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Biopriming of maize hybrid COH(M) 5 seed with liquid biofertilizers for enhanced germination and vigour

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An investigation was carried out with COH(M) 5 maize hybrid seed to standardize seed biopriming with liquid biofertilizers (*Azospirillum* and phosphobacteria). To optimize the concentration and duration of biopriming, seeds were bioprimed with liquid biofertilizers such as *Azospirillum* and phosphobacteria (Concentration: 10, 15, and 20%; Duration: 6, 12, 18, and 24 h). The seeds were also hydroprimed for 12, 24, 36, and 48 h for standardization. The nonprimed seed formed the control. Biopriming with liquid *Azospirillum* 20% concentration for 12 h expressed high values for all the parameters studied namely speed of germination, germination, root length, shoot length, dry matter production, total dry matter production and vigour index (G × SL) which accounted for 82, 20, 26, 24, 28, 58, and 59% increase over nonprimed seed. Phosphobacteria 20% concentration biopriming for 12 h was found to improve the speed of germination, germination, root length, shoot length, dry matter production, total dry matter production and vigour index (G × SL). The increases over nonprimed seed for these parameters were 35, 12, 34, 61, 66 and 62%.

Key words: Biopriming, liquid biofertilizers, *Azospirillum,* phosphobacteria, germination, vigour.

INTRODUCTION

Maize (*Zea mays* L.) is the third important cereal crop next to rice and wheat in the World. It is considered as the “Queen of Cereals”. Since, maize is an industrial important crop, the demand for maize seed is more. On realizing the importance of maize in seed industry, the private seed companies are now concentrating more on maize hybrid development and because of continuous research many hybrids were also developed. For existence of any variety or hybrid, timely supply of quality seed is foremost requirement. Maize is cultivated over an area of about 159 000 000 ha with a production of about 817 million tonnes and productivity of 5 tonnes of grain ha^{-1} in 2009 (en.wikipedia.org/wiki/maize).

By 2020 AD, the requirement of maize in various sectors will be around 100 million tonnes, of which the poultry sector demand alone will be 31 million tonnes. It is a very difficult task for our agriculturists to increase the maize production from the present level of 34 to 100 million tonnes (Seshaiah, 2000). The only option is to increase the maize productivity per unit area of land and time, which can be achieved through selection of genotype and application of proper production management techniques.

COH(M) 5 is one of the newly released high yielding single cross maize hybrid by the Department of Millets, Tamil Nadu Agricultural University, Coimbatore. It is resistant to downy mildew and moderately resistant to stem borer.

Higher production and productivity of crop is possible only through use of good quality seeds and proper management practices. Good quality seeds imply vigour, uniformity and structural soundness besides its genetic and physical purity.To provide higher quality seeds, many researchers have developed new technologies called “Seed

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Table 1. Biopriming agents, their concentrations and duration of priming.

Biopriming agent	Concentration (%)	Duration of soaking (h)
Nonprimed seed	-	-
Water	-	6, 12, 18 and 24
Azospirillum	10	6, 12, 18 and 24
	15	
	20	
Phosphobacteria	10	6, 12, 18 and 24
	15	
	20	

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 a common seed treatment to increase the rate and uniformity of emergence and crop establishment in most 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 seed treatment system that integrates the biological and physiological aspects of enhancing growth, disease control and increase in yield, involves coating the seed with biological agents and incubating the seed under warm, moist conditions. Seed may be planted moist or dried for storage.

Excessive and continuous use of chemical fertilizers coupled with pesticides and fungicides have damaged the soil health which causes deleterious effects on crop cultivation and productivity. Now-a-days, the chemical fertilizers are replaced by environment friendly biofertilizers. Most of the biofertilizers manufactured in India are solid carrier based and generally suffer from shorter shelf life, poor quality, high contamination and low field performance (Hedge, 2002). At present, the carrier based biofertilizers are replaced by liquid formulations.

Many scientists and researchers are now recommending liquid formulations of biofertilizers (Gomathy et al., 2007; Thamizh and Thangaraju, 2007; Martin and Maria, 2009), since they spread well and mix uniformly without any sticking agent over the seed surface (Rice and Olsen, 1992).

Liquid biofertilizers are developed only recently. Scientists have researched about the standardization of liquid formulations and compared their performance for productivity with the inorganic fertilizers (Thamizh and Thangaraju, 2006; Gomathy et al., 2008a, b, 2009). But, studies on the effect of seed treatment with liquid biofertilizers on the germination and seedling vigour are very negligible. Hence, study was undertaken to standardize the optimum concentration and duration for seed biopriming using liquid *Azospirillum* and phosphobacteria.

MATERIALS AND METHODS

Genetically pure fresh seeds of hybrid maize COH(M) 5 obtained from Agricultural Research Station, Tamil Nadu Agricultural University, Bhavanisagar, Erode District of Tamil Nadu formed the base material for this study. The liquid biofertilizers such as *Azospirillum* and phosphobacteria collected from the Department of Agricultural Microbiology were also used for this study. The details on biopriming agents, their concentrations and duration of priming are given in Table 1.

Five hundred seeds were soaked twice in the volume of the respective concentration in each of the bio-priming agents. For hydropriming, the seeds were soaked in water for 6, 12, 18, and 24 h. The non-primed seeds formed the control. After the soaking duration, the seeds were removed from the solutions and shade dried at room temperature for assessing the seed quality parameters. The experiment was carried out with 4 replications in factorial completely randomised design (CRD).

Germination

The germination test was conducted by following the procedure outlined by ISTA (1999) using paper (between papers) medium. Four replicates of 100 seeds each were germinated in a germination room maintained at 25 ± 2°C temperatures and 95 ± 5% relative humidity (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.

Speed of germination

Four replicates of 100 seeds each were used to test the speed of germination of seeds from different treatments. The seeds showing radicle protrusion were counted daily from the third day after sowing

Table 2. Effect of seed biopriming using *Azospirillum* on speed of germination of hybrid maize COH(M) 5.

Biopriming treatment (T)	Soaking duration in h (D)				
	6	12	18	24	Mean
Nonprimed seed	3.8	3.8	3.8	3.8	3.8
Hydropriming	5.8	5.8	5.3	5.0	5.5
Azospirillum 10%	5.2	5.1	5.5	6.0	5.4
15%	4.6	5.9	4.9	5.7	5.3
20%	4.2	6.9	5.8	5.5	5.6
Mean	4.7	5.5	5.0	5.2	
		T	D	T × D	
SEd		0.05	0.04	0.10	
CD (P = 0.05)		0.10	0.09	0.21	

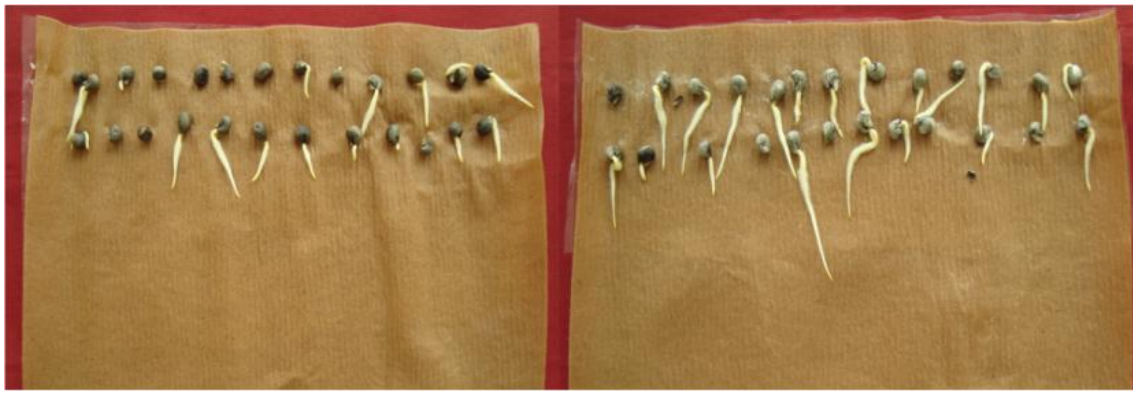

Figure 1. Speed of germination at 48 h of germination as influenced by *T. viride* biopriming.

until the seventh day. From the number of seeds germinated on each day, the speed of germination was calculated using the following formula and the results were expressed in number (Maguire, 1962).

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

Where, 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, 10 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 the tip of the leaf and the mean values were expressed in centimetre.

Drymatter production

The 5 normal seedlings were placed in a paper cover and dried in the shade for 24 h and then, they were kept in an oven maintained at 103 ± 2°C for 16 ± 1 h. 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 formulae and the mean values were expressed in whole number (Abdul-Baki and Anderson, 1973). Vigour index = Germination percentage × (Root + Shoot length).

Table 3. Effect of seed biopriming using *Azospirillum* on root and shoot length of hybrid maize COH(M) 5.

Biopriming treatment (T)	Root length (cm)					Shoot length (cm)				
	Soaking duration in h (D)					Soaking duration in h (D)				
	6	12	18	24	Mean	6	12	18	24	Mean
Nonprimed seed	17.9	17.9	17.9	17.9	17.9	7.9	7.9	7.9	7.9	7.9
Hydropriming	20.4	21.6	20.1	19.3	20.3	8.6	8.7	8.1	8.1	8.4
*Azospirillum*10%	21.5	21.7	20.1	20.2	20.8	8.9	8.7	8.1	9.2	8.7
15%	21.2	21.9	21.3	21.8	21.5	8.5	9.3	8.6	9.2	8.9
20%	20.7	22.5	21.1	19.9	21.0	8.3	9.8	9.0	8.8	9.0
Mean	20.3	21.1	20.1	19.8		8.4	8.9	8.3	8.6	
		T	D	T x D			T	D	T × D	
SEd		0.09	0.08	0.18			0.07	0.06	0.14	
CD (P = 0.05)		0.18	0.16	0.37			0.14	0.13	0.30	

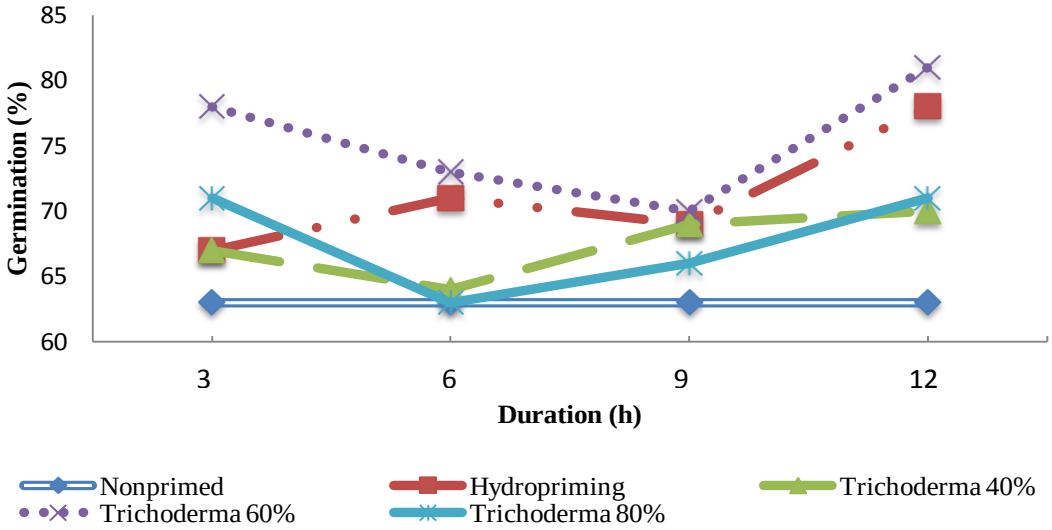

Figure 2. Germination of bhendi seed bioprimed with *T. viride.*

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 *Azospirillum*

Statistically significant variation was observed for speed of germination, germination, root and shoot length, dry matter production and vigour index due to priming treatment, duration of biopriming and its interaction effect. The seeds bioprimed with *Azospirillum* 20% for 12 h registered higher speed of germination (6.9) (Table 2) and germination (95%) (Figure 1) than nonprimed seed. An increase of 20% was noticed for germination due to *Azospirillum* biopriming over nonprimed seed. *Azospirillum* 20% bioprimed seed for 12 h measured the longest root (22.5 cm) and shoot (9.8 cm) shortest root and shoot was observed in nonprimed seed (17.9 and 7.9 cm, respectively) (Table 3 and Figure 2).

Seeds bioprimed with 20% *Azospirillum* for 12 h produced higher dry matter production (0.95 g 5 seedlings^{-1}) which was followed by *Azospirillum* 20% for 24 h (0.91 g 5 seedlings^{-1}). The dry matter production was lower (0.73 g 5 seedlings^{-1}) in *Azospirillum* 20% 6 h which was on a par with nonprimed seed (0.74 g 5 seedlings^{-1}) (Table 4)

This treatment also registered more vigour (3069) when compared to other treatments. The vigour index value of control was 1935 (Figure 3).

Table 4. Effect of seed biopriming using *Azospirillum* on dry matter production of hybrid maize COH(M) 5.

Biopriming treatment (T)	Dry matter production (g 5 seedlings^{-1})				
	Soaking duration in h (D)				
	6	12	18	24	Mean
Nonprimed seed	0.74	0.74	0.74	0.74	0.74
Hydropriming	0.82	0.84	0.83	0.84	0.83
Azospirillum 10%	0.83	0.88	0.79	0.89	0.85
15%	0.85	0.80	0.83	0.81	1.42
20%	0.73	0.95	0.82	0.91	0.85
Mean	0.79	1.32	0.80	0.84	
		T	D	T × D	
SEd		0.38	0.33	0.75	
CD (P = 0.05)		0.77	0.69	1.53	

Table 5. Effect of seed biopriming using phosphobacteria on speed of germination of hybrid maize COH(M) 5.

Biopriming treatment (T)	Soaking duration in h (D)				
	6	12	18	24	Mean
Nonprimed seed	5.1	5.1	5.1	5.1	5.1
Hydropriming	5.6	6.0	5.9	5.4	5.8
Phosphobacteria 10%	5.9	6.0	6.1	5.1	5.8
15%	5.3	6.4	6.3	5.9	5.9
20%	5.7	6.9	5.6	5.8	6.0
Mean	5.5	6.1	5.8	5.4	
		T	D	T × D	
SEd		0.06	0.06	0.13	
CD (P = 0.05)		0.13	0.12	0.27	

Seed biopriming with phosphobacteria

The speed of germination, germination, root and shoot length, dry matter production and vigour index were significantly influenced by biopriming treatment, duration of biopriming and their interaction. The results indicated the better performance of phosphobacteria 20% for 12 h with respect to speed of germination (6.9). The lowest speed of germination of 5.1 was noticed in nonprimed seed which was on a par with phosphobacteria 10% for 24 h and 15% for 6 h (5.3) (Table 5).

Seeds primed with phosphobacteria at 20% concentration for 12 h also recorded higher germination (95%) which showed an increase of 25% over nonprimed seed (Figure 4).

Seeds bioprimed with phosphobacteria at 20% for 12 h measured longer root (25.7 cm) and shoot (12.9 cm) compared to nonprimed seed (19.2 and 8.0 cm, respectively) (Table 6 and Figure 5). The biopriming involving phosphobacteria 20% for 12 h registered higher dry matter production (0.99 g 5 seedlings^{-1}) and this was on a par with 15% for 18 h (0.97 g 5 seedlings^{-1}). The dry matter production of hydroprimed seed was 0.71 g 5 seedlings^{-1} (6 h) and phosphobacteria 10% for 12 h (0.73 g 5 seedlings^{-1}) which were on a par with each other (Table 6). Compared to nonprimed seed (2258), the phosphobacteria biopriming at 20% for 12 h registered better vigour index (3667) (Figure 6).

In the present study, seed biopriming with phosphobacteria 20% for 12 h was found to be the best biopriming treatment for improving the seed germination and seedling vigour of COH(M) 5 maize hybrid (Table 7). Similar increase in the seedling growth due to liquid phosphobacteria seed treatments was reported by Ponnusamy (1993) in neem, Vijaya kumari (2003) in neem, kapok and amla, Gomathy et al. (2007) in maize, Yousry et al. (1978) in pea, and Mahfouz and Sharaf-Eldin (2007) in fennel. Rice and Olsen (1992) suggested that, liquid formulation was an effective method of seed inoculation of biofertilizer. The relative enhancement of germination and seedling vigour might be attributed to the role of phosphorus solubilising bacteria known as

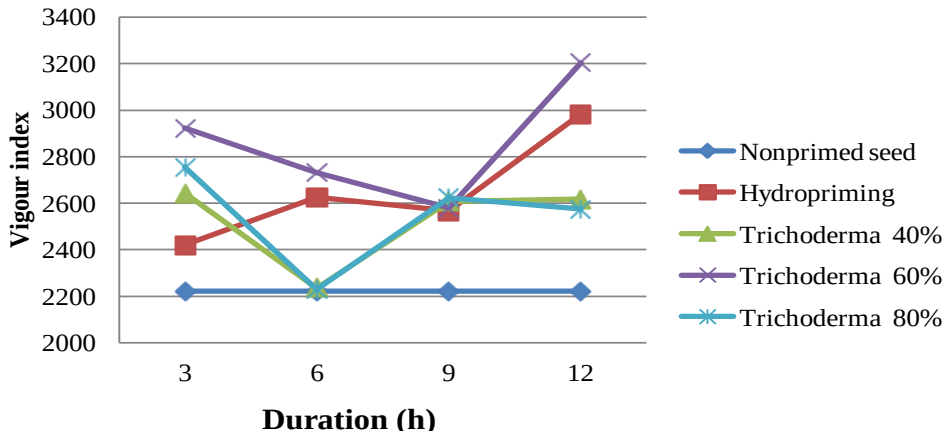

Figure 3. Vigour index of bhendi bioprimed seeds with *T. viride.*

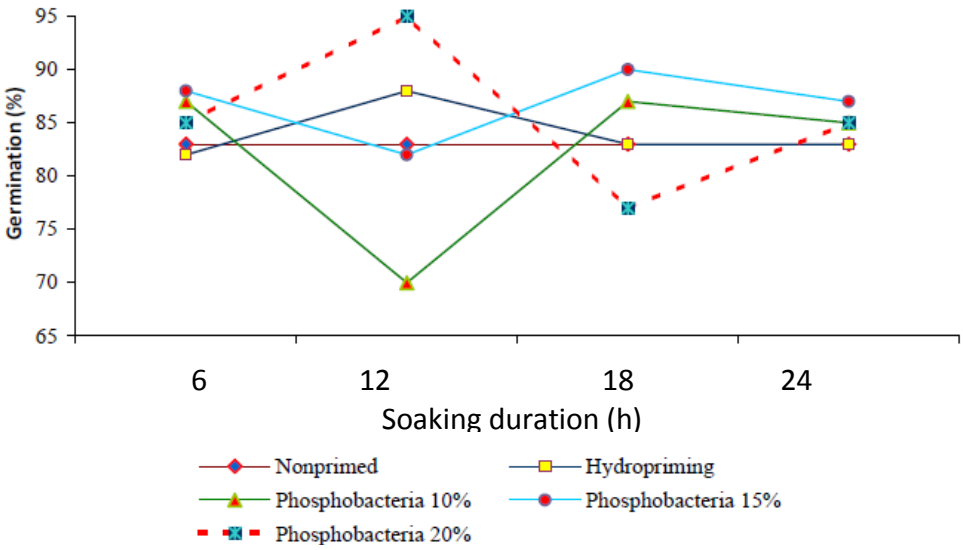

Figure 4. Germination of maize hybrid seed bioprimed with phosphobacteria.

Figure 5. Seedling vigour at 7th day of germination as influenced by phosphobacteria biopriming.

Table 6. Effect of seed biopriming using phosphobacteria on root and shoot length of hybrid maize COH(M) 5.

Biopriming treatment (T)	Root length (cm)					Shoot length (cm)				
	Soaking duration in h (D)					Soaking duration in h (D)				
	6	12	18	24	Mean	6	12	18	24	Mean
Nonprimed seed	19.2	19.2	19.2	19.2	19.2	8.0	8.0	8.0	8.0	8.0
Hydropriming	23.0	24.2	23.5	23.2	23.5	11.4	11.5	11.2	11.3	11.3
Phosphobacteria 10%	24.1	22.8	23.2	24.9	23.7	10.4	11.2	11.4	11.7	11.1
15%	24.2	22.7	23.8	23.6	23.6	10.9	11.5	11.1	12.3	11.4
20%	24.7	25.7	24.6	23.8	24.7	11.9	12.9	11.0	10.9	11.7
Mean	23.0	22.9	22.8	22.9		10.5	11.0	10.5	10.8	
		T	D	T × D			T	D	T × D	
SEd		0.11	0.10	0.22			0.06	0.05	0.12	
CD (P = 0.05)		0.22	NS	0.45			0.12	0.11	0.25	

Table 7. Effect of seed biopriming using phosphobacteria on dry matter production of hybrid maize COH(M) 5.

Biopriming treatment (T)	Drymatter production (g 5 seedlings^{-1})				
	Soaking duration in h (D)				
	6	12	18	24	Mean
Nonprimed seed	0.82	0.82	0.82	0.82	0.82
Hydropriming	0.71	0.76	0.79	0.77	0.75
Phosphobacteria 10%	0.89	0.73	0.84	0.91	0.84
15%	0.89	0.86	0.97	0.84	0.89
20%	0.83	0.99	0.83	0.89	0.88
Mean	0.83	0.83	0.85	0.85	
		T	D	T × D	
SEd		0.01	0.01	0.01	
CD (P = 0.05)		0.02	0.02	0.02	

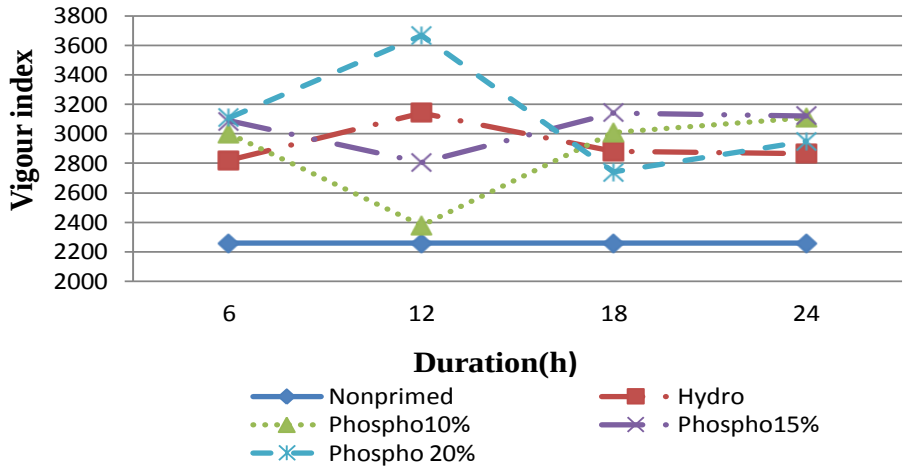

Figure 6. Vigour index of maize hybrid seed bioprimed with phosphobacteria.

phosphobacteria in enhancing the solubilisation of insoluble phosphorus and making it available to the germinating seed with consequent enhancement in the metabolic activity which resulted in higher germination (Cooper, 1979).

The improvement in seed germination and seedling

vigour parameters under laboratory conditions due to seed biopriming with liquid *Azospirillum* 20% for 12 h could be possible because of the production of germination accelerating and growth promoting substances by the liquid *Azospirillum*. Similar observations were reported by Morgenstern and Okon (1987), they reported that, auxin, gibberellins and cytokinin are synthesised and produced when the seeds were inoculated with *Azospirillum*. Tien et al. (1979), Cacciari et al. (1989) and Tiwary et al. (1998) also clearly established the production of gibberellins and cytokinins. Because of this Azospirillum inoculation, Okon and Kapulnik (1986) noticed larger proportion of younger roots and seminal root elongation, which resulted in increased size and number of root hairs (Kapulnik et al., 1985). In addition, the *Azospirillum* colonies efficiently in the root hairs which improved the water uptake (Sarig et al., 1988) in the early stages of growth. The results of the present study are in agreement with the findings of Ramamoorthy et al. (2000) in rice. They reported that, seed biofortication with *Azospirillum* enhanced seedling vigour encompassing speed of germination, seedling length and dry weight of high vigour in low vigour seed lots.

According to Kavitha (2011) seed biopriming with liquid *Azospirillum* 20% for 12 h or liquid phosphobacteria 15% biopriming for 12 h was found to be the best seed biopriming treatment for rice seed to enhance the germination rate, total germination percentage, seedling growth and vigour. Bhendi seeds bioprimed with liquid phosphobacteria 20% for 12 h or *Azospirillum* 15% for 12 h also resulted in higher germination percentage and seedling vigour (Mariselvam, 2012).

Conclusion

It is summarized from this study that, seed biopriming with liquid *Azospirillum* 20% for 12 h or liquid phosphobacteria 20% for 12 h was found to be the best biopriming treatment for improving the seed germination and seedling vigour of COH(M) 5 maize hybrid.

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