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Standardization of Bio-Priming Technique with Bio Control Agents on Maize under Laboratory and Green House Condition

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A B S T R A C T

An investigation was undertaken by taking seed samples of maize in laboratory and green house of the AICRP on Seed Technology Research, NSP (crops), at Department of Seed science and technology, Ouat, Bhubaneswar, to find out the best bio control agent using seed priming technique, under laboratory and green house condition. The experiment material consists of three biocontrol agents seven treatment with four replications that is T 1-Seed priming with *Trichoderma viride*(10%), T 2- Seed priming with *Trichoderma viride* (20%), T 3- Seed priming with *Trichoderma harzianum* (10%) for one hour, T4-Seed priming with *Trichoderma harzianum* (20%), T5-Seed priming with *Pseudomonas fluorescens* (10%), T6-Seed priming with *Pseudomonas fluorescens*(20%), T7-untreated /control with 12 hours of soaking. The treatments were also evaluated for their effect on seed quality parameters like seedling length, Field emergence seedling dry weight, seedling vigour index. The occurrence of seed rot and seedling infection was also observed in laboratory and green house condition. Seed biopriming with *T. harzianum* 10% for 12 hours was found best in increasing the germination, seedling length, seedling dry weight, SVI-I, SVI-II by 94%, 27.85cm, 0.316g, 2564.4 & 29.71 respectively and reducing the seed rot and seedling infection by 82% and 78% respectively. Under green house condition also, seed biopriming with *T. harzianum* 10% for 12 hours was best with highest field emergence 88%, plant height 28.6cm, seedling length 34.9cm, dry weight 2.75g, SVI - I 3070.4, SVI-II 241.9 and minimum seedling blight 10.3% and seedling damping off 11.25%.

Introduction

Maize (*Zea maize* L.) is one of the most important cereal crops of the world and contributes to food security in most of the developing countries. Globally, maize is known as queen of cereals because it has the highest genetic yield potential among the cereals. It is cultivated on nearly 150 million hectares in about 160 countries having wider

diversity of soil, climate, biodiversity and management practices that contributes 36% (782 million tonnes) in the global grain production. India is one of the top 10 maize producers in the world; it contributes around 2-3% of the total maize produced globally and is one of the top five maize exporters in the world contributing almost 14% of the total maize exported to different countries around the world. Biopriming means biological seed treatment. A new technique combining the biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects (seed hydration) of disease control. So it is an ecological approach using selected fungal antagonists against the soil and seed borne pathogens. Common biocontrol agents used are-*Trichoderma viride*, *T. harzianum* & *Pseudomonas fluorescence*. Biocontrol agent *T. harzianum* was applied which resulted in better control of *Fusarium verticillioides* and *F. fumonisins* in maize (Nayaka *et al.,* 2010). *T. harzianum* is a good biocontrol agent against a wide range of plant pathogens, and it has ability to reduce *F. verticillioides* colonization under greenhouse conditions (Ferrigo *et al.,* 2014). Biopriming was found to improve the speed of germination, germination, root length, shoot length, dry matter production, total dry matter production and vigour index as compared to non primed seed in maize (Vanangamudi *et al.,* 2013). Thus the present experiment was carried to find the effective biocontrol agents which can reduce seed borne diseases and give higher yield in maize with following objectives-

To standardize the bio-priming technique using bio control agents on maize under laboratory and green house condition. To study and evaluate various seed quality parameters like seedling length or field emergence, seedling dry weight, seedling vigor index and occurrence of seed rot and seedling infection of treatments in laboratory and green house condition.

Materials and Methods

Thus the present experiment "Standardization of bio-priming technique with bio control agents on maize under laboratory and green house condition" was conducted in laboratory and in the green house of the AICRP on Seed Technology Research, NSP (crops), OUAT, Bhubaneswar.

Effect of biopriming in laboratory condition

The effect of different concentrations of biocontrol agents for biopriming on the seed quality was studied taking the seeds of maize var Mayurbhanj local. The seeds were first examined under magnivision and healthy seeds without any discoloration or abnormality were separated. The seeds were surface sterilized and then subjected to priming with different biocontrol agents and concentration taking 400 seeds for each treatment. The concentration of biocontrol agents was 10% and 20% which was prepared by thoroughly mixing 20g & 40g of biocontrol agent formulation in 200ml sterile water, separately. The seeds were soaked in suspension of biocontrol agents separately for 12hours followed by drying.

The seeds of the different treatments were kept in separate polythene packets and subjected to germination test following between paper method(BP) (ISTA,1985).

Hundred treated seeds were placed equidistantly between two layers of kraft papers which are soaked in water, which are again covered by another layer of wet non-absorbent or wax paper. The entire set is rolled, tagged and kept inside the germinator at 30±2°C in upright position. The experiment was carried out with four replication in Completely Randomized Block Design(CRD) and the final count was taken on the 7th day.

Treatment details

T 1-Seed priming with *Trichoderma viride (10%)*

T 2- Seed priming with *Trichoderma viride (20%)*

T 3- Seed priming with *Trichoderma harzianum (10%)*

T4-Seed priming with *Trichoderma harzianum (20%)*

T5-Seed priming with *Pseudomonas fluorescens(10%)*

T6-Seed priming with *Pseudomonas fluorescens (20%)*

T7-untreated /control

Note: Soaking duration 12hours

The observation were taken for germination%, speed of germination, seedling length, seedling vigour index – I, seed rot and seedling infection. Number of seeds germinated daily was counted till completion of germination.

Germination%

Germination %=Total no. of normal seedlings/no. of seeds sown x100

Speed of germination

Speed of Germination=X1/n1+(X2-X1)/n2+(X3- X2)/n3…… (Xn-Xn-1)/nn Where X1, X2, X3…=Number of seedling germinated on n1, n2, n3 ….nth day.

Seedling length

Seedling length measured using a plastic meter scale from shoot tip to root tip of the seedling

Seedling vigour Index(SV-I)

Seedling Vigour Index SVI- I = Germination % X Length of seedling in cm. The root and shoot portion of each seedling collected, excluding the cotyledons, was taken for determining the dry weight following oven dry method.

Seedling dry weight

Seedling dry weight- Seedling dry weight was recorded by oven drying a single seedling for 6hours in the hot air oven, weighed in electronic weighing balance and expressed in g/seedling.

Seedling vigour index(SV-II)

SVI - II = Germination% X Seedling dry weight in g.

Seed rot

Percent incidence of seed rot was calculated on the basis of total number of seed sown.

Seed and Seedling infection

Development of fungal colonies were noted and are calculated on the basis of total number of seed sown.

Effect of biopriming in Green house condition

Effect of biopriming on occurrence of seed borne diseases was studied under green house condition. The study was under taken in the green house of the AICRP on Seed Technology Research, NSP (crops), OUAT, Bhubaneswar. First of all sterilized soil was taken in the plastic pots and the primed seeds were sown. The pots were irrigated on alternate day with sterile water.

The experiment was carried out with four replication in Completely Randomized Block Design(CRD). The pots were kept inside the green house for 30 days to take observation.

Treatment details

T 1-Seed priming with *Trichoderma viride(10%)*

T 2- Seed priming with *Trichoderma viride (20%)*

T 3- Seed priming with *Trichoderma harzianum(10%)*.

T4-Seed priming with *Trichoderma harzianum(20%)*

T5-Seed priming with *Pseudomonas fluorescens(10%)*

T6-Seed priming with *Pseudomonas fluorescens(20%)*

T7-untreated /control

Note: Soaking duration 12hours

The observations included are- field emergence %, speed of germination, plant height, seedling length, seedling vigour index I & II, seedling blight and seedling damping off.

Field emergence%

The total number of plants emerged from soil in each treatments was counted and plant emergence percentage was calculated.

Plant height

Plant height was measured using a plastic meter scale as height of the plant for soil level in the pot to the tip of the main branch

Seedling blight and seedling damping off

Occurrence of different seed borne diseases was recorded. Observation were taken during 30 days of growth of seedling. The seedling sowing symptoms like, brown discoloration, rotting closer to the soil level and blightening of seedling were noted. Seedling damping off and seedling blight was calculated on the basis of total number of seed sown.

Results and Discussion

Effect of biopriming on seed quality and seedling health

Seed biopriming with *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescens* with two different concentration were evaluated under both laboratory and green house condition and compared with untreated control.

Effect of biopriming on seed quality and seedling health under laboratory condition

The treated seeds were subjected to germination test following standard between paper method. The treatments were also evaluated for their effect on seed quality parameters like seedling length, seedling dry weight, seedling vigour index. The occurrence of seed rot and seedling infection was also observed. All the treatments significantly improved seed germination over control (Table1).

The data revealed that highest germination was in seed priming with *T. harzianum* 10% (94%) which was at par with *T. harzianum* 20% and significantly higher than other treatments and over control (77.7%). The next best treatment was *T. viride* 20% &*T. viride* 10% with 89.25% and 89% germination respectively and followed by *P. fluorescens* 20% and 10%. Biopriming with *P. fluorescens* 20% could enhance 10.3% germination over

control. All the biopriming treatments significantly improved the seedling length over control. Highest seedling length was rerecorded in *T. viride* 10% i.e 30.78cm whereas seedling length in control was 22.54cm. But there was no significant difference in all the biopriming treatments & as per the seedling length is concerned.

There was significant enhancement of seedling dry weight in all treatments over control. Seed biopriming with *T. harzianum* 10% enhanced the dry weight of seedling (g/seedling) to 0.316g, which was at par with seed biopriming with *T. harzianum* 20%.

The next best treatment in enhancing the seedling dry weight was *T. viride* 10% which was at par with *T. viride* 20%. The minimum increase in seedling dry weight was recorded in *P. fluorescens* 10% and *P. fluorescens* 20%, but it was increasing the seedling dry weight two times as compared to control.

There was significant enhancement in seedling vigour index (SVI-I & SVI-II), in all the treatments due to the enhancement in germination, seedling length and dry weight.

The maximum enhanced SVI-I was recorded in seed biopriming with *T. viride* 10% (2739.1) and the minimum was control (1756.6). Similarly highest SVI-II was recorded in seed biopriming with *T. harzianum* 10% (29.71) and lowest was in control (6.57).

All the treatments had significantly reduced the seedling infection and seed rot over control. Seed biopriming with *T. harzianum* 10% had minimum seedling infection i.e 4.75%, which was at par with *T. harzianum* 20%. The second best treatment was *T. viride* 10% and *T. viride* 20% followed by *P. fluorescens* 10% and *P. fluorescens* 20%. The maximum seedling infection was recorded in control i.e 21.5%. Seed biopriming with *T. harzianum* 10% shows maximum reduction in seed rot i.e. 1.75%. The next best treatment in reduction of seed rot was *T. harzianum* 20% followed by *P. fluorescens* 10%. The maximum seed rot was recorded in control i.e.9.75%.

Effect of bio-priming on seedling growth and health under green house condition

The bioprimed seeds were sown in pot cultures under green house condition and the seedlings were allowed to grow for 30days to ascertain the effect of biopriming with *T. viride, T. harzianum* and *P. fluorescence*.

All the bio-priming treatments resulted in better field emergence as compared to control (Table 2). The highest field emergence was recorded in *T. harzianum10%* (88%) which was at par with *T. harzianum 20%*, while 80.5% field emergence was recorded in *T. viride* 10% followed by *T. viride 20%*. Biopriming with *P. fluorescens 10%* and *P. fluorescens 20%* also increased the field emergence over control.

The minimum field emergence was recorded in control is 67.5%. There was no significant increase in speed of germination in biopriming treatment with *T. viride, T. harzianum* and *P. fluorescens* as compared to control. 53 Plant height was maximum in *T. harzianum* 10% i.e 28.6cm. Plant height in control was 14.95cm.

There was 52.2% increase in plant height in *T. harzianum* 10% over control. *T. harzianum* 20% was at par with *T. harzianum* 10% in increasing the plant height. *T. viride* 10%, *T. viride* 20%, *P. fluorescens 10%, P. fluorescens 20%* had no significant difference in increasing the plant height, but there was significant increase in plant height as compared to control.

Table.1 Effect of biopriming on seed quality enhancement under laboratory condition

Sl. No.	Treatment	Germination %	Seedling length (cm)	Dry wt. (g)	SVI-I	SVI-II	Seedling infection (%)	Seed rot (%)
1	T.V(10%)	89 (9.43)	30.78	0.238	2739.1	21.15	8.5(2.90)	4.75(2.16)
2	T.V(20%)	89.5 (9.45)	28.19	0.209	2515.2	18.69	8.25(2.87)	4.25(2.05)
3	T.H(10%)	94 (9.70)	27.29	0.316	2564.4	29.71	4.75 (2.16)	1.75(1.31)
4	T.H(20%)	92.5 (9.60)	27.85	0.275	2570.7	25.33	5.75 (2.36)	3 (1.72)
5	P.f(10%)	86 (9.27)	27.58	0.173	2370.5	14.83	11(3.31)	3.75(1.93)
6	P.f(20%)	85.5 (9.26)	27.84	0.174	2385.2	14.91	11.75(3.42)	4.5(2.10)
7	DRY	77.5 (8.82)	22.54	0.085	1756.6	6.57	21.5(4.63)	9.75(3.11)
	SE(m)±	0.073	1.066	0.021	99.66	1.994	0.147	0.135
	CD	0.214	3.134	0.063	293.10	5.863	0.433	0.397

*Figures in the parenthesis are square root transformed value

Table.2 Effect biopriming on seed quality enhancement under greenhouse condition

Sl. No.	Treatment	Field emergence (%)	Speed of Germination	Plant height (cm)	Seedling length (cm)	Dry wt. (g/seedling)	SVI-I	SVI-II	Seedling blight (%)	Damping off (%)
		1	2	3	4	5	6	7	8	9
1	T.V(10%)	80.5(63.80)	11.25	23.60	28.0	2.21	2251.0	178.2	16.5(4.06)	15(3.84)
2	T.V(20%)	77.5(61.68)	8.01	22.60	26.6	1.13	2061.9	87.6	15(3.86)	14.75(3.84)
3	T.H(10%)	88 (69.75)	6.15	28.65	34.9	2.75	3070.4	241.9	10.3(3.17)	11.25(3.35)
4	T.H(20%)	86 (68.06)	6.75	27.80	29.4	2.37	2530.6	203.9	12.0(3.43)	11.5 (3.36)
5	P.f(10%)	74.5(59.68)	8.23	21.10	25.1	1.48	1870.7	110.3	14.5(3.73)	15 (3.86)
6	P.f(20%)	73.75(59.22)	6.04	21.95	26.6	1.15	1959.1	84.5	14.0(3.74)	16.5 (4.05)
7	DRY	67.5 (55.26)	3.17	14.95	19.5	0.37	1307.0	25.3	27.5(5.26)	25 (4.99)
	SE(m)±	0.703	NS	1.917	1.459	0.161	115.392	13.308	0.207	0.194
	CD	2.068	NS	5.638	4.291	0.476	339.383	39.140	0.610	0.571

*Figures in the parenthesis in column1 are the angular transformed value
*Figures in column 8 & 9 are square are square root transformed value

All the treatment had significantly increased the seedling length over control. Highest seedling length was recorded in biopriming with *T. harzianum* 10% i.e 34.9cm followed by *T. harzianum* 20%. The lowest seedling length was recorded in control (19.5cm).

Biopriming with *T. harzianum* 10% recorded highest seedling dry weight (g/seedling) i.e 2.75g which was at par with *T. harzianum* 10%. The second best treatment was *T. viride* 10% followed by *T. viride* 20%, *P. fluorescens* 10% and *P. fluorescens* 20%. *P. fluorescens* 20% recorded minimum increase in seedling dry weight over conrol. The lowest seedling dry weight was found in control i.e 0.37g.

There was significant enhancement in seedling vigor index (SVI-I &SVI-II). Biopriming with *T. harzianum* 10% recorded highest enhancement in both SVI-I and SVI-II i.e. 3070.4 and 241.9 respectively. The lowest SVI-I and SVI-II were observed in control. Seedling diseases like seedling blight and damping off were observed in all the treatment along with control. But there was significant reduction in seedling blight and seedling damping off. Maximum seedling blight and damping off was recorded in the control pots.

Among all the treatments *T. harzianum* 10% show minimum seedling blight incidence(10.3%) followed by *T. harzianum* 20% (i.e. 12%), *P. fluorescens* 10% and *P. fluorescens* 20% reduced the seedling blight incidence i.e 49% & 47.3% over control.

Biopriming with *T. viride 10% and T. viride 20%* also reduced the seedling blight incidence i.e. 45.5% and 40%over control. Maximum seedling blight incidence was recorded in control (27.5%).

Biopriming with *T. harzianum 10%* and *T. harzianum* 20% recorded lowest seedling damping off (11.25 and 11.5 respectively). Second best treatment in reducing seedling damping off was *T. viride* 20%, *T. viride* 10% and *P. fluorescens* 10% i.e. 14.75%,15% & 15% respectively. Biopriming with *P. fluorescens* 20% also reduced the seedling damping off up to 34% compared to control. Maximum seedling damping off was found in control pots i.e 25%.

Two concentrations of biocontrol agents were used to standardize the concentration of biocontrol agent with soaking seed in biocontrol agent suspension for 12 hours and drying to evaluate their performance under laboratory condition as well as in green house condition.As earlier Kalaivani,2010, found that themaize seeds bioprimed with 60% *T. viride* for 12h showed higher germination (88%) with an increase of 7% over nonprimed seed and 6% over hydropriming. She has also reported that the seeds bioprimed with *P. fluorescens* at 80% for 12h showed greater germination and vigour.

It was found that *T. harzianum* 10% concentration was best in improving the seed quality parameters. Higher concentration had no better result in improving the seed quality parameters. *T. harzianum* 10% had highest germination (94%), seedling length (27.85cm), dry weight (0.316g), SVI-I (2564.4) and SVI-II (29.71). *T. harzianum* 10% was found to be more effective in reducing the seedling infection (4.75%) and seed rot (1.75%). Many workers had reported that biopriming with *T. harzianum* resulted in increase in germination, root length, shoot length, seedling vigor index as well as effective against seed borne diseases. Lopez *et al.,* 2009 has reported that *Trichoderma sp* synthesis auxin such indole acetaldehyde which induce plant root and shoot growth.

Bioprimed seeds with *T. viride*, *T. harzianum* and *P. fluorescens* with two different concentrations were also evaluated under

green house condition, to validate the result, biocontrol agents as well as seed were exposed to field condition, they may be stressful due to variation in soil environment and atmospheric condition differently. Biopriming *with T. harzianum* 10% resulted in higher field emergence, increased plant height, seedling length, dry weight with reduced incidence of seedling blight and seedling damping off. Seedling vigour index was found to be enhanced due to increase in field emergence, seedling length and seedling dry weight. But there was no significant increase in speed of germination in the treatments over control. Seedling blight and seedling damping off was observed but the incidence of these disease were reduced than control. Seedling damping off in maize. Which can be effectively controlled by biopriming with *T. harzianum* as reported by earlier workers like- Okoth and Siameto, 2010. Seedling blight in maize can be controlled by biopriming with *T. harzianum* was reported by Hend. A. Alwathnani *et al.,* 2012 and Chandra Nayaka *et al.,* 2010.

From the present investigation it was concluded that biopriming of seed with biocontrol agent like *T. harzianum* is very effective in reducing the seed borne diseases as well as improving seed quality and crop quality leading to higher production.

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