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Biopriming with bacterial inoculants on seed quality parameters of maize hybrid COH (M) 6 seeds

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Abstract
The present study was conducted to assess the effect of bio-priming with different bacterial suspension *viz. Bacillus amyloliquefaciens* VB7(MH348121), *Bacillus licheniformis* COEH6 (MG241257), *Brachybacterium paraconglameratum* YEBPT2 (MK263736) on performance of seed quality parameters such as speed of germination, germination per cent, length of root, shoot, dry matter production and vigour index. Results revealed that every single examined parameter influenced in improving seedling performance on bioprimed seeds with *Bacillus amyloliquefaciens* VB7 5% concentration which was on par with 1% *Bacillus amyloliquefaciens* VB7 biopriming treatment compared to control, hydropriming and other treatments.

Keywords: Biopriming, *Bacillus amyloliquefaciens VB7*

Introduction
Maize (*Zea mays*) is the third most important cereal in the world. It is called as "queen of cereals" with highest productivity among cereals. Maize grain contains significant quantity of nicotinic acid, vitamin A, vitamin E and phosphorus.
In modern agriculture, usage of hybrid is predominant. Farmers prefer hybrids than varieties because of their faster growth, higher yield and better adaptability to various environmental conditions. The maize hybrid COH (M) 6 is popular among the farmers because of its higher yield potential. The quality seeds are essential for sustainable crop production and food security. Though maize plants are bigger, stronger and more vigorous, the time from sowing to seedling establishment is crucial when seeds are exposed to wide range of environmental stresses. To overcome such kind of stress certain seed quality enhancement has to be given before taking up sowing.
Seed priming is a controlled hydration process that involves exposing the seeds to low water potential that restricts germination, but permits pre-germinative physiological and biochemical changes to occur (Heydecker and Coolbear, 1977) [8]. 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) [9]. Bio-priming is a process of biological seed treatment that refers combination of seed hydration (physiological aspects) and inoculation (biological aspects) to protect seed (Callan *et al.*, 1997) [6].
Biopriming is a new technique of seed enhancement in which the seeds are soaked in the bacterial suspension for precalculated period of time to allow the bacterial imbibition into the seed (Abuamsha *et al.*, 2011) [2]. Seed priming with bio-inoculants often results in more rapid growth and increased seedling vigour. Seed priming creates ideal conditions for the bacterial inoculation and colonization in the seed (McQuilken *et al.*, 1998) [11].
There is a need of optimizing the concentration of bio-inoculants liquid formulations for bio-priming of maize hybrid seeds. Hence, this study was aimed to optimize the dosage of three different bio-inoculants *viz. Bacillus amyloliquefaciens* VB7, *Bacillus licheniformis* COEH6 and *Brachybacterium paraconglameratum* PT2 based on several seedling parameters.

Material and Methods
A laboratory experiment on enhancement of seed quality parameters and optimization of concentration with different bio-control agents through biopriming in maize COH (M) 6 was conducted in the Department of Seed Science and Technology, Tamil Nadu Agriculture University, Coimbatore during 2019. Seeds were obtained from Department of Millets, TNAU, Coimbatore. Bacterial suspension of three bio-inoculants *viz. Bacillus amyloliquefaciens* VB7 (MH348121), *Bacillus licheniformis* COEH6 (MG241257),

Brachybacterium paraconglameratum YEBPT2 (MK263736) were obtained from Department of Plant Pathology TNAU, Coimbatore. Seeds of maize hybrid COH(M) 6 were bioprimed with three bio-inoculants at 1%, 5%, 10%, 15% and 20% for 12 hrs of soaking duration. Soaking solution ratio was 1:2. Seeds were also hydroprimed for 12 hours. After 12 hours of soaking, solution was drained out and the seeds were shade dried to bring back the original moisture content. Four hundred seeds (100×4) were subjected to germination test for evaluating seed quality parameters along with control.The experiment was carried out with four replications in Completely Randomized Design (CRD).

Table 1: Treatment details

Treatments	Concentration (%)	Duration of soaking	Volume of soaking (v:v)
Control	-	-	-
Hydropriming	-	12 hrs	1:2
Brachybacteriumparaconglameratum PT2	1, 5, 10, 15, 20		
Bacillus amyloliquefaciens VB7	1, 5, 10, 15, 20		
Bacillus licheniformis COEH6	1, 5, 10, 15, 20		

According to ISTA rules of seed testing (ISTA, 1999), standard germination test was conducted by between paper method. At the end of seventh day after sowing, germination was calculated and expressed in percentage. According to (Maguire, 1962) [10], speed of germination was calculated. Ten normal seedlings were randomly selected and root length and shoot length were measured. For dry matter production seeds were removed and the seedlings were dried in shade for 24 h followed by drying in hot air oven at 103±2º C for 16±1 hour. Dried seedlings were weighed and expressed in g per 10 seedlings. Vigour index was computed using the following formula and the mean values were expressed in whole number (Abdul-Baki and Anderson, 1973) [1].

Vigor index = Germination percentage × Seedling dry weight.

Analysis of Variance for Completely Randomized Design was calculated and the percent values were transformed to angular (arcsine) values before analysis (Panse and Sukhatme, 1985) [13].

Results and Discussion

"Bacillus amyloliquefaciens" a gram positive bacterium is used as safe biocontrol agents and Plant Growth Promoting Rhizobacteria and it has the ability to quickly colonize the root in a variety of crops. Statistically significant differences were observed on seed quality parameters *viz.* speed of germination, germination (%), dry matter production, vigour index on bio-priming treatments.

Among the three bacterial bio-inoculants, *Bacillus amyloliquefaciens* @ 1% treatment showed highest value for all the seed quality parameters followed by the same bacteria @ 5%. The results revealed that benefits of biopriming was highest at 1% and 5% concentration compared to 10, 15 and 20% concentrations of all the bacterial inoculants.

Table 2: Standardization of different concentration of bacterial inoculants and its effect on seed quality parameters in maize COH (M)

Treatments		Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	DMP (g/10 seedlings)	Vigour index
T0	-Control (unprimed)	6.5	90(71.57)	15.0	7.1	0.47	42.5
T1	–Hydropriming	6.9	93(74.66)	16.8	8.8	0.48	44.6
T2	*B.paraconglameratum*-1%	7.1	91(72.54)	16.8	7.7	0.50	45.7
T3	*B.paraconglameratum*-5%	7.6	92(73.57)	16.8	8.2	0.49	45.1
T4	*B.paraconglameratum*-10%	7.4	90(71.57)	16.9	7.6	0.51	46.1
T5	*B.paraconglameratum*-15%	7.0	89(70.63)	17.0	7.5	0.48	43.1
T6	*B.paraconglameratum*-20%	6.7	86(68.03)	16.1	7.3	0.46	39.9
T7	*B.amyloliquefaciens*-1%	7.8	95(77.08)	18.7	9.1	0.59	56.1
T8	*B.amyloliquefaciens*-5%	8.1	96(78.46)	19.4	9.4	0.64	61.4
T9	*B.amyloliquefaciens*-10%	7.3	90(71.57)	17.5	9.0	0.53	47.7
T10	*B.amyloliquefaciens*-15%	7.3	88(69.73)	17.0	8.2	0.52	45.8
T1	*B.amyloliquefaciens*-20%	7.1	86(68.03)	16.7	7.7	0.53	45.9
T12	*B.licheniformis*- 1%	7.5	91(72.54)	16.4	7.4	0.57	51.6
T13	*B.licheniformis*- 5%	7.6	94(75.82)	16.5	7.1	0.54	50.5
T14	*B.licheniformis*- 10%	7.3	90(71.57)	15.4	7.5	0.50	45.1
T15	*B.licheniformis*- 15%	6.5	87(68.87)	15.3	7.0	0.52	45.0
T16	*B.licheniformis*- 20%	6.4	85(67.21)	15.1	6.8	0.46	39.1
Mean		7.1	90(71.72)	16.7	8.0	0.52	46.8
SE(d)		0.23	2.124	0.55	0.40	0.048	4.57
CD(p=0.05)		0.47	4.266	1.11	0.81	0.098	9.18

Higher speed of germination (8.1) and germination (96%) was recorded in *B.amyloliquefaciens* 5% treatment which was on par with *B.amyloliquefaciens* 1% treatment (7.8) and (95%) over unprimed (6.5) & (90%) and hydroprimed seeds (6.9) & (93) respectively. Statistically significant variations were observed for root length and shoot length due to biopriming. The longest root length (19.4 cm) and shoot length (9.4 cm) were observed in *B.amyloliquefaciens* 5% treatment which was on par with treatment (18.7cm) & (9.1cm) over control (15.0cm) & (7.1cm) and hydroprimed seeds (16.8cm) & (8.8cm) (Table 2). Dry matter production 0.64 g per 10 seedlings and vigour index (61.4) was significantly influenced by biopriming was recorded in treatment which was on par with treatment over control and hydroprimed seeds. (Table 2)

These results are similar to the findings of earlier workers who have indicated several improvement in seed germination due to PGPR and increased synthesis of IAA, the high lipid band, which would have triggered the activity of specific

enzymes such as amylase, which is essential for starch synthesis that promoted early germination and brought an increase in availability of starch absorption. Increase in seedling vigour is due to increase in auxin synthesis (Buensanteai *et al*., 2008) [5].

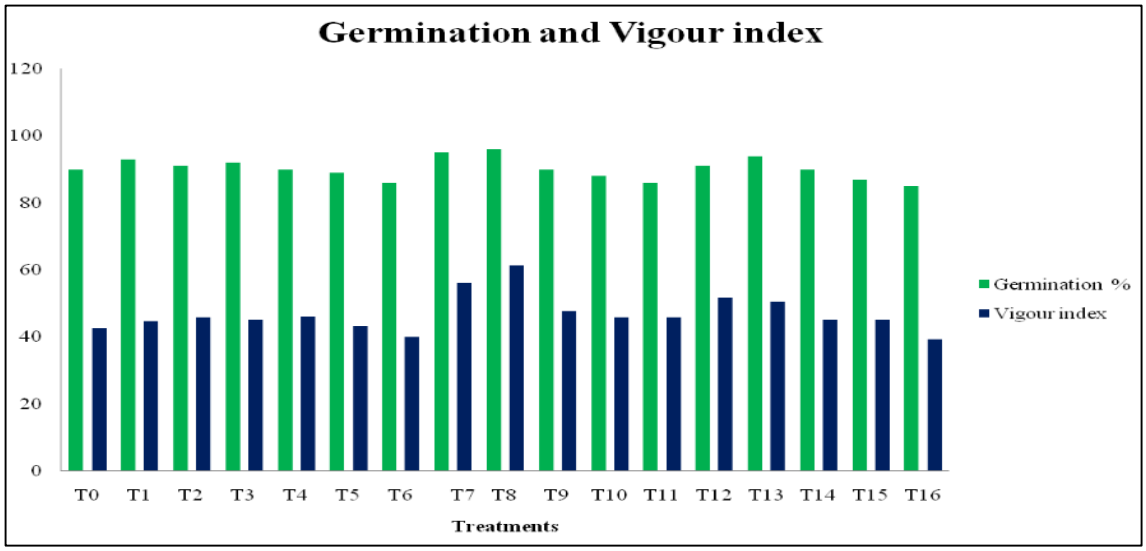

Fig 1: Influence of different biopriming treatments on germination percentage and vigour index

Agrawal and Agrawal (2013) [3] reported that in tomato seeds increase in germination per cent and other seed quality parameters by *Bacillus sp* is due to Plant Growth Promoting Rhizobacteria (PGPR) by production of indole acetic acid (IAA) which is an important mechanism for plant growth stimulation in tomato seedlings. (Fernando *et al*., 2005) [7] identified the metabolites of indole- 3-acetic acid (IAA) and abscisic acid (ABA) in both the strains of *Bacillus sp*, the metabolites of AP-3 increased production of root hairs, while the metabolites of PRBS-1 stimulated outgrowth of lateral roots in soybean. The potential of using selected strains of *Bacillus subtilis*is revealed in the biological control of seed pathogens, as well as in promoting soybean growth.

Spaepen *et al* (2007) [15] found that several *Bacillus sp*. are potentially able to produce auxin that promotes root proliferation and nutrient uptake. Tsavkelova *et al*., (2006) [16] found that *Bacillus sp*. involves the modulation of plant development through phytohormones production. Sathya *et al*., 2016 [14] reported that biopriming with six per cent *Bacillus amyloliquefaciens* and polymer coating @ 10 ml kg^{-1} of chilli seed was found higher for speed of germination, germination percentage, shoot length, root length, vigour index, dry matter production compared to other treatments and this would have occurred by better synthesis of auxin.

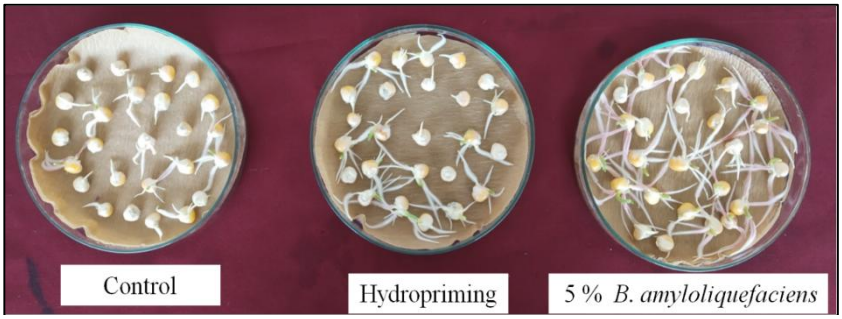

Fig 2: Speed of germination influenced by 5% *Bacillus amyloliquefaciens* VB7 bioprimed seeds compared to hydroprimed and control seeds on 4th day.

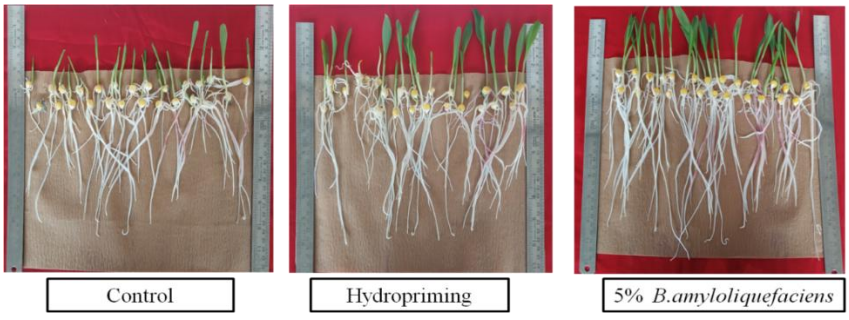

Fig 3: Seedling vigour influenced by 5% *Bacillus amyloliquefaciens* VB7 bioprimed seeds compared to hydroprimed and control seeds on 7th day.

In sunflower seeds biopriming treatments with *Pseudomonas flourescens* promoted quick and uniform germination as well as better plant growth (Moeinzadeh *et al*., 2010) [12]. Germination and improved seedling establishment obtained through seed priming with PGPR in *Rosa sp* (Anitha*et al*., 2013) [4]. Similarly, promotion in seedling parameters and yields of various crop in response to inoculation with PGPR were reported by many scientists.

Conclusion

The present study showed that bioprimed seeds with 5% *Bacillus amyloliquefaciens* VB7 recorded maximum germination per cent, seedling length, dry matter production and vigour index, which was on par with 1% *Bacillus amyloliquefaciens* VB7. So, we can conclude 1% and 5% *Bacillus amyloliquefaciens* can be effectively used for biopriming treatment among different treatments for maize COH (M) 6 seeds.

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