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Management of Dry Root Rot of Mungbean Caused by *Macrophomina phaseolina* Through Organic Amendments, Plant Extracts and Bio-agents

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ABSTRACT

Background: Mungbean [*Vigna radiata* (L.) Wilczek], commonly recognized as green gram, is the most significant and advanced pulse crop grown in Rajasthan. Dry root rot is one of the important diseases of mungbean caused by *M. phaseolina,* which is responsible for 30 to 40 per cent disease incidence with huge yield loss during seedling to mature stages.

Methods: The experiments were conducted for two consecutive years during *kharif* 2020 and 2021 for managing the disease through organic amendments, plant extracts and bio-agents *viz., Trichoderma harzianum* (10 g/kg seeds), garlic extract (15%), neem cake (0.5 t/ha) and mustard cake (0.5 t/ha) alone and in combinations through seed as well as soil applications.

Result: Our investigations cleared that the maximum disease reduction and highest seed yield were reported by treating the seeds with *T. harzianum* @10 g/kg seed + soil application of mustard cake @ 0.5 t/ha followed by seed treatment with *T. harzianum* @ 10 g/kg seed + soil application of neem cake @ 0.5 t/ha. In view of consumer health, present findings may be helpful for growers to get extra benefits by producing organic mungbean.

Key words: Bio-agents, Dry root rot, *M. phaseolina,* Mungbean, Organic amendment, Plant-extract.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek], vernacularly known as green gram, is the most important and nutritious pulse crop grown in Rajasthan and India. Mungbean has also been cultivated as a green manure crop to fix impressive nitrogen *via* reciprocal nitrogen fixation, which contributes to soil fertility. The seeds are extremely nutritious; contain around 62.6% carbohydrates, 23.9% protein, 1.15% fat, 5.27% crude fiber and 3.32% ash (Afzal *et al.,* 2008).

Several abiotic and biotic factors affect the growth and development of mungbean leading to qualitative and quantitative yield losses. Diseases are most damaging and major limiting factors that cause the largest economic losses in profitable cultivation of this crop in Rajasthan. Amongst fungal diseases, dry root rot of mungbean caused by *M. phaseolina* is one of the important soil borne diseases.

Warm climatic conditions and water stress encourage dry root rot. It has become a serious mungbean and urdban disease in Myanmar, as well as a spreading disease in South Asian nations such as India, Pakistan and Thailand (Dambal *et al.,* 2019; Khan and Shuaib, 2007). In recent years, higher levels of susceptibility to dry root rot in mungbean have resulted from more appropriate water stress at some point throughout the crop year as a result of atypical and lower rainfall and extended temperatures, resulting in higher economic losses.

The pathogen overwinters in soil or crop residues as microsclerotia for up to 3 years (Su *et al*., 2001). The microsclerotia act as the source of primary inoculum (Gahlot, 2018). Its life cycle consists of four phases, namely germination phase, penetration phase, parasitic phase and saprophytic phase (Dhingra and Sinclair, 1978). The pathogen spreads from one plant to its adjacent plants through the soil. It spreads through the vascular system within the infected plants (Gangopadhyay *et al.,* 1970). During infection, *M. phaseliona* produces toxins such as botryodilodin and phaseolinone which help the pathogen to infect susceptible plants from soil reservoirs, particularly over the winter (Abbas *et al.,* 2019). After germination, the pathogen attacks the basal portion of the plant and produces characteristic symptoms like irregular necrotic lesions on the root sarcastic extending towards hypocotyls and turn into a black color that finally causes rotting. Roots of the affected plants get rotten and become blackish, leading

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eventually to the death of the plants and resulting to complete yield loss. However, if it occurs after the initiation of flowering, some mature pods with seeds may be obtained. Eradication of this soil-borne pathogen is difficult because of its polyphagous nature and its survival in the soil through its resting structures. The leaves of the infected plant turn yellow and drop within 2-3 days. The infected plants become feeble and when dry, exhibit dark ashy lesions on the stem. The lesions joint together and develop larger patches on branches leading to plants wilted shortly and ultimately dying in the early stages (Khan *et al.,* 2017).

MATERIALS AND METHODS

Evaluation of treatments under pot conditions

The pot culture experiment was conducted in 20 cm (9×12 inches) diameter earthen pots. The sand sorghum inocula of highly pathogenic isolate (Durgapura) of *M. phaseolina* was mixed with the soil thoroughly at 20 g kg^{-1} soil and transferred to earthen pots containing sterilized soil. As mentioned earlier, the pathogen infested soil was allowed for stabilization of inoculum for 72 hours before sowing of mungbean seeds. The pathogen was multiplied on sterilized sorghum grains side by side. For seed dressing with the bioagents, the desired quantity of talc-based formulation of bio-agent was added to 50 g mungbean seeds (cv. RMG-62) in 100 ml Erlenmeyer flask and shaken thoroughly to give uniform coating of the preparations. For soil application, the desired amount of talc-based formulation of test bio-agent was mixed well in upper 10 cm soil of pots. The organic manure (mustard and neem cake) were thoroughly mixed as per recommended dose of nitrogen supplied by each manure in each pot @ 10 per cent (w/w) of soil before one month of sowing (Lumsden *et al.*, 1983). The seeds treated with organic amendments were sown in pots containing *M. phaseolina* inoculated soil. The inoculum was added @ 20 g in each pot and mixed thoroughly upto 5-7 cm depth in the pot. In each pot 10 seeds were sown according to treatment. Ten mungbean seeds were sown in each pot. Each treatment was replicated thrice. Surface sterilized seed sown without organic manure with inoculated pot served a check. The pots were irrigated usually on alternate day with uniform quantity of water.

Evaluation of treatments under field conditions

A field experiment was conducted for two consecutive years during *kharif* 2020 and 2021 to manage dry root rot through bio-agents, mustard cake and neem cake using mungbean variety RMG-62 with spacing of 30 cm × 10 cm under RBD with three replications. Talc based formulations of *T. harzianum* and garlic extract were used as seed treatment (ST) and soil application (SA) as well as in combinations of ST + SA. The bio-agent and plant extract were used for combined treatment of seed and soil. Mustard cake and neem cake were used @ 0.5 tonnes/ha before sowing. The effect of bio-agent as seed treatment alone as well as in combinations with soil application and farm yard manure was tried. The trial was conducted under artificial soil inoculation conditions. For this purpose, inoculum multiplied on sand+sorghum grains was applied at 50 g per plot (4 × 3 m^2) before sowing and mixed thoroughly on top surface of soil. Standard agronomic practices were followed to raise the crop.

RESULTS AND DISCUSSION

Evaluation of organic amendments, bio-agents and plant extracts under pot conditions

The results (Table 1) revealed that among different treatments, seed treatment with *Trichoderma harzianum* @ 10 g/kg seed+soil application with mustard cake @ 0.5 t/ha

Table 1: Integrated management of dry root rot through organic amendments, bio-agents and plant extracts under pot conditions.

Treatments	% Disease incidence*		Pooled*	% Disease reduction
	2020	2021		
ST with *Trichoderma harzianum* @ 10 g/kg seed	26.67 (31.07)	29.44 (32.84)	28.06 (31.96)	47.28
ST with garlic extract @ 15%	35.66 (36.65)	37.03 (37.46)	36.35 (37.06)	31.71
Soil application with neem cake @ 0.5 t/ha	31.11 (33.88)	35.55 (36.58)	33.33 (35.24)	37.38
Soil application with mustard cake @ 0.5 t/ha	29.44 (32.84)	34.44 (35.92)	31.94 (34.38)	39.99
ST with *Trichoderma harzianum* @ 10g /kg seed+ Soil application with neem cake @ 0.5 t/ha	17.78 (24.92)	19.44 (26.13)	18.61 (25.54)	65.04
ST with *Trichoderma harzianum* @ 10 g/kg seed+ Soil application with mustard cake @ 0.5 t/ha	16.20 (23.64)	18.70(25.60)	17.45 (24.67)	67.2
ST with garlic extract @ 15%+ Soil application with neem cake @ 0.5 t/ha	25.74 (30.47)	28.24 (32.09)	26.99 (31.28)	49.29
ST with garlic extract @ 15% + Soil application with mustard cake @ 0.5 t/ha	22.40 (28.24)	26.48 (30.93)	24.44 (29.60)	54.09
Control	51.38 (45.77)	55.08 (47.90)	53.23 (46.83)	0.0
C.D. at 5%	3.23	3.75	5.40	-
SEm±	1.08	1.25	1.66	-
CV (%)	6.56	6.87	7.84	-

*Figures given in parenthesis are angular transformed.

was gave the lowest disease incidence in 2020, 2021 and at pooled level (16.20, 18.70 and 17.45%, respectively) whereas the least effective treatment was seed treatment with garlic extract @15%, which revealed 35.66, 37.03 and 36.35 per cent disease. Maximum per cent disease reduction (67.20%) was recorded with seed treatment with *Trichoderma harzianum* @ 10 g/kg seed+soil application with mustard cake@ 0.5 t/ha followed by seed treatment with *Trichoderma harzianum* @ 10 g/kg seed+soil application with neem cake @ 0.5 t/ha (65.04%) while minimum per cent disease reduction (31.71%) was recorded seed treatment with garlic extract @ 15%.

Evaluation of organic amendments, bio-agents and plant extracts under filed conditions

Before sowing the seeds, the bio-agent and plant extract were employed with organic amendments. In this study, the effect of bio-agent as a seed treatment alone and in conjunction with soil application of organic amendment was investigated. At harvest, measurements were taken of root and shoot length, dry weight, disease incidence and grain yield. The root, shoot lengths and dry weight of mungbean plants (Table 2) significantly increased due to the bio-agent and plant extract used as seed treatment enriched with organic amendment (@ 0.5 t/ha) before sowing of seeds. The maximum root lengths, shoot lengths and dry weight was recorded in treatment, T_6 (Seed treatment with *Trichoderma harzianum*@ 10g kg^{-1} seed+ Soil application with mustard cake @ 0.5 t/ha),which showed root lengths of 18.65 cm, shoot lengths of 45.60 cm and root dry weight of 2.75 g, shoot dry weight of 12.80 g, while minimum root, shoot lengths and dry weight were observed in T_2 (seed treatment with garlic extract @ 15%), which revealed 13.15 cm, 37.3 cm, 1.45 g and 8.50 g, respectively.

In field management study (Table 3), per cent disease incidence was monitored after 20, 30 and 40 days during *Kharif* seasons 2020 and 2021. Pooled analysis of 20,30 and 40 days after sowing, revealed that maximum per cent disease reduction 65.74%, 68.04% and 68.49% was recorded, respectively with T_6 (Seed treatment with *Trichoderma harzianum*@ 10 g kg^{-1} seed + Soil application with Mustard cake @ 0.5 t/ha). It was found significantly superior in all the treatments. This was followed by T_5 treatment (Seed treatment with *Trichoderma harzianum* @ 10 g kg^{-1}seed + Soil application with Neem cake @ 0.5 t/ha) which showed 61.30%, 62.03% and 65.96% disease reduction, respectively. The moderate per cent disease reduction was observed with T_8 treatment (Seed treatment with garlic extract @ 15% + Soil application with mustard cake @ 2 t ha^{-1}) which showed 55.32%, 52.62% and 56.10% after 20, 30 and 40 days after sowing, respectively. T_2 (Seed treatment with garlic extract @15%), was found least effective due to show minimum per cent disease reduction 19.55%, 19.59% and 22.46% after 20, 30 and 40 days after sowing, respectively.

The highest per cent grain yield (Table 4) was increased (170.44%) over control in T_6 (Seed treatment with

Table 2: Effect of bio-agent, plant-extract and organic amendment on the plant growth and dry weight of mungbean grown in *M phaseolina* inoculated pots.

Treatment	Shoot length (cm)		Pooled	Per cent increase over control	Root length (cm)		Pooled	Per cent increase over control	Shoot dry weight (g)		Pooled	Root dry weight (g)		Pooled
	2020	2021			2020	2021			2020	2021		2020	2021	
T_1- ST with *Trichoderma harzianum* @ 10 g/kg seed	42.2	41.6	41.9	33.65	15.8	15.2	15.50	64.02	10.80	9.90	10.35	2.10	2.00	2.05
T_2- ST with garlic extract @ 15%	37.8	36.8	37.3	18.98	13.4	12.9	13.15	39.15	8.80	8.20	8.50	1.50	1.40	1.45
T_3- Soil application with Neem cake @ 0.5 t/ha	38.9	38.2	38.55	22.97	13.9	13.4	13.65	44.44	9.40	8.90	9.15	1.80	1.70	1.75
T_4- Soil application with Mustard cake @ 0.5 t/ha	40.1	39.2	39.65	26.48	14.5	14.1	14.30	51.32	9.80	9.40	9.60	1.90	1.80	1.95
T_5- ST with *Trichoderma harzianum* @ 10 g/kg seed+ Soil application with Neem cake @ 0.5 t/ha	45.2	44.9	45.05	43.70	18.3	17.9	18.10	91.53	12.20	11.90	12.05	2.60	2.50	2.60
T_6- ST with *Trichoderma harzianum* @ 10 g/kg seed+ Soil application with Mustard cake @ 0.5 t/ha	45.9	45.3	45.60	45.45	18.8	18.5	18.65	97.35	13.10	12.50	12.80	2.80	2.70	2.75
T_7- ST with Garlic extract @ 15%+ Soil application with Neem cake @ 0.5t/ha	43.6	42.7	43.15	37.64	16.7	16.1	16.40	73.54	11.10	10.90	11.00	2.30	2.10	2.20
T_8- ST with Garlic extract @ 15% + Soil application with mustard cake@ 0.5t/ha	44.2	43.8	44.00	40.35	17.1	16.8	16.95	79.37	11.70	11.20	11.45	2.40	2.30	2.35
T_9- Control	31.8	30.9	31.35	0.0	9.8	9.1	9.45	0.0	6.90	6.60	6.75	1.20	1.10	1.15
C.D. at 5%	4.58	4.89	3.94	-	1.87	1.96	1.76	-	1.44	1.26	1.78	0.28	0.30	0.35
SEm±	1.51	1.62	1.19	-	0.62	0.65	0.53	-	0.47	0.42	0.54	0.09	0.10	0.11
CV (%)	6.39	6.94	4.13	-	6.98	7.54	4.96	-	7.89	7.24	7.48	7.82	8.65	7.42

Table 3: Integrated management of dry root rot through organic amendment, bio-agents and plant extracts under field conditions.

Treatments	Per cent disease incidence at									(%) Disease reduction		
	20 days		Pooled	30 days		Pooled	40 days		Pooled	20	30	40
	2020	2021	20 days	2020	2021	30 days	2020	2021	40 days	days	days	days
T_1- ST with *Trichoderma harzianum* @ 10 g/kg seed	11.10	13.33	12.22	17.76	18.67	18.22	21.67	23.33	22.50	45.83	46.08	49.57
	(19.40)	(21.40)	(20.43)	(24.91)	(25.55)	(25.25)	(27.68)	(28.85)	(28.30)			
T_{2-} ST with garlic extract @ 15%	17.38	19.65	18.15	25.38	28.95	27.17	32.55	36.64	34.60	19.55	19.59	22.46
	(24.61)	(26.27)	(25.47)	(30.22)	(32.52)	(31.39)	(34.77)	(37.23)	(36.01)			
T_3- Soil application with neem cake @ 0.5t/ha	14.65	17.80	16.23	22.17	26.04	24.11	28.50	31.33	29.92	27.62	28.65	32.94
	(22.49)	(24.93)	(23.72)	(28.04)	(30.67)	(29.38)	(32.24)	(34.02)	(33.14)			
T_4- Soil application with mustard cake @ 0.5t/ha	12.85	16.06	14.46	21.67	24.50	23.09	27.38	29.95	28.67	35.90	31.67	35.75
	(20.95)	(23.60)	(22.31)	(27.72)	(29.65)	(28.69)	(31.52)	(33.14)	(32.35)			
T_5- ST with *Trichoderma harzianum* @ 10 g/kg seed+ Soil application with Neem cake @ 0.5 t/ha	7.60	9.85	8.73	12.33	13.33	12.83	14.17	16.20	15.19	61.30	62.03	65.96
	(15.98)	(18.25)	(17.14)	(20.50)	(21.34)	(20.98)	(22.04)	(23.70)	(22.91)			
T_6- ST with *Trichoderma harzianum* @ 10 g/kg seed+ Soil application with Mustard cake @ 0.5 t/ha	6.95	8.50	7.73	9.92	11.67	10.80	13.45	14.67	14.06	65.74	68.04	68.49
	(15.18)	(16.90)	(16.11)	(18.32)	(19.86)	(19.16)	(21.43)	(22.46)	(22.01)			
T_7- ST with Garlic extract @ 15%+ Soil application with neem cake @ 0.5t/ha	10.67	12.38	11.52	16.00	17.38	16.69	19.50	22.07	20.79	48.94	50.60	53.40
	(19.04)	(20.58)	(19.83)	(23.55)	(24.56)	(24.10)	(26.12)	(27.93)	(27.10)			
T_8- ST with garlic extract @ 15% + Soil application with mustard cake@ 0.5 t/ha	9.50	10.65	10.08	15.40	16.62	16.01	18.67	20.50	19.59	55.32	52.62	56.10
	(17.89)	(19.01)	(18.49)	(23.08)	(23.99)	(23.57)	(25.56)	(26.88)	(26.25)			
T_9- Control	20.45	3.047	22.56	31.25	36.33	33.79	41.44	47.80	44.62	0.0	0.0	0.0
	(26.85)	(29.76)	(28.32)	(33.96)	(37.04)	(35.51)	(40.05)	(43.72)	41.89			
C.D. at 5%	2.67	3.56	1.59	3.50	4.23	2.48	4.94	4.65	2.60	-	-	-
SEm±	0.88	1.01	0.48	1.16	1.40	0.75	1.63	1.54	0.79	-	-	-
CV (%)	12.36	11.82	5.00	10.49	11.28	5.22	11.71	9.88	4.35	-	-	-

Table 4: Integrated management of dry root rot through organic amendment, bio-agents and plant extracts on grain yield of mungbean under field conditions.

Treatments	Grain yield (q ha^{-1})		Pooled	Increased yield over
	2020	2021	yield	control (%)
T_1- ST with *Trichoderma harzianum* @ 10 g/kg seed	7.43 (15.80)	7.08 (15.41)	7.26 (15.62)	100.55
T_2- ST with garlic extract @ 15%	5.71 (13.76)	4.39 (12.08)	5.05 (12.95)	39.50
T_3- Soil application with neem cake @ 0.5 t/ha	6.25 (14.45)	5.95 (14.10)	6.10 (14.29)	68.51
T_4- Soil application with mustard cake @ 0.5 t/ha	6.89 (15.21)	6.41 (14.61)	6.65 (14.94)	83.70
T_5- ST with *Trichoderma harzianum* @ 10 g/kg seed + Soil application with Neem cake @ 0.5 t/ha	9.55 (17.99)	9.05 (17.49)	9.30 (17.75)	156.91
T_6- ST with *Trichoderma harzianum* @ 10 g/kg seed + Soil application with mustard cake@ 0.5t/ha	10.12 (18.54)	9.46 (17.90)	9.79 (18.22)	170.44
T_7- ST with garlic extract @ 15% + Soil application with neem cake @ 0.5 t/ha	8.23 (16.66)	7.61 (16.00)	7.92 (16.34)	118.78
T_8- ST with Garlic extract @ 15% + Soil application with mustard cake @ 0.5t/ha	8.86 (17.30)	7.67 (16.07)	8.27 (16.69)	128.45
T_9- Control	4.04 (11.60)	3.20 (10.29)	3.62 (10.95)	0.0
C.D. at 5%	1.23	1.216	0.592	
SEm±	0.41	0.40	0.18	
CV (%)	9.48	10.30	7.56	

Trichoderma harzianum@ 10 g kg^{-1}seed+ Soil application with Mustard cake @ 0.5 t/ha)followed by in T_5 (Seed treatment with *Trichoderma harzianum*@ 10 g kg^{-1} seed+ Soil application with Neem cake @ 0.5t/ha)treatment (156.91%). The lowest grain yield increment was recorded (39.50%) in T_2 (Seed treatment with garlic extract @ 15%). Although, every treatment had positive influence on the grain yield of mungbean. Our results are in agreement with findings of Choudhary and Ashraf, (2019); Meena *et al.,* (2016); Sayyad *et al.,* (2015); Rafi *et al.* (2016) and Anis *et al.* (2010) who found effectiveness of combined effect of bio-priming of seeds with *T. harzianum* spore suspension and amendment of soil with mustard cake @ 1% for the growth of leguminous and non leguminous crop plants and for the reduction of root infecting fungi like *M. phaseolina*, *Fusarium* spp. followed by *R. meliloti* primed seeds in combination with cotton, almond and black seed cakes amendment, respectively as compared to control *i.e*. untreated seeds and soil. Shahid and Khan (2016) and Kumari *et al.* (2012) evaluated different fungal and bacterial antagonists *viz*., *T. harzianum, T. reesei, A. niger* and *B. subtilis* for management of root rot of mungbean. Among the tested bio agents, seed treatment of *T. harzianum* @ 4 g/kg seed was most effective and resulted in 51 per cent decreased in root rot severity and 31 per cent increased in the yield.

CONCLUSION

The highest disease reduction and maximum seed yield were reported in seed treatment with *T. harzianum* @10 g/kg seed + soil application of mustard cake @ 0.5 t/ha followed by seed treatment with *T. harzianum* @ 10 g/kg seed + soil application of neem cake @ 0.5t/ha. The maximum root lengths, shoot lengths and dry weight was recorded in treatment, T_6 (seed treatment with *Trichoderma harzianum* @ 10 g kg^{-1} seed+ soil application with mustard cake @ 0.5 t/ha). Maximum grain yield was recorded with T_6 (Seed treatment with *Trichoderma harzianum*@ 10 g kg^{-1} seed+soil application with mustard cake @ 0.5 t/ha). All the treatments were significantly differing with each other and found superior over control. In lieu of consumer health, present findings may be helpful for growers to get extra benefits by producing organic mungbean.

Conflict of interest: .

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