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Field effect of seed bio priming on leaf blight (*Macrophomina phaseolina*) of Black gram

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Abstract
Two year's field experiment was conducted to see the effect of seed biopriming on incidence of leaf blight in black gram at college farm, NMCA, NAU, Navsari. Seed biopriming with five different bioagents *viz*., *T. viride, T. harzianum, T. virens, P. fluorescens* and *B. subtilis* were taken as bioprimed treatments while, hydroprimed seeds and untreated seeds were acted as treated and absolute control. Data on average Per cent disease control was taken in present experiment. All the treatments were found to be effective in reducing the leaf blight over control. The highest Per cent disease control was obtained in seed biopriming with *T. harzianum* (57.14%) followed by *T. viride* (53.03%), *P. fluorescens* (50.00%) and all the other treatments over control at 65 DAS. Thus, seed biopriming with *T. harzianum, T. viride* or *P. fluorescens* @10 gm talc base formulation/kg seeds was recommended to manage leaf blight significantly.

Keywords: Leaf blight, black gram, management, seed biopriming

Introduction

Black gram (*Vigna mungo* L.) is one of the most important pulse crops all over the world. It is commonly referred as the Urd bean or black lentil. It is widely cultivated in India, Pakistan, Iran, East Africa, South East Asia and Greece (Ahmed *et al*., 2015) [2]. It is major dietary protein of the vegetarian population of the world in India and highly priced among other pulses. Black gram grows normally in 90-120 days and is very nutritious as it contains high levels of Protein, Potassium, Calcium and Iron helping the reduction of cholesterol and supporting the blood circulation (Khairnar *et al*., 2019) [4]. In India, it is around 4.40 million hectares with a production of 2.15 million tones and a productivity of 546 kg ha^{-1}. It is affected by number of diseases caused by fungi, bacteria and viruses.

Like other crops, black gram is attacked by many diseases during seed germination to seed production and maturity. Over many fungal pathogens, few viral, bacterial and nematode species are known to attack black gram resulting into substantial yield losses (Agarwal, 2011) [1]. Most of the fungal diseases such as *Macrophomina* leaf blights, anthracnose, *Alternaria* leaf spot and root rot causing severe losses are seed borne in nature in black gram. To increase the production of black gram qualitatively and quantitatively, farmer requires healthy quality seeds with high percentage of germination and purity. Hence, it necessitates the eradication of seed borne inoculum through various seed treatments and through the enforcement of proper domestic and international quarantine acts and procedures. Seed treatment is the oldest practice in plant protection and now, this is an attractive delivery system for either fungal or bacterial bioprotectants. The uses and expectations of seed treatments are greater today due to the impact of environmental regulations that have either banned or restricted the use of number of highly toxic fungicides such as organomercurials because of their residual toxicity. Seed treatments with bioagents provide economical and relatively nonpolluting delivery systems for protective materials compared to other field application systems. Bioprotectants applied to seeds may not only protect seeds but also may colonize and protect roots and increase the plant growth. However, biological agents have tended to be somewhat less effective and more variable than chemical seed treatments. Thus, seed treatment systems that will enhance efficacy of biological agents are needed and "biopriming" is one such attempt being made in this direction. Seed treatment with bio-control agents along with priming agents may serve as an important means of managing many of the soil and seed-borne diseases, the process often known as bio-priming (Taylor and Harman, 1990) [11]. Thus the study was conducted to check the efficassy of various biopriming agents on the seed germination and seedling vigour by controlling the seed mycoflora by Paper towel method in lab condition.

Materials and Methods

A field trial was conducted to study the effect of seed biopriming on intensity of leaf blight in black gram for two seasons. The seeds of GU-1 were collected from Pulse Research Station, N.A.U., Navsari and sown after biopriming treatment as mentioned below. Concept of seed biopriming of (Taylor and Harman, 1990) [11] was adopted and seed biopriming was done in black gram as follow

1. 10 g bioagent (10^8 cfu/gm) was suspended in two liter sterilized distilled water.
2. 1 kg collected seeds were suspended in above solution and left to soak for 8 hrs.
3. Soaked seeds were then drawn out and spread over the blotter paper for drying.
4. Such seeds were used for sowing immediately for testing the efficacy in field.
5. Seeds with hydration and without any treatment served as control.

Treatment includes Talc base formulation of *T. viride, T. harzianum, T. virens, P. fluorescens* and *B. subtilis,* Seeds with hydration priming and absolute control. Talc based formulations of *Trichoderma* spp., *Pseudomonas* spp. And *Bacillus* spp. containing 10^8 cfu/gm used in seed biopriming was the products of the Department of Plant Pathology, N.A.U., Navsari.

Observation taken

Percent Disease Intensity of each type of fungal disease was also recorded by selecting 10 plants/plot in each treatment by observing three trifoliate leaves first of base, second of middle and third of upper portion of the selected plant starting from the initiation up to harvest each at 15 days interval of the crop using suitable standard scale of 0-6, (Kumar *et al.*, 1969) [6] as 0- Leaves disease free, 1- Leaves area covered up to 5%, 2- Leaves area covered up to 6-10%, 3- Leaves area covered up to 11-25%, 4- Leaves area covered up to 26-50%, 5- Leaves area covered up to 51-75% and 6- Leaves area covered up to 76-100%.

Formula for calculating per cent disease intensity employed was

$$\text{PDI} = \frac{\Sigma \text{ of ratings of infected leaves observed}}{\text{No. of leaves observed x Maximum disease score}} \times 100$$

Results and Discussion

Results obtained on the incidence of leaf blight disease at 35 DAS, 50 DAS and 65 DAS in field for two different seasons are presented as here under

35 Days after sowing

During the *Kharif* season of first year the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 35 DAS. It was significantly lower in seed biopriming with *T. harzianum* (0.93%) as compared to the rest of the treatments and found statistically at par with *T. viride* (1.30%). Moreover, next best in order of merit were *P. fluorescens* (2.22%), *T. virens* (2.31%), *B. subtilis* (2.41%) and primed seeds with hydration (3.80%). The maximum per cent disease intensity was observed in the control (7.50%).

During the *Kharif* season of second year the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 35 DAS. It was significantly lower in seed biopriming with *T. harzianum* (1.02%) as compared to the rest of the treatments and found statistically at par with *T. viride* (1.48%). Moreover, next best in order of merit were *P. fluorescens* (2.31%), *T. virens* (2.41%), *B. subtilis* (2.50%) and primed seeds with hydration (3.89%). The maximum per cent disease intensity was recorded in the control (7.59%).

The pooled data of both the year indicated that the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 35 DAS. It was significantly lower in seed biopriming with *T. harzianum* (0.97%) as compared to the rest of the treatments and found statistically at par with *T. viride* (1.38%). Moreover, next best in order of merit were *P. fluorescens* (2.26%), *T. virens* (2.36%), *B. subtilis* (2.45%) and primed seeds with hydration (3.84%). The maximum per cent disease intensity was recorded in the control (7.54%). Maximum per cent disease control of Macrophomina leaf blight was recorded in *T. harzianum* (87.11%) was followed by *T. viride* (81.59%), *P. fluorescens* (69.93%), *T. virens* (68.7%), *B. subtilis* (67.48%) and primed seeds with hydration (49.07%).

50 Days after sowing

During the *Kharif* season of first year the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 50 DAS. It was significantly lower in seed biopriming with *T. harzianum* (8.15%) as compared to the rest of the treatments and found statistically at par with *T. viride* (9.07%) and *P. fluorescens* (10.28%). Moreover, next best in order of merit were the seed bioprimed with *T. virens* (11.67%), *B. subtilis* (11.85%) and primed seeds with hydration (13.15%). The maximum per cent disease intensity was observed in the control (22.04%).

During the *Kharif* season of second year the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 50 DAS. It was significantly lower in seed biopriming with *T. harzianum* (8.24%) as compared to the rest of the treatments and found statistically at par with *T. viride* (9.17%) and *P. fluorescens* (10.37%). Moreover, next best in order of merit were *T. virens* (11.76%), *B. subtilis* (11.94%) and primed seeds with hydration (13.24%). The maximum per cent disease intensity was recorded in the control (22.13%).

The pooled data of both the year indicated that the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 50 DAS. It was significantly lower in seed biopriming with *T. harzianum* (8.19%) as compared to the rest of the treatments and found statistically at par with *T. viride* (9.12%). Moreover, next best in order of merit were *P. fluorescens* (10.32%), *T. virens* (11.71%), *B. subtilis* (11.89%) and primed seeds with hydration (13.19%). The maximum per cent disease intensity was observed in the control (22.08%). Maximum per cent disease control of Macrophomina leaf blight was recorded in *T. harzianum* (62.89%) was followed by *T. viride* (58.70%), *P. fluorescens* (53.24%), *T. virens* (46.96%), *B. subtilis* (46.12%) and primed seeds with hydration (40.25%).

65 Days after sowing

During the *Kharif* season of first year the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 65 DAS. It was significantly lower in seed biopriming with *T. harzianum* (12.96%) as compared to the rest of the treatments and found statistically at par with *T. viride* (14.26%) and *P. fluorescens* (15.98%). Moreover, next best in order of merit were *T. virens* (17.22%), *B. subtilis* (17.31%) and primed seeds with hydration (18.15%). The maximum per cent disease intensity was observed in the control (30.37%).

During the *Kharif* season of second year the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 65 DAS. It was significantly lower in seed biopriming with *T. harzianum* (13.15%) as compared to the rest of the treatments and found statistically at par with *T. viride* (14.35%) and *P. fluorescens* (15.19%). Moreover, next best in order of merit were *T. virens* (17.59%), *B. subtilis* (18.33%) and primed seeds with hydration (13.24%). The maximum per cent disease intensity was recorded in the control (30.56%).

The pooled data of both the year indicated that the per cent disease intensity of Macrophomina leaf blight was significantly reduced in all treatments over control at 65 DAS. It was significantly lower in seed biopriming with *T. harzianum* (13.05%) as compared to the restof the treatments and found statistically at par with *T. viride* (14.30%) and *P. fluorescens* (15.23%). Moreover, next best in order of merit were *T. virens* (17.40%), *B. subtilis* (17.63%) and primed seeds with hydration (18.24%). The maximum per cent disease intensity was recorded in the control (30.46%). Maximum per cent disease control of Macrophomina leaf blight was recorded in *T. harzianum* (57.14%) was followed by *T. viride* (53.03%), *P. fluorescens* (50.00%), *T. virens* (42.85%), *B. subtilis* (42.09%) and primed seeds with hydration (40.12%).

From the above results, it is very clear that seed biopriming with *T. harzianum*, *T. viride* or *P. fluorescens* was significantly manage leaf blight (*M. phaseolina*). Thus, seed biopriming proved as reliable and potent method for getting better quality and high yield of black gram.

Seed treatments with *T. harzianum* (Mohamedy *et al*., 2006, Kumari *et al*. 2012 and Ashwini and Giri, 2014), *P. fluorescens* or *T. harzianum* (Srinivas *et al*., 2006) and seed bacterization with *P. fluorescens* (Minaxi and Saxena, 2010) [8] were reported as very effective to get maximum seed germination, seedling vigour index and management of *M. phaseolina* with an increase in yield in black gram.

The results revealed by earlier workers gave the confirmation and possible reasons of the results of present investigation in a very clear way. Seed priming changes the physiology of the seeds that enhances the seed germination, seedling vigour (Khan, 1992) [5], along with solubilization of molybdenum which gives rise to maximization of nodulation index in legume crops (Kumar *et al*., 2004) [7]. Thus, seed priming ultimately gives better crop stand with more productive plants (Rashid *et al*.; 2004) [9]. Along with these additions of bio agents during seed priming gives an additional dimension to seed priming for proper colonization of the bio agents to the seeds (Khan, 1992) [5]. Moreover, Brown (1974) [3] reported that seeds treated with bacterial inoculants *Pseudomonas* and *Bacillus* were found to inhibit the growth of plant pathogens in addition to bring about changes in plant growth and significant increase in crop yield. Sridar *et al*. (1991) [10] reported *B. subtilis* isolates were known to produce extracellular antibiotics that were inhibitory to some plant pathogens. However, the production of metabolites such as siderophore (a source providing iron) and chitinase (a source providing protection against pathogenic fungi) by *P. fluorescens* BAM-4 (Minaxi and Saxena, 2010) [8] and are responsible for antibiosis and in inducing the systemic resistance in plants and overcoming the pathogen attack in the management of seed borne as well as soil borne diseases in black gram.

Table 1: Effect of seed biopriming on leaf blight (*M. phaseolina*) of blackgram

Sr.No	Treatment	PDI (Leaf blight: *M. phaseolina*)											
		35DAS				50DAS				65DAS			
		2019	2020	Pooled	PDC	2019	2020	Pooled	PDC	2019	2020	Pooled	PDC
1	*T.viride*	6.43 (1.30)	6.80 (1.48)	6.61 (1.38)	81.59	17.45 (9.07)	17.53 (9.17)	17.48 (9.12)	58.70	22.17 (14.26)	22.24 (14.35)	22.20 (14.30)	53.03
2	*T.harzianum*	5.51 (0.93)	5.78 (1.02)	5.64 (0.97)	87.11	16.58 (8.15)	16.67 (8.24)	16.62 (8.19)	62.89	21.08 (12.96)	21.24 (13.15)	21.15 (13.05)	57.14
3	*T.virens*	8.65 (2.31)	8.85 (2.41)	8.75 (2.36)	68.71	19.91 (11.67)	19.98 (11.76)	19.94 (11.71)	46.96	24.49 (17.22)	24.79 (17.59)	24.64 (17.40)	42.85
4	*P.fluorescens*	8.54 (2.22)	8.73 (2.31)	8.63 (2.26)	69.93	18.68 (10.28)	18.77 (10.37)	18.72 (10.32)	53.24	22.97 (15.98)	22.90 (15.19)	22.93 (15.23)	50
5	*B.subtilis*	8.89 (2.41)	9.04 (2.50)	8.90 (2.45)	67.48	20.07 (11.85)	20.14 (11.94)	20.10 (11.89)	46.12	24.56 (17.31)	25.04 (17.96)	24.80 (17.63)	42.09
6	Hydrated seeds	11.17 (3.80)	11.28 (3.89)	11.22 (3.84)	49.07	21.24 (13.15)	21.32 (13.24)	21.27 (13.19)	40.25	25.18 (18.15)	25.32 (18.33)	25.24 (18.24)	40.12
7	Absolute control	15.83 (7.50)	15.94 (7.59)	15.88 (7.54)	-	27.98 (22.04)	28.05 (22.13)	28.01 (22.08)	-	33.38 (30.37)	33.50 (30.56)	33.43 (30.46)	-
S.Em±		0.45	0.42	0.30	-	0.93	0.98	0.67	-	1.08	1.09	0.76	-
CD at 5%		1.37	1.29	0.89	-	2.86	3.03	1.97	-	3.33	3.36	2.24	-
CV%		8.30	7.62	7.95	-	7.92	9.37	8.14	-	7.53	7.54	7.53	-
YT				NS				NS				NS	

Data outside the paranthesis are arcsin transformed data; Data inside the paranthesis are original values, PDI-Percent disease intensity; PDC-Percent disease control

Conclusion

Two year's field experiments clearly suggests the usefulness of seed biopriming in black gram by *T. harzianum*, *T. viride* or *P. fluorescens* for the management of Macrophomina leaf blight in south Gujarat. This will improve plant health and gives healthy produce. This saves huge qualitative and quantitative losses by very simple, ecofriendly and cost effective method and hence *T. harzianum*, *T. viride* or *P. fluorescens* @ 10 gm talc base formulation/kg seeds is recommended for the ecofriendly management of Macrophomina leaf blight significantly.

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