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Biopriming is a new technique of seed treatment that integrates biological 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 the soil and seed borne pathogens. Hence, during 2014 and 2018 *in vitro* as well as field trial was conducted for the plant growth promoting activity of tomato seed and management of damping-off of tomato disease respectively. Plant growth promoting activity was found highest when biopriming of tomato seed with *Trichoderma harzianum*@10gm/lit. water containing 10^8 cfu/ml, seed germination (95.33 %), shoot length (6.33 cm) and root length (10.64 cm) and root colonization (6.33 x 10^{-4} cfu/ml). In biopriming of tomato seed with different bioagents, seed biopriming with *T. harzianum* @10gm/kg seed containing 10^8cfu/gm (13.72 %) was found significant among all the treatments and reduced damping-off tomato.

Key words: PGPR, biopriming, *Trichoderma harzianum, d*amping-off , tomato

INTRODUCTION

Biopriming, in which specific biological control agents are incorporated into the seed priming process, can be very effective in suppressing disease, *viz*., *Trichoderma* spp. incorporated into a solid matrix priming process increased the rate of seedling emergence and suppressed pre and post-emergence damping-off caused by *Pythium* spp. (Harman and Taylor,1988; Harman *et al*. 1989 & Osburn and Schroth, 1988. Biopriming is a new technique of seed treatment that integrates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects (seed hydration) 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 the soiland seedborne pathogens. Biological seed treatments may provide an alternative to chemical control. Seed priming, osmopriming and solid matrixpriming were used commercially in many horticultural crops, as a tool to increase speed and uniformity of germination and improve final stand. However, if seeds are infected or contaminated with pathogens, fungal growth can be enhanced during priming, thus resulting in undesirable effects on plants. Therefore, seed priming alone or in combination with low dosage of fungicides and/or biocontrol agents has been used to improve the rate and uniformity emergence of seed and reduce damping-off disease. Biopriming has great promise for enhancing the efficacy, shelf life, and consistent performance of biological control agents (Callan *et al*. 1997)**.** The management of soil borne pathogens has traditionally relied on the use of cultural practices such as crop rotation and no-tillage, and the application of fungicides and fumigants (Reeleder, 2003 ; Martin, 2003). Fungicide movement in soil is often limited by the soil matrix or pore availability and soil leaching and chemical breakdown by microbial communities also alter fungicide efficacy (Paulitz, 1997). Fungicides are commonly applied multiple times for plant disease control in conventional agriculture

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systems, but these synthetic pesticides have also been shown to negatively affect the health of the ecosystem (Ecobichon, 2001;Agrios, 2005). Most fungicides also require a restricted-entry period post-application before workers can return to the treated crop, and a similar harvest interval is also commonly required between last application and harvest of the crop. Additionally, heavy utilization of fungicides greatly increases production costs for growers and producers and can result in the development of resistant pathogen populations. Overall public awareness of the environmental impact of chemical pesticides in agriculture has been shifting the focus away from high-energy input agroecosystems towards more ecologically sustainable systems. In these systems, synthetic chemicals are at the least minimized, and biologically-based processes are used for both nutrient supply and pathogen control (Tilman *et al.* 2002). Hence, the present investigation was carried out on the following aspects to generate information on management of damping-off of tomato through biopriming.

MATERIALS AND METHODS

Biopriming of tomato seed with different bioagents against damping-off
In vitro study

In vitro study to check the efficacy of seed biopriming on the seed germination and seedling vigour by controlling the seed borne mycoflora was carried out by Paper towel method.

Method of seed biopriming (Taylor and Harman, 1990).

1. 50 tomato (GT-2) seeds were taken.
2. One liters suspension of talc based formulations (10gm/lit) of respective bioagents containing 10^8CFU/gm was prepared in sterilized distilled water.
3.Tomato seeds was then mixed in the above suspension and kept for 2 hrs.
4. Soaked seeds was then drawn out from the solution and spread over the blotter paper for drying.
5. Such seeds were used immediately for testing the efficacy *in vitro* or for sowing purpose in field trial.
6. Seeds with hydration and without any treatment served as control.

Talc based formulations of *Trichoderma* sp. and *Pseudomonas* sp. containing 10^8CFU/gm were used in seed biopriming was the products of the Biopesticide unit, Department of Plant Pathology, N.A.U., Navsari. Trial was conducted using Controlled Randomized Design led with eight treatments and three replications.

Treatment details

Treatment No.	Name of bioagents (NAU, isolates)
T1	*Trichoderma viride*
T2	*Trichoderma harzianum*
T3	*Trichoderma fasciculatum*
T4	*Trichoderma virens*
T5	*Pseudomonas fluorescens*
T6	*Bacillus subtilis*
T7	Seeds with hydration priming
T8	Control (without treatment)

Observations

Total numbers of normal seedlings/pot were recorded at 10 days after sowing and per cent germination was calculated by using following formulae.

1.Germination Percentage (%) =

$$\frac{\text{Total number of germinated seed}}{\text{Total number of seed sown}} \times 100$$

2. Shoot length (cm)
3. Root length (cm)
4. Root colonization (cfu/g).

Field study

A field trial was conducted for the management of tomato damping-off disease for two seasons during 2014-2015, at N. M. College of Agriculture, Research Farm, N.A.U., Navsari. The tomato variety GT-2 seeds were collected from Regional Horticultural Research Station, N.A.U., Navsari and sown after biopriming treatment as mentioned earlier. The seed rate was 100 seeds per plot sown in 1 m x 1m plot size on ridge bed. Trial were conducted using Randomized Block Design led with eight treatments and three replications.

The observations on the pre-emergence mortality were taken by observing the germinated and

total sown seeds in each row in a plot. The possibility of the cause of the pre-emergence mortality due to seed infecting fungi in respective treatment

Treatment details

Treatment No.	Name of bioagents
T1	*Trichoderma viride*
T2	*Trichoderma harzianum*
T3	*Trichoderma fasciculatum*
T4	*Trichoderma virens*
T5	*Pseudomonas fluorescens*
T6	*Bacillus subtilis*
T7	Seeds with hydration priming
T8	Control (without treatment)

was confirmed by observing the ungerminated/ rotted seeds after digging out from the plots through naked eye or microscopic observations. The percentage of diseased ungerminated seeds was be recorded after 10 days of sowing and per cent pre-emergence mortality was calculated. The observations on the post-emergence mortality was taken by observing the numbers of rotted or damping off of seedlings after 10 days of sowing. The numbers of died seedlings recorded during observations were used to calculate Per cent post-emergence mortality.

1. Germination Percentage= Total number of germinated seed X 100 Total number of seed sown
2. Per cent pre-emergence mortality=No. of diseased ungerminated seeds / plot x 100
Number of sown seeds/ plot
Numbers of rotted or damping off of seedlings were recorded after 10 days of sowing and calculated using following formulae.
3. Per cent post- emergence mortality = Number of seedling died / plot x 100 Number of total seedling / plot

RESULTS AND DISCUSSION

Biopriming of tomato seed with different bioagents against damping-off

In vitro study

In vitro study conducted to check the efficacy of seed biopriming on the seed germination, root length, shoot length and root colonization by controlling the seed mycoflora was carried out by Paper towel method. Results revealed (Table.1) that per cent seed germination, root length, shoot lengths and root colonization were significantly increased in all the treatments tested over control.

Germination (%)

Per cent germination was significantly higher in the seed biopriming with *T. harzianum* (95.33 %) as compared to the rest. Next best in order of merit was *P. fluorescens* (91.00 %) followed by *T. fasciculatum* (90.33 %), *T. viride* (81.33 %), *T. virens* (74.33 %), *B. subtillis* (73.67%) and primed seeds with hydration (65.67 %). Lower seed germination was observed in control (45.67 %).

Shoot length (cm)

Shoot length was significantly longer in all the treatments as compared to the control. Among these, significantly longer shoot (6.33 cm) was recorded in seeds treated with *Trichoderma harzianum* than the other treatments tested but it was statistically at par with *T. viride* (5.99 cm). Next best treatment in order of merit was *Pseudomonas fluorescens* (5.81 cm) followed by *T. fasciculatum* (4.18 cm), *Bacillus subtillis* (4.18 cm) and seed with hydration priming gave (3.63 cm) shoot length. The lowest shoot length (1.02 cm) was recorded in the untreated control.

Root length (cm)

Root length was significantly longer in all the treatments as compared to the control. Among these, significantly higher root (10.64 cm) was recorded in seeds bioprimed with the *Trichoderma harzianum* than the other treatments tested. Next best treatment in order of merit was *T. viride* (9.13 cm) followed by *T. virens* (7.09 cm), *Pseudomonas fluorescens* (6.99 cm), *T. fasciculatum* (5.24 cm), *Bacillus subtillis* (4.84 cm) and seed with hydration priming gave (4.15 cm) shoot length. The lowest root length (2.03 cm) was recorded in the untreated control.

Root colonization (cfu/ml)

The root colonization of bioprimed seed was maximum with the seed treatment *Trichoderma harzianum* (6.33 population at 10^{-4} dilution). Next best treatment in order of merit was *P. fluorescens* (5.33 population at 10^{-4} dilution) followed by *T. viride* (5.00 population at 10^{-4}dilution) and *T. fasciculatum* (4.67 population at 10^{-4} dilution). The least root colonization was recorded in seed bioprimed with *T. virens* (3.33 population at 10^{-4} dilution) and *B. subtillis* (3.33 population at 10^{-4} dilution).

Effect of seed biopriming on damping-off of tomato in field condition

Field study

A field study for management of damping-off of tomato disease through seed biopriming was carried out during 2014 and 2015.

Table 1: Exffect of seed biopriming on tomato seed germination, root length, shoot length and root colonization

Treatment (Navsari Isolates) (10gm/lit. seed containing 10^8cfu/gm)	Germination (%)	Shoot length (cm)	Root length(cm)	Percent root Colonization (cfu/ml) X 10^{-4}
Trichoderma harzianum	95.33	6.33	10.64	6.33
Trichoderma viride	90.33	5.99	9.13	5.00
Trichoderma fasciculatum	81.33	4.18	5.24	4.67
Trichoderma virens	74.33	3.89	7.09	3.33
Bacillus subtilis	73.67	4.18	4.84	3.33
Pseudomonas fluorescens	91.00	5.81	6.99	5.33
Seeds with hydration priming	65.67	3.63	4.15	00
Control (without treatment)	45.67	1.02	2.03	0.00
S.Em ±	0.58	0.12	0.15	0.26
C. D. at 5%	1.75	0.36	0.45	0.79
C.V. %	1.32	4.75	4.19	13.04

Table 2: Effect of seed biopriming on damping-off of tomato during 2014 in field condition

Treatment (Navsari Isolates) (10gm/kg seed containing 10^8CFU/gm)	Percent Disease Incidence (%)		
	Germination (%)	Per cent pre-emergence mortality	Per cent post- emergence mortality
Trichoderma harzianum	74.94*(92.00)**	16.08*(8.00)**	13.43*(5.43)**
Trichoderma viride	70.45(88.00)	20.15(12.00)	15.10(6.81)
Trichoderma fasciculatum	68.80(84.00)	23.44(16.00)	16.75(8.33)
Trichoderma virens	62.03(78.00)	27.97(22.00)	20.96(12.82)
Bacillus subtilis	62.03(78.00)	27.97(22.00)	22.04(14.10)
Pseudomonas fluorescens	69.74(88.00)	20.26(12.00)	17.53(9.10)
Seeds with hydration priming	62.73(79.00)	27.27(21.00)	21.90(13.92)
Control (without treatment)	49.60(58.00)	40.40(42.00)	55.58(67.85)
S.Em ±	3.28	1.42	1.18
C. D. at 5%	9.96	4.32	3.57
C.V. %	8.75	9.70	8.89

*Figures outside parenthesis are arcsine (angular) transformed values
** Figures indicate original values

2014

Per cent seed germination was significantly increased in all the treatments over control. It was significantly higher in seed biopriming with *T. harzianum* (74.94 %) as compared to the rest but was statistically at par with *T. viride* (70.45 %), *P. fluorescens* (69.74 %) and *T. fasciculatum* (68.80 %). Next best treatment in order of merit was

Table 3: Effect of seed biopriming on damping-off of tomato during 2015 in field condition

Treatment (Navsari Isolates) (10gm/kg seed containing 10^8CFU/gm)	Germination Percentage	Percent Disease Incidence (%)	
		Per cent pre-emergence mortality	Per cent post- emergence mortality
Trichoderma harzianum	72.65*(91.00)**	18.26*(10.00)**	14.00*(5.86)**
Trichoderma viride	69.17(85.00)	22.78(15.00)	15.05(7.05)
Trichoderma fasciculatum	64.16(81.00)	25.74(19.00)	17.10(8.64)
Trichoderma virens	60.00(75.00)	29.94(25.00)	21.79(13.79)
Bacillus subtilis	60.67(76.00)	29.24(24.00)	23.07(15.36)
Pseudomonas fluorescens	66.43(84.00)	23.57(16.00)	18.35(9.92)
Seeds with hydration priming	60.00(75.00)	30.00(25.00)	22.87(15.11)
Control (without treatment)	47.39(54.00)	42.71(46.00)	59.43(74.07)
S.Em ±	3.00	1.39	1.11
C. D. at 5%	9.10	4.23	3.36
C.V. %	8.31	8.70	8.01

*Figures outside parenthesis are arcsine (angular) transformed values
** Figures indicate original values

primed seed with hydration (62.73 %) followed by *Bacillus subtillis* (62.03 %) and *T. virens* (62.03 %). The lowest seed germination was recorded in the control (49.60 %).

Per cent pre-emergence damping-off was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *T. harzianum*(16.08 %) as compared to the rest but was statistically at par with *T. viride* (20.15 %) followed by *P. fluorescens* (20.26 %). Next best treatment in order of merit was *T. fasciculatum* (23.44 %) followed by primed seed with hydration (27.27 %), *Bacillus subtillis* (26.97 %) and *T. virens* (27.97 %). The highest per cent pre-emergence damping-off was recorded in the control (40.40 %).

Per cent post emergence damping-off was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *T. harzianum* (13.43 %) as compared to the rest but it was statistically at par with *T. viride* (15.10 %). *T. fasciculatum* (16.75 %) and *P. fluorescens* (17.53 %). Next best treatment in order of merit was *T. virens* (20.96 %) followed by primed seed with hydration (21.90 %) and *Bacillus subtillis* (22.04 %).. The highest per cent post-emergence damping-off was recorded in the control (55.58 %). (The highest per cent of post emergence damping-off was recorded in the control (55.58 %) (Table.2)

2015

Per cent seed germination was significantly increased in all the treatments over control. It was significantly higher in seed biopriming with *T. harzianum* (72.65 %) as compared to the rest but was statistically at par with *T. viride* (69.17 %), *P. fluorescens* (66.43 %) and *T. fasciculatum* (64.16 %). Next best treatment in order of merit was *Bacillus subtillis* (60.67 %) which was followed by *T. virens* (60.00 %) and primed seed with hydration (60.00 %). The lowest seed germination was recorded in the control (47.39 %).

Per cent pre-emergence damping-off was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *T. harzianum* (18.26 %) as compared to the rest. Next best treatment in order of merit was *T. viride* (22.78 %) followed by *P. fluorescens* (23.57 %), *T. fasciculatum* (25.74 %), *Bacillus subtillis*

(29.24 %), *T. virens* (29.94 %) and(29.94 %) and primed seed with hydration (30.00 %). The highest per cent pre-emergence damping-off was recorded in the control (42.71 %). Per cent post emergence damping-off was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *T. harzianum* (14.00 %) as compared to the rest but it was statistically ar par with *T. viride* (15.05 %) and *T. fasciculatum* (17.10 %). Next best treatment in order of merit was *P. fluorescens* (18.35 %) followed by *T. virens* (21.79 %), primed seed with hydration (22.87 %) and *Bacillus subtillis* (23.07 %). The highest per cent of post emergence damping-off was recorded in the control (59.43 %) (Table.3).

First report of biopriming was reported in South Gujarat region on green gram done by Deshmukh, during 2013, he found that seed biopriming with *T. harzianum*, *T. viride* or *P. aeruginosa* @10 gm talc base formulation/kg seeds proved very effective not only to get better seed germination, seedling vigour, plant growth, root growth, root nodules, yield parameters and yield but also to manage significantly leaf spot (*A. alternata*), Leaf blight (*M. phaseolina*) and anthracnose (*C. capsici*). The results of the experiment are quite confirmative with the result of Vandana and Priya (2014) found higher seed germination (73.33 %) and disease control (66.53 %) in tomato against soil-borne pathogen with the seed biopriming of *T. harzianum*. Khanna *et al*. (2003) treated the seeds of mungbean with *T. harzianum* strains T-2 and found maximum seed germination, root length, fresh weight and dry weight of seedlings and increased in yield due to seed treatment *in vitro*. Arif *et al*. (2004) found that mungbean seed soaked for 8 hours in water (seed priming with hydration) emerged earlier with higher germination (30.1 %) as compared to non-soaked seed (14.7 %). Mugilan *et al*. (2011) who found treatment of *P. striata* recorded highest seed germination (97%), root length (8.5 cm) and shoot length (22.5 cm) of chilli. Manikandan *et al*. (2010) also reported plant growth promoting activity of *P. fluorescens* (Pf1) in tomato seed. While, application of *T. viride* and *P. fluorescens* reduced the population of *P. aphanidermatum* in tomato, from the above results, it was very clearly indicated that seed biopriming with *T. harzianum, T. viride* or *P. fluorescens* @10gm/kg seed containing (10^8CFU/gm) very effective to get better for seed germination, seedling vigour, plant growth, root growth and disease management.

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), along with solubilization of Molybdenum which gives rise to maximization of nodulation index in legume crops (Johanson, 2004). Thus, seed priming ultimately gives better crop stand with more productive plants (Rashid *et al*. 2004). 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). 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 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 *Pseudomonas* sp. was also observed by Minaxi and Saxena (2010) 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. The work done on seed biopriming in tomato here is the first time report in South Gujarat condition.

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