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Biopriming of Maize Hybrid Seeds with Biocontrol Agents for Improving Germination and Vigour

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An investigation was carried out with COH (M) 5 hybrid maize seed to standardize the optimum concentration and duration of biopriming with talc based formulations of Trichoderma viride and Pseudomonas fluorescens individually. The duration for hydropriming was also standardized. The results revealed that biopriming with TNAU talc formulations of Trichoderma viride and Pseudomonas fluorescens @ 80% concentration for 6 h and 12h, respectively was found to enhance the germination rate, total germination percentage, seedling growth and vigour of COH (M) 5 hybrid maize seeds.

Key words: *Trichoderma viride*, *Pseudomonas fluorescens*, Talc product, Biopriming, Maize hybrid seeds, Enhanced germination and vigour.

Maize (*Zea mays* L.) is the world's third important cereal crop, next to rice and wheat. Considered as the "Queen of Cereals", it is grown in an area of 8.26 million ha^{-1} in India with a total production of 19.3 million tonnes, and the average productivity is 2337 kg ha^{-1}. Tamil Nadu accounts for an area of 3.08 lakh ha with a production of 0.24 million tonnes recording an average productivity of 4 tonnes ha^{-1} in Tamil Nadu.

To provide higher quality seeds, many researchers have developed new technologies called seed quality enhancement techniques. The main objective of this technique is to optimize the application of seed treatment products by improving the technical quality of seeds. In the last two decades, seed priming, an effective seed invigouration method has become common to increase the rate and uniformity of emergence and crop establishment in most of the vegetable and flower crops especially, in advanced countries.

Seed priming is a controlled hydration process that involves exposing seeds to low water potentials that restrict germination, but permits pregerminative physiological and biochemical changes to occur (Heydecker and Coolbear, 1977; Bradford, 1986; Khan, 1992). Upon rehydration, primed seeds may exhibit faster rate of germination, more uniform emergence, greater tolerance to environmental stresses and reduced dormancy in many species (Khan, 1992).

Biopriming, a new technique of seed treatment that integrates biological and physiological aspects of disease control, was recently used as alternative method for controlling many seed and soil borne pathogens (Harman and Taylor, 1988; Harman *et al*., 1989; Callan *et al*., 1991; Jahn and Plus, 1998; EL. Mohamedy, 2004; EL. Mohamedy *et al*., 2006).

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Application of biological control using antagonistic microorganisms against seed and root rot pathogens proved to be successfull in controlling many crop diseases (Adams 1990; Callan *et al*., 1991; Farzana and Ghaffar, 1991). Coating seeds with biocontrol agents such as Trichoderma spp., Bacillus subtilis and *Pseudomonas fluorescens* were the most effective treatments for controlling seed and soil borne pathogens (Abd El-Kareem, 2002; Adams,1990; EL. Mohamedy, 2004; EL. Mohamedy *et al*., 2006; Harman *et al*., 1989; Lacicova and Pieta, 1996; Ragab *et al*., 1999).

Biopriming of maize hybrid COH(M) 5 seeds with talc based commercial formulations (TNAU) of the biocontrol organisms *viz., Trichoderma viride* and *Pseudomonas fluorescens* has been taken up and the results are presented.

Materials and Methods

Genetically pure, fresh seeds of hybrid maize COH (M) 5 were obtained from Agricultural Research Station, Bhavanisagar, Tamil Nadu Agricultural University. Talc based commercial products of the biocontrol agents viz., *Trichoderma viride* and *Pseudomonas fluorescens* were obtained from the Department of Plant Pathology, TNAU, Coimbatore. The laboratory studies were carried out at the Department of Seed Science and Technology, TNAU, Coimbatore.

The wet product for priming (100% concentration) was prepared by mixing the talc based commercial products of the biocontrol agents with equal volume of water. This was further diluted to prepare 40, 60 and 80% concentrations.

The details on biopriming agents, their concentrations and duration of soaking are given below:

Biopriming agents	Concentration (%)	Duration of soaking (h)
Nonprimed seed	-	-
Water	-	6, 12, 18 and 24
Trichoderma viride	40	6, 12, 18 and 24
	60	
	80	
Pseudomonas fluorescens	40	6, 12, 18 and 24
	60	
	80	

Five hundred seeds were soaked in double the volume of respective concentration of the biopriming solution followed by soaking in water for 6,12,18 and 24h for hydropriming. Later, the seeds were shade dried at ambient temperature for assessing the seed quality parameters. The experiment was conducted with four replications in factorial completely randomised design (CRD).

Germination

F our replic at es of 100 s eeds each wer e germinated in a germination room maintained at 25±2°C temperature and 95±5% RH. At the end of seventh day of sowing, the number of normal seedlings in each replication was counted and the germination was calculated and expressed in percentage (ISTA, 1999).

Speed of germination

Four replicates of hundred seeds each were used to study this parameter. The seeds showing radicle protrusion were counted daily from third day after sowing until seventh day. From the number of seeds germinated on each day, the speed of germination was calculated using the following formula (Maguire, 1962) and the results are expressed in number.

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots + \frac{X_n - X_{n-1}}{Y_n}$$

X_1- Number of seeds germinated at first count

X_2- Number of seeds germinated at second count

X_n- Number of seeds germinated on n^{th} day

Y_1- Number of days from sowing to first count

Y_2- Number of days from sowing to second count

Y_n- Number of days from sowing to n^{th} count

Root length

At the time of germination count, ten normal seedlings were selected at random from each replication and used for measuring the root length of seedlings. Root length was measured from the point of attachment of seed to the tip of primary root. The mean values were calculated and expressed in centimetre.

Shoot length

The seedlings used for measuring root length were also used for measuring shoot length. The shoot length was measured from the point of attachment of seed to tip of the leaf and the mean values were expressed in centimetre.

Dry matter production

The five normal seedlings were placed in a paper cover and shade dried for 24 h and oven dried at 103±2°C for 16±1h. The dried seedlings were weighed and the mean values were expressed in g 5 seedlings^{-1}.

Vigour index

Vigour index values were computed using the following formula and the mean values were expressed in whole number (Abdul-Baki and Anderson, 1973).

Vigour index = Germination percentage x (Root + Shoot length).

Statistical analysis

The data obtained from different experiments were analysed for the 'F' test of significance following the methods described by Panse and Sukhatme (1985).

Results and Discussion

Seed biopriming with Trichoderma viride

The speed of germination, germination percentage, root and shoot length, and vigour index were significantly influenced by biopriming treatment, duration and its interaction. Seed biopriming with *T. viride* product (80%) for 6 h recorded a high speed of germination of 8.6 when compared to non primed seeds (5.3) (Table 1).

Plate 1. Speed of germination at 4th day of germination as influenced by T. viride biopriming

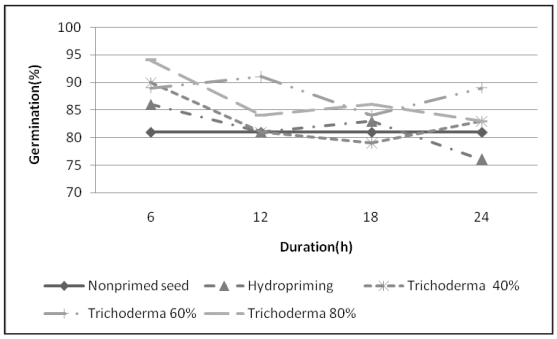
Nonprimed seeds

Seeds bioprimed with *T. viride* product 80% for 6h

Seeds bioprimed with talc product of *T. viride* @ 80% for 6h registered the highest germination of 94% which was 13% higher than non primed seeds. The lowest germination of 81% was observed in the seeds hydroprimed for 12 h after biopriming with 40% of *T. viride* product.

The longest root was recorded in seed bioprimed with *T. viride* product at 80% for 6 h (26.7cm root length) when compared to non primed seed (16.3 cm). The longer shoot was measured in the seeds primed for 18 h (12.5cm), which was on a par with priming for 24 h (12.6cm).

Fig 1. Germination percentage of maize hybrid seed bioprimed with *T. viride*



Biopriming involving *T. viride* product at 60% for 6 h registered higher drymatter production (0.75 g 5 seedlings^{-1}) and this was on a par with that of 80% for 6 h (0.74 g 5 seedlings^{-1}). The drymatter production of non primed seed was 0.53 g 5 seedlings^{-1}.

Seeds bioprimed with *T. viride* product at 80% for 6 h registered more vigour index value (3873), when compared to control (2057). The percentage of increase over non primed seed was 88 (Fig.2).

Fig 2. Vigour index of maize hybrid seeds bioprimed with *T.viride*

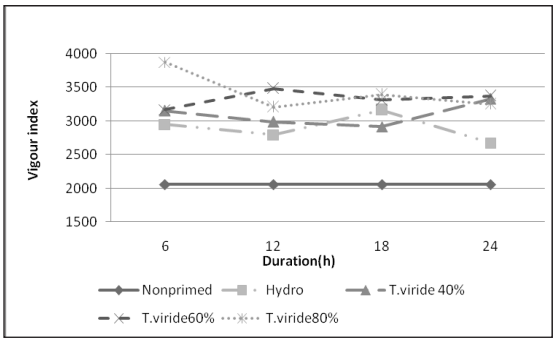
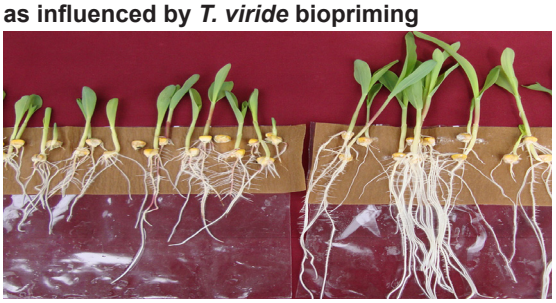


Plate 2. Seedling vigour at 7th day of germination as influenced by *T. viride* biopriming



Nonprimed seeds

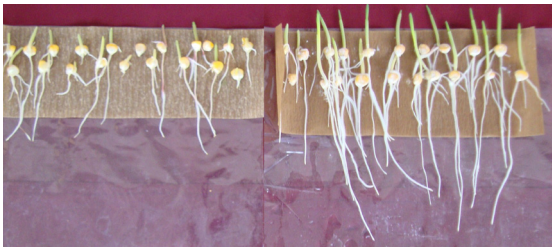
Seeds bioprimed with *T. viride* product 80% for 6h

Seed biopriming with P. fluorescens

Seeds bioprimed with *P. fluorescens* product at 80 % for 12 h registered the highest speed of germination of 9.8 when compared to non primed seed (6.9) (Table 2).

Seeds bioprimed with *P. fluorescens* product at 80% for 12 h outperformed other treatments recording 95 % germination. The germination of non primed seed was the lowest (75%).

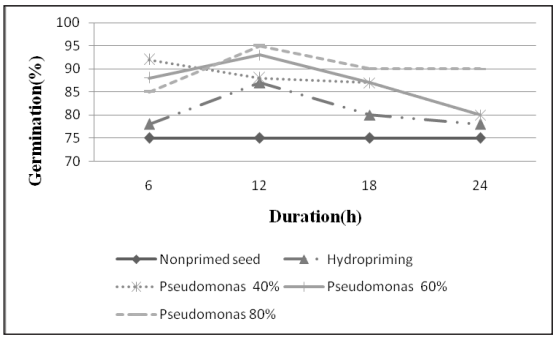
Plate 3. Speed of germination at 4th day as influenced by *P. fluorescens* biopriming



Nonprimed seeds

Seeds bioprimed with *T. viride* product 80% for 6h

Fig 3. Germination of hybrid maize seed bioprimed with *P. fluorescens*



The longest root of 27.4 cm (Table 5) was measured with *P. fluorescens* product used at 80% biopriming for 12 h, which was on a par with *P. fluorescens* 80% biopriming for 24 h (27.3 cm). The root length was the shortest in the seeds bioprimed with *P. fluorescens* product at 40% for 18 h (24.9 cm). The lesser root length was observed in non primed seeds (19.6 cm).

P. fluorescens product at 80% for 12 h registered higher shoot length (19.5 cm) which was on par with 80 %, 40 % and 60 % product treatments for 18 h (19.0 cm), 24 h (18.9 cm), 24 h (18.7 cm) and hydropriming for 24 h (18.7cm) respectively. The shoot length of non primed seed was 14.0cm (Table 2).

The drymatter production in case of seeds primed with *P. fluorescens* product @ 80 % was more (0.96g. 5 seedlings^{-1}), which was on par with 60 % (0.95g. 5 seedlings^{-1}) and 40% (0.94g. 5 seedlings^{-1}) product treatments.

The drymatter production of nonprimed seed was0.87 g. 5 seedlings^{-1} and this was on par with hydropriming (0.90 g. 5 seedlings^{-1}). Among the priming duration, the drymatter production ranged from 0.90 g.5 seedlings^{-1} (6 h, 18 h) to 0.97 g.5 seedlings^{-1} (12 h) (Table 2).

Seed bioprimed with *P.fluorescens* product at 80 % for 12 h registered more vigour index of 4456. The lesser vigour index of 2530 was recorded in non primed seeds.

The significant enhancement in speed of germination, germination percentage and seedling

Table 1. Effect of seed biopriming with *T. viride* talc product (TNAU) on COH (M) 5 maize hybrid

Biopriming treatments (T)	Speed of germination					Root length (cm)					Shoot length (cm)					Drymatter production (g 5 seedlings-1)				
	Soaking duration in h (D)																			
	6	12	18	24	Mean	6	12	18	24	Mean	6	12	18	24	Mean	6	12	18	24	Mean
Nonprimed	5.3	5.3	5.3	5.3	5.3	16.3	16.3	16.3	16.3	16.3	9.1	9.1	9.1	9.1	9.1	0.53	0.53	0.53	0.53	0.53
Hydropriming	8.2	7.2	7.3	6.8	7.4	22.0	23.1	25.5	23.3	23.5	12.3	11.4	12.6	11.9	12.0	0.65	0.64	0.63	0.63	0.64
Trichoderma product 40%	7.4	8.0	7.9	8.4	7.9	22.0	23.8	24.1	26.5	24.1	13.0	13.1	12.8	13.6	13.1	0.63	0.61	0.64	0.57	0.61
60%	7.9	7.9	7.4	5.8	7.2	23.1	24.3	25.7	24.7	24.4	12.5	14.0	13.7	13.2	13.3	0.75	0.56	0.66	0.59	0.64
80%	8.6	8.2	7.4	7.0	7.8	26.7	24.0	25.0	24.0	25.0	14.5	14.2	14.5	15.2	14.6	0.74	0.57	0.62	0.61	0.63
Mean	7.4	7.3	7.0	6.7		22.0	22.3	23.3	23.0		12.3	12.4	12.5	12.6		0.66	0.58	0.62	0.59	
	T		D		T x D	T		D		T x D	T		D		T x D	T		D		T x D
SEd	0.05		0.04		0.10	0.08		0.07		0.16	0.08		0.07		0.16	0.005		0.004		31.86
CD (P=0.05)	0.10		0.90		0.20	0.16		0.14		0.32	0.16		0.14		0.32	0.010		0.009		64.39

Table 2. Effect of seed biopriming with *P. fluorescens* talc product (TNAU) on COH (M) 5 maize hybrid

Biopriming treatments (T)	Speed of germination					Root length (cm)					Shoot length (cm)					Drymatter production (g 5 seedlings-1)				
	Soaking duration in h (D)																			
	6	12	18	24	Mean	6	12	18	24	Mean	6	12	18	24	Mean	6	12	18	24	Mean
Nonprimed	6.9	6.9	6.9	6.9	6.9	19.6	19.6	19.6	19.6	19.6	14.0	14.0	14.0	14.0	14.0	0.87	0.87	0.87	0.87	0.87
Hydropriming	7.9	8.6	7.8	7.7	8.0	22.4	23.4	22.9	25.1	23.4	14.3	15.4	14.3	18.7	15.7	0.90	0.94	0.89	0.86	0.90
Pseudomonas product 40%	8.4	8.5	8.6	7.8	8.3	25.1	25.7	24.9	25.5	25.3	15.6	16.5	18.3	18.9	17.3	0.96	0.98	0.89	0.93	0.94
60%	8.9	8.8	9.3	8.1	8.8	26.6	26.6	25.4	25.6	26.1	16.9	16.7	17.2	18.7	17.4	0.85	1.00	0.97	0.97	0.95
80%	7.5	9.8	8.3	8.2	8.5	26.4	27.4	26	27.3	26.8	16.2	19.5	19.0	18.1	18.2	0.92	1.06	0.87	1.00	0.96
Mean	7.9	8.5	8.2	7.7		24.0	24.5	23.8	24.6		15.4	16.4	16.6	17.7		0.90	0.97	0.90	0.92	
	T		D		T x D	T		D		T x D	T		D		T x D	T		D		T x D
SEd	0.06		0.05		0.11	0.11		0.09		0.22	0.20		0.17		0.40	0.26		0.23		0.52
CD (P=0.05)	0.11		0.10		0.23	0.22		0.19		0.44	0.40		0.36		0.80	0.05		0.04		NS

vigour due to seed biopriming with *T.viride* talc product observed in the present study might be attributed to the activation of pregerminative metabolic activities before the protrusion of radicle as reported by Bradford (1986), Taylor and Harman (1990); and Mc Donald (2000). However, biopriming with biocontrol agents had a dual action such as control of disease causing pathogens during germination and enhancement of germination percentage and vigour by integrating the biological and physiological changes during germination (Callan *et al*., 1991; Jahn and Plus, 1998; EL. Mohamedy, 2004; EL. Mohamedy *et al*., 2006).The observations of current investigation are also in agreement with the findings of Sunil Kumar *et al*., (2007), who reported a positive effect of Trichoderma biopriming in increasing the fresh and dry weight of root and shoot in tomato. Similar observations were made in, lentil (Sultana and Hossain, 1999), tomato (Srivastava *et al*., 2010) and soybean (Begum *et al*., 2010). Seed biopriming with pure culture of T.harzianum increased the germination from 18.2 to 35.2 per cent and vigour index from 36.0 to 66.4 per cent among the three varieties of maize (Chandra Nayaka *et al*., 2010). Kleifeld and Chet (1992) proposed that T.harzianum would enhance seed germination and growth of bean, radish, tomato, pepper and cucumber.

Fig 4. Vigour index of hybrid maize seed bioprimed with *P.fluorescens*

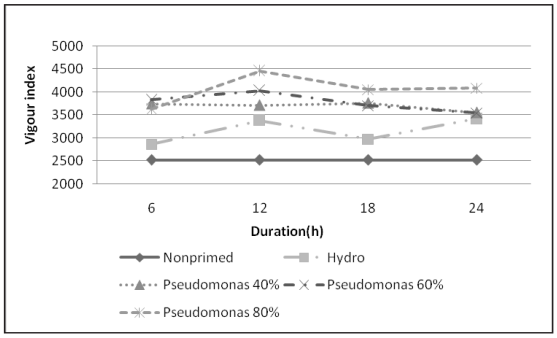
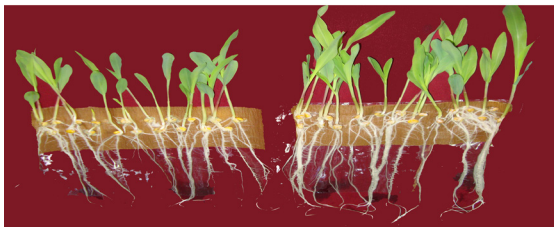


Plate 4. Seedling growth at 7th day of germination as influenced by *P.fluorescens biopriming*



Nonprimed seeds

Seeds bioprimed with *T. viride* product 80% for 6h

It is also evident from the present study that seed biopriming with *P. fluorescens* talc product promoted germination, seedling growth and vigour of maize. Earlier works in pearl millet (Umesha *et al*., 1998 and Niranjan Raj *et al*.,2004); sorghum (Raju *et al*.,1999) and rice (Praveen Kumar *et al*.,2001) support the present findings. The enhancement in the seedling growth can be attributed to suppression of deleterious microorganisms and pathogens; production of plant growth regulators such as gibberellins, cytokinins and indole acetic acid; increased availability of minerals and other ions; and more water uptake as reported by Van Loon *et al*., (1998) and Ramamoorthy *et al*., (2001).The results of the present investigation are also in agreement with Sunil Kumar *et al*. (2007), who stated that Pseudomonas had a positive effect on root development in tomato. Begum *et al*. (2010) reported that in soybean, bioprimed seed with *P.aeruginosa* resulted in enhancement of seed germination ranging from 32.4 to 60.7% as compared to hydroprimed control. Strains of *P.fluorescens* appear to be outstanding in this context, because, in addition to induced resistance, they also promote growth and development of plants (Chen *et al*., 2000; Ongena *et al*., 2000; Ramamoorthy *et al*., 2001; Desai *et al*., 2002; Gnanamanickam *et al*.,2002).

The beneficial effects of biopriming observed in this study might be the result of a synergism since priming confers benefits such as completion of early germination phases, increasing the population of bioprotectants, rapid and uniform seedling emergence, facilitation of uptake of water and nutrients, protection against pathogens, potential defense responses such as early oxidation burst, incorporation of various phenolic compounds and polymers to the cell wall and secretion of phytoalexins as evidenced from previous studies (Musa *et al*., 1999; 2001; Mathre *et al*., 1999; Conrath *et al*., 2002). These findings could also be attributed to either direct suppression of pathogens or indirectly through the production of growth hormones; increased uptake, solubilization and translocation of less available minerals (Windham *et al*., 1986; Tronsmo and Hjeljord, 1988; Compant *et al*., 2005; Harman, 2005). Furthermore, some reports indicate that Trichoderma and Pseudomonas are plant growth promoting fungus (Windham et al., 1986) and bacteria (Pandey et al., 2005), respectively. According to Kavitha (2011) as quoted by Vanangamudi *et al*.(2012), seed biopriming with *T.viride* talc product at 60% for 18 h or P. fluorescens at 60 % for 12 h was the best seed treatment for rice seed to enhance the germination rate, total germination percentage, seedling growth and vigour. Bhendi seeds bioprimed with *T.viride* talc product at 60% for 12 h or *P.fluorescens* talc product at 60 % for 12 h also resulted in higher germination percentage and seedling vigour (Mariselvam, 2012).

From the studies on standardization of biopriming the seeds of maize hybrid COH(M) 5, it is concluded that biopriming with talc based product (TNAU) of *T.viride* or *P. fluorescens* at 80 % concentration for 6 h and 12 h, respectively could be the best seed biopriming treatments to enhance the germination percentage and seedling vigour.

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