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Field effect of seed biopriming on *Alternaria* leaf spot of Green gram

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Abstract
Two years field experiment was conducted to see the effect of seed biopriming on incidence of *Alternaria* leaf spot in green gram at college farm, NMCA, NAU, Navsari. Seed biopriming with six different bioagents *viz*., *T. viride, T. harzianum, T. fasciculatum P. fluorescens-I, P. fluorescens-II, P. Aeruginosa* 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 *Alternaria* leaf blight by 46.54 to 55.81 Per cent over absolute control. The highest Per cent disease control was obtained in seed biopriming with *T. harzianum* (55.81 %) followed by *T. viride* (55.28%), *P. aeruginosa* (55.03%) and all the other treatments over control at 65 DAS. Thus, seed biopriming with *T. harzianum, T. viride* or *P. aeruginosa* @10 gm talc base formulation/kg seeds was recommended to manage *Alternaria* leaf spot significantly.

Keywords: Alternaria leaf spot, green gram, management, seed biopriming

Introduction

Greengram [*Vigna radiata* (L.) Wilczek] is an important pulse crop locally known as mung bean, golden gram, mung or moong (John, 1991) [5] and considered to be native of India, Central Asia and grown widely in number of Asian countries including Africa, USA and Australia (Agrawal, 1989) [1]. The composition of greengram seed is approximately 25.0 to 28.0 per cent protein, 1.0 to 1.5 per cent oil, 3.5 to 4.5 per cent fiber, 4.5 to 5.5 per cent ash and 62.0 to 65.0 per cent carbohydrate on a dry weight basis (Singh *et al*., 1970; Tsou *et al*., 1979) [23, 27].

Like other crops, greengram is attacked by many diseases during seed germination to seed production and maturity. Over 35 fungal pathogens, few viral, bacterial and nematode species are known to attack greengram resulting into substantial yield losses (Agrawal, 1989) [1]. Among these, seed borne fungal diseases are important in reducing the yield and seed quality of greengram (Sinnha and Prasad, 1981) [24]. Most of the fungal diseases such as anthracnose, *Alternaria* leaf spot, *Macrophomina* leaf blights and root rot causing severe losses are seed borne in nature in greengram. Hence, seeds of moong have been reported to play an important role in the dissemination of various pathogens (Rangaswami and Prasad, 1960 and Rayen, 1961) [18, 20]. The seed infecting fungi not only damages the quantity but also quality of seed. Similarly, nutritional status of greengram seeds is likely to be hampered by depleting the protein content and by the disturbance of the reducing sugar as well as starch content due to seed mycoflora (Bilgrami, *et al*. 1978) [3]. To increase the production of Greengram 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 organo mercurials because of their residual toxicity. Seed treatments with biagents provide economical and relatively non-polluting delivery systems for protective materials compared to other field application systems. Bio protectants 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) [26].

Alternaria leaf spot caused by *A. alternata* was one of the important and serious diseases occurring in serious proportion (30.9 to 32 PDI) was earlier observed and recorded by Patel, 2003. He also proved *Alternaria* leaf spot as an external and internal seed borne disease during *Kharif* from south Gujarat. Considering the serious ness of the problem faced by the farmer, present field study was conducted to see the effect of seed biopriming on incidence of *Alternaria* leaf spot in green gram to find out suitable biopriming agent.

Materials and Methods

A field trial was conducted to study the effect of seed biopriming on incidence of *Alternaria* leaf spot in green gram for two seasons. The seeds of GM-3 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 [26] was adopted and seed biopriming was done in green 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* (TV-1), *T. harzianum* (TH-1), *T. fasiculatum* (TF- 1), *P. fluorescens*-I (PF-1), *P. fluorescens*-II (PF-2), *P. aeruginosa* (PA-1), Seeds with hydration priming and absolute control. Talc based formulations of *Trichoderma* spp. and *Pseudomonas* 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

The observation Per cent Disease Intensity of *Alternaria* leaf spot disease was 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 standard graded scale of 0-5, Mathur *et al*., 1972 [10] as 0 – no infection, 1- 1 to 20 % area infected, 2- 21 to 40 % area infected, 3- 41 to 60 % area infected, 4- 61 to 80 % area infected and 5- Above 80 % area infected.

Formula for calculating per cent disease intensity employed was:

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

Results and Discussion

Results obtained on the incidence of *Alternaria* leaf spot disease at 35 DAS and 65 DAS in field for two different seasons are presented as hereunder

35 Days after sowing (Table-1)

During the *Kharif* season of first year the PDI of *Alternaria* leaf spot was significantly reduced in all the treatments over control at 35 DAS. It was significantly lower in seed biopriming with *T. harzianum* (2.67%) as compared to the rest but was statistically at par with *T. viride* (2.77%), *P. aeruginosa* (2.86%) and *T. fasiculatum* (2.96%). *P. fluorescens*- II (4.77%) followed by *P. fluorescens*-I (5.06%) and primed seeds with hydration (5.8%) were found comparatively moderately effective in reducing the disease. The highest *Alternaria* leaf spot was recorded in the control (10.95%).

During the *Kharif* season of second year, the PDI of *Alternaria* leaf spot was also significantly reduced in all the treatments over control at 35 DAS. It was significantly lower in seed biopriming with *T. harzianum* (2.59%) as compared to the rest but was statistically at par with *T. viride* (2.69%), *P. aeruginosa* (2.79%) and *T. fasiculatum* (2.89%). *P. fluorescens*- II (4.71%) followed by *P. fluorescens*-I (5.01%) and primed seeds with hydration (5.76%) were found comparatively poor in their efficassy against the disease. The highest *Alternaria* leaf spot was recorded in the control (10.96%).

The pooled data of both the year indicated that the PDI of *Alternaria* leaf spot was significantly reduced in all the treatments over control at 35 DAS. It was significantly lower in seed biopriming with *T. harzianum* (2.63%) as compared to the rest but was statistically at par with *T. viride* (2.73%), *P. aeruginosa* (2.83%) and *T. fasiculatum* (2.93%). *P. fluorescens*- II (4.74%) followed by *P. fluorescens*-I (5.04%) and primed seeds with hydration (5.78%) were found comparatively poor in reducing the disease. The highest *Alternaria* leaf spot was recorded in the control (10.96%).

Maximum control of *Alternaria* leaf spot was recorded in *T. harzianum* (76.00%) followed by *T. viride* (75.09%), *P. aeruginosa* (74.18%), *T. fasiculatum* (73.27%), *P. fluorescens*- II (56.75%), *P. fluorescens*-I (54.01%) and primed seeds with hydration (47.26%). (Table -1, Fig -1).

Table 1: Effect of seed biopriming on leaf spot (*A. alternata*) of greengram at 35 DAS

S. No.	Treatment	PDI (Leaf spot : *A. alternata*)			
		First Year	**Second year**	**POOLED**	**PDC**
1	*T.viride*	9.57 (2.77)	9.45 (2.69)	9.51 (2.73)	75.09
2	*T.harzianum*	9.40 (2.67)	9.27 (2.59)	9.33 (2.63)	76.00
3	*T.fasiculatum*	9.91 (2.96)	9.79 (2.89)	9.85 (2.93)	73.27
4	*P.fluorescens*-I	13.00 (5.06)	12.94 (5.01)	12.97 (5.04)	54.01
5	*P.fluorescens*-II	12.61 (4.77)	12.54 (4.71)	12.57(4.74)	56.75
6	*P.aeruginosa*	9.74 (2.86)	9.62 (2.79)	9.68 (2.83)	74.18
7	Seeds with hydration priming	13.94 (5.80)	13.89 (5.76)	13.91 (5.78)	47.26
8	Control (without treatment)	19.32 (10.95)	19.33 (10.96)	19.33 (10.96)	
S.Em.±		0.75	0.76	0.48	

C.D. at 5%	2.26	2.31	1.37	
C.V. %	10.65	10.96	10.8	
YT	-	-	NS	

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

65 Days after sowing (Table-2)

During the *Kharif* season of first year, the PDI of *Alternaria* leaf spot was significantly reduced in all the treatments over control at 65 DAS. It was significantly lower in seed biopriming with *T. harzianum* (12.97%) as compared to the rest but was statistically at par with *T. viride* (13.12%), *P. aeruginosa* (13.20%), *T. fasiculatum* (13.27%), *P. fluorescens*- II (15.29%) and *P. fluorescens*-I (15.29%). Poor performance in disease control was observed in primed seeds with hydration (15.59%). The highest *Alternaria* leaf spot was recorded in the control (28.13%).

During the *Kharif* season of second year, the PDI of *Alternaria* leaf spot was significantly reduced in all the treatments over control at 65 DAS. It was significantly lower in seed biopriming with *T. harzianum* (11.80%) as compared to the rest but was statistically at par with *T. viride* (11.95%), *P. aeruginosa* (12.02%), *T. fasiculatum* (12.10%), *P. fluorescens*- II (14.10%) and *P. fluorescens*-I (14.10%) Poor performance in reduction of the disease was observed in primed seeds with hydration (14.40%). The highest Alternaria leaf spot was recorded in the control (27.95%)

The pooled data of both the years indicated that the PDI of *Alternaria* leaf spot was significantly reduced in all the treatments over control at 65 DAS. It was significantly lower in seed biopriming with *T. harzianum* (12.39%) as compared to the rest but was statistically at par with *T. viride* (12.54%), *P. aeruginosa* (12.61%) and *T. fasiculatum* (12.68%). Poor performance in reduction of the disease was observed in *P. fluorescens*- II (14.69%), *P. fluorescens*-I (14.69%) and primed seeds with hydration (14.99%). The highest Alternaria leaf spot was recorded in the control (28.04%).

Maximum control of *Alternaria* leaf spot was recorded in *T. harzianum* (55.81%) followed by *T. viride* (55.28%), *P. aeruginosa* (55.03%), *T. fasiculatum* (54.78%), *P. fluorescens*- II (47.61%), *P. fluorescens*-I (47.61%) and primed seeds with hydration (46.54%) (Table -2, Fig -1).

Table 2: Effect of seed biopriming on leaf spot (*A. alternata*) of greengram at 65 DAS

S. No.	Treatment	PDI (Leaf spot : *A. alternata*)			
		First Year	Second year	POOLED	PDC
1	*T.viride*	21.24 (13.12)	20.22 (11.95)	20.74 (12.54)	55.28
2	*T.harzianum*	21.11 (12.97)	20.09 (11.80)	20.61 (12.39)	55.81
3	*T.fasiculatum*	21.36 (13.27)	20.35 (12.10)	20.86 (12.68)	54.78
4	*P.fluorescens*-I	23.02 (15.29)	22.05 (14.10)	22.54 (14.69)	47.61
5	*P.fluorescens*-II	23.02 (15.29)	22.05 (14.10)	22.54 (14.69)	47.61
6	*P.aeruginosa*	21.30 (13.20)	20.29 (12.02)	20.80 (12.61)	55.03
7	Seeds with hydration priming	23.26 (15.59)	22.30 (14.40)	22.78 (14.99)	46.54
8	Control (without treatment)	32.03 (28.13)	31.92 (27.95)	31.97 (28.04)	-
S.Em.±		0.68	0.70	0.43	-
C.D. at 5%		2.10	2.14	1.27	-
C.V. %		5.58	6.00	5.79	-
YT		-	-	NS	-

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

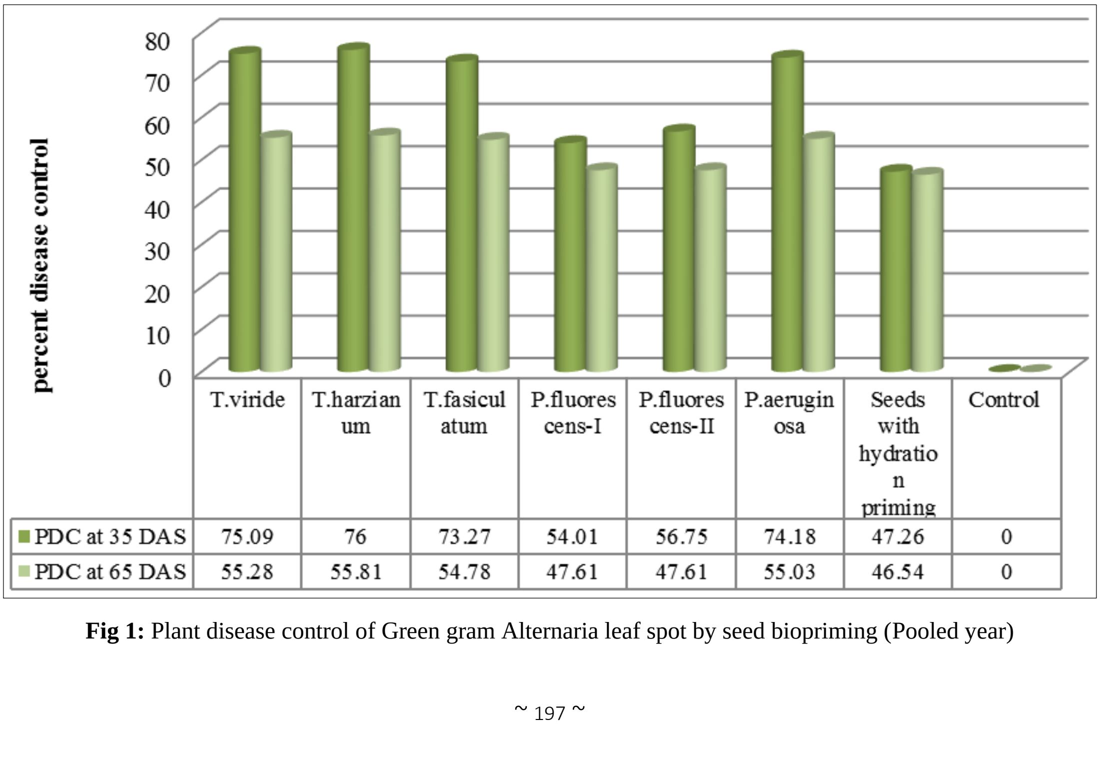

Fig 1: Plant disease control of Green gram Alternaria leaf spot by seed biopriming (Pooled year)

There was no any report on seed biopriming in greengram but seed priming (hydration) in green gram was studied earlier by earlier by Rashid *et al*., (2004) [19], Arif *et al*., (2004) [2], Khan *et al*., (2008) [7] and Umair *et al* (2010) [28]. They obtained more or less similar results as observed in the present study. Seed treatments with *T. harzianum* (Rajeswari, *et al*., 1999 and Pooja *et al*., 2003.) [17, 16], *P. fluorescens* or *T. harzianum* (Sarkar and Bhattacharya, 2008) [21] and seed bacterization with *P. fluorescens* (Minaxi and Saxena, 2010a) [11] or *P. aeruginosa* (Minaxi and Saxena, 2010b) [12] were reported as very effective to get maximum seed germination, seedling vigour index and management of *M. phaseolina* with an increase in yield in greengram. Biopriming proved very useful for better seed germination, seedling vigour and disease management in groundnut (Malathi and Doraisamy, 2004) [9], pea (Mohamedy and Baky, 2008) [3], maize (Nayaka *et al*., 2008) [14], soybean, chilli, tomato and brinjal (Someshwar and Sitansu, 2010) [25]. Seed priming changes the physiology of the seeds that enhances the seed germination, seedling vigour (Khan, 1992) [6], along with solubilization of Molybdenum which gives rise to maximization of nodulation index in legume crops (Johanson, 2004 and Kumar *et al*., 2004) [4, 8]. Thus, seed priming ultimately gives better crop stand with more productive plants (Rashid *et al*.; 2004) [19]. Along with these additions of bioagents during seed priming gives an additional dimension to seed priming for proper colonization of the bioagents to the seeds (Khan, 1992) [6]. 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, 2010a) [11] and the production of siderophore and hydrogen cyanide (HCN) on chrome azurol S, extracellular chitinase enzyme and an important antibiotic, phenazine-I in *in vitro* by *P. aeruginosa* RM-3 was also observed by Minaxi and Saxena (2010b) [12] 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 green gram. The work done on seed biopriming in green gram here is the first time report and was more or less similar with the results obtained in other crops by these workers.

Conclusion
Two years field experiments clearly suggest the usefulness of seed biopriming in greengram by *T. harzianum*, *T. viride* or *P.aeruginosa* for the management of *Alternaria* leaf spot in south Gujarat. This will improves plant health and gives healthy produce. This saves huge qualitative and quantitative losses by very simple, eco-friendly and cost effective method and hence Seed biopriming with *T. harzianum*, *T. viride* or *P. aeruginosa* @10 gm talc base formulation/kg seeds is recommended for the ecofriendly management of *Alternaria* leaf spot significantly.

Conflict of intrest
The authors declare that there is no conflict of interests regarding the publication of this paper.

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