 (55.52)	11.47	2061.76	18.84
7.	Control	58.10 (49.58)		61.10 (51.33)		1749.24	
	S.E.±	0.557		1.198		37.93	
	CD at 1%	2.346		5.046		159.7	

(Figures in parentheses indicates arc sin transformed values)

Table 4: Efficacy of seed biopriming with bioagents on incidence of seed borne *Penicillium* spp. *(*Artificially inoculated to seed) and their effect on seed germination and seedling vigour index of mungbean.

Sr. No.	Treatment	Incidence of *Penicillium* spp. (%)	Reduction in incidence over Control (%)	Seed germination (%)	Increase in seed germination over Control (%)	Seedling Vigour Index (SVI)	Increase in SVI over Control (%)
1.	*Trichoderma viride*@ 10 g/kg seed	19..03 (25.82)	66.07	68.12 (55.52)	17.24	2243.06	25.90
2.	*Trichoderma harzianum*@ 10 g/kg seed	18.02 (25.08)	67.85	72.11 (58.03)	24.13	2281.46	28.05
3.	*Pseudomonas fluorescens* @ 10 g/kg seed	21.04 (27.26)	62.50	67.11 (54.92)	15.51	2171.61	21.88
4.	*Trichoderma viride +Pseudomonas fluorescens* @ 5g each/kg seed	17.03 (24.33)	69.64	73.12 (58.68)	25.86	2354.92	32.21
5.	*Trichoderma harzianum + Pseudomonas fluorescens* @ 5g each/kg seed	15.03 (22.77)	73.21	74.12 (59.32)	27.58	2425.04	36.11
6.	*Bacillus subtilis*	49.08 (44.40)	12.50	64.11 (53.11)	10.34	2061.43	15.72
7.	Control	56.09 (48.42)		58.10 (49.58)		1781.62	
	S.E.±	0.541		1.154		37.10	
	CD at 1%	2.280		4.857		156.22	

(Figures in parentheses indicates arc sin transformed values)

Table 5: Efficacy of seed biopriming with bioagents on incidence of seed borne *Aspergillus flavus* (Artificially inoculated to seed) and their effect on seed germination and seedling vigour index of mungbean.

Sr. No.	Treatment	Incidence of *A. flavus* (%)	Reduction in incidence over Control (%)	Seed germination (%)	Increase in seed germination over Control (%)	Seedling Vigour Index (SVI)	Increase in SVI over Control (%)
1.	*Trichoderma viride*@ 10 g/kg seed	17.03 (24.33)	70.17	73.12 (58.68)	17.74	2160.25	28.04
2.	*Trichoderma harzianum*@ 10 g/kg seed	15.03 (22.77)	73.68	74.12 (59.33)	19.35	2226.71	32.51
3.	*Pseudomonas fluorescens*@ 10 g/kg seed	19.01 (25.82)	66.66	71.11 (57.39)	14.51	2092.48	24.02
4.	*Trichoderma viride +Pseudomonas fluorescens*@ 5g each/kg seed	14.02 (21.95)	75.43	75.12 (59.97)	20.96	2278.79	35.06
5.	*Trichoderma harzianum + Pseudomonas fluorescens*@ 5g each/kg seed	12.02 (20.26)	78.94	77.12 (61.32)	24.19	2422.36	43.57
6.	*Bacillus subtilis*	49.08	14.03	69.13	11.29	1903.17	12.80

		(44.40)		(56.15)			
7.	Control	57.10 (49.00)		62.10 (51.92)		1686.31	
	S.E.±	0.529		1.213		35.83	
	CD at 1%	2.230		5.107		150.8	

(Figures in parentheses indicates arc sin transformed values

Table 6: Efficacy of seed biopriming with bioagents on incidence of seed borne *Colletotrichum* spp (Artificially inoculated to seed) and their effect on seed germination and seedling vigour index of mungbean.

Sr. No.	Treatment	Incidence of *Colletotrichum*. spp (%)	Reduction in incidence over Control (%)	Seed germination (%)	Increase in seed germination over Control (%)	Seedling Vigour Index (SVI)	Increase in SVI over Control (%)
1.	*Trichodera viride*@10g/kg seed	20.02 (26.55)	64.21	70.12 (56.76)	18.64	2167.60	21.23
2.	*Trichodera harzianum*@10g/kg seed	17.03 (24.34)	69.64	71.12 (57.40)	20.33	2218.35	24.07
3.	*Pseudomonas fluorescens* @10g/kg seed	21.04 (27.26)	62.50	69.15 (56.15)	16.94	2159.25	20.76
4.	*Trichoderma viride* +*Pseudomonas fluorescens* @5g each/kg seed	16.02 (23.56)	71.42	73.12 (58.68)	23.72	2294.82	28.34
5.	*Trichoderma harzianum* + *Pseudomonas fluorescens* @5g each/kg seed	15.03 (22.77)	73.21	74.11 (59.32)	25.42	2374.95	32.82
6.	*Bacillus subtilis*	48.08 (43.43)	14.28	65.11 (53.70)	10.16	1990.31	11.31
7.	Control	56.09 (48.42)		59.10 (50.16)		1787.98	
	S.E.±	0.537		1.166		36.30	
	CD at 1%	2.260		4.905		152.82	

(Figures in parentheses indicates arc sin transformed val

CONCLUSIONS

Among all the seed biopriming treatments, seed biopriming with *Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5 g each/kg seed recorded lowest seed mycoflora, increasing seed germination and seedling vigour index in naturally infected seed as well as artificially inoculated seed with all seed borne *Fusarium oxysporum, Colletotrichum lindemuthianum, Alternaria alternata, Aspergillus niger, Aspergillus flavus,* and *Penicillium* spp.

FUTURE SCOPE

The finding of study support the use of novel biopriming agents: Investigate new biocontrol agents, such as plant growth-promoting rhizobacteria (PGPR), *Trichoderma* spp., and *Bacillus* spp., for enhanced efficacy. Develop optimized formulations and delivery methods for biopriming agents, including seed coatings, pellets, or liquid applications. Biopriming's impact on seed quality and vigor: Study effects on seed germination, emergence, and overall seed health. Investigating biopriming's efficacy against emerging seed-borne pathogens: Monitor and adapt biopriming strategies to address evolving pathogen population.

Among all the seed biopriming treatment, seed biopriming with *Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5g each/kg seed recorded highest pot emergence and lowest incidence of disease. Among all the seed biopriming treatments, seed biopriming with *Trichoderma harzianum Pseudomonas fluorescens* @ 5g each/kg seed was found most effective in pot tested characters *viz.*, seed germination, seed emergence, and seedling vigour

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