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Optimization of liquid phospho bacteria required for maize seeds

M. GOMATHY, M. THANGARAJU AND C. SARATHAMBAL
Department of Agricultural Microbiology, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

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The survival of the *Bacillus cultures* on the seeds of the maize was tried in different combinations. One ml inoculum with one ml adhesive combination or sterile water showed better results followed by 1.5 ml inoculum with 0.5 ml adhesive or sterile water. In 1.5 ml inoculum and 0.5 ml adhesive combination, adherence and survival population of phosphobacterial cells were maximum (6.5×10^5 cfu/seed) for the treatment of sporulated inoculum with rice gruel.

Key words : Phosphobacteria, Liquid inoculum, Maize

INTRODUCTION

Generally 5-30% yield increase have been recorded from various crops by phosphate solubilizing bacterial inoculation particularly in crops, *viz*. paddy, wheat, potato and soybean either through seed bacterization or soil application (Datta *et al.*, 1982). The adherence and survival of sporulated *Bacillus* culture on the seeds of blackgram, greengram, soybean, maize and paddy showed maximum population in all seeds when inoculated with rice gruel (Sumathy, 2001). Seed treatment of cumbu and blackgram with the mixed inoculants of *Azospirillum* and *phosphobacteria* / *Rhizobium* and phosphobacteria, respectively revealed of individual organisms survived up to 24h on the seeds (Poonguzhali, 2002).

MATERIALS AND METHODS

The sporulated inoculum of Bacillus sp. (PB-1) and its log phase cells were used for the study.

The treatments details for maize of this experiment are as follows:

0.5% of inoculum:

T_1 - 0.5ml of vegetative cell + 1.5ml of sterile water, T_2- 0.5ml of vegetative cell + 1.5ml of rice gruel, T_3- Direct seed application of vegetative cells as such (2 %), T_4- 0.5ml of sporulated inoculum+1.5ml of sterile water, T_5- 0.5ml of sporulated inoculum+1.5ml of rice gruel, T_6 - Direct seed application of sporulated inoculum as such (2 %)

1 % of inoculum:

T_1 - 1ml of vegetative cell + 1ml of sterile water, T_2-1ml of vegetative cell + 1ml of rice gruel, T_3 - Direct seed application of vegetative cells as such (2 %), T_4 - 1ml of sporulated inoculum + 1ml of sterile water, T_5- 1 ml of sporulated inoculum + 1 ml of rice gruel, T_6 - Direct seed application of sporulated inoculum as such (2 %)

1.5% of inoculum:

T_1 - 1.5ml of vegetative cell + 0.5ml of sterile water, T_2 - 1.5ml of vegetative cell + 0.5ml of rice gruel, T_3- Direct seed application of vegetative cells as such (2 %), T_4-0.5ml of sporulated inoculum + 0.5ml of sterile water, T_5 - 1.5ml of sporulated inoculum + 0.5ml of rice gruel, T_6- Direct seed application of sporulated inoculum as such (2 %)

The seeds were coated with the inoculum by preparing slurry. A sample size of 20 seeds were taken in each treatment for examining the survival and adherence at 0, 12, 24, 36, 48, 60 and 72 hr of inoculation at room temperature. The number of cells adhered per seed was calculated using serial dilution and plating technique and the results were expressed as cfu/seed.

RESULTS AND DISCUSSION

Application of inoculum to the seeds of host plants is still the usual practice with carrier based bacterial inoculants (Graham Weiss *et al.*, 1987). Just prior to sowing, mixing of inoculant with seeds is followed. Sometimes to improve stickiness adhesive is added. (Fages, 1994; Jauhri, 2001). The results of seed treatment of maize using vegetative cells and sporulated *Bacillus cultures* are given in Table 1, 2 and 3.

The results of 0.5 ml inoculum and 1.5 ml adhesive combination are presented in Table 1.

Sporulated inoculum with rice gruel showed maximum number of population (3.5×10^5 cfu/seed) and adherence were noticed even after 60th hr whereas without adhesive the population drastically reduced after

24th hr.

One ml inoculum and one ml adhesive combination results are presented in Table 2. Rice gruel was found to be a good adhesive agent. Maximum population of 8.5 x 105 cfu/seed was noticed in one mi sporulated medium plus one mi rice gruel followed by vegetative cells and rice gruel combination.

In case of 1.5 ml inoculum and 0.5 ml adhesive combination (Table 3) adherence and survival population of phospho bacterial cells were maximum (6.5 x 105 cfu/ seed) for the treatment of sporulated inoculum with rice gruel. Statistically there was significant difference between the treatments. Sporulated inoculum performed better than vegetative cells.

The inoculation of sporulated inoculum along with rice gruel favored the adherence of the regenerated cells oil the seeds. The results also supported the earlier discussion that the spores could germinate at nutrient

Table 1 : Survival of 0.5 per cent liquid *Bacillus megaterium* var *phosphaticum* (PB-1) on the seeds of maize (CO-1)

Treatments	Population (cfu / seed)						
	0hr	12 hr	24hr	36 hr	48hr	60 hr	72hr
0.5ml vegetative cell +	2.0 x105	1.5 x104	2.5 x103	-	-	-	-
l.5ml sterile water	(5.3010)	(4.1761)	(3.3979)				
05 ml vegetative cell +	2.0 x105	1.0 x104	1.0 x103	1.0 x101	0.5 x101	-	-
1.5ml rice gruel	(5.4771)	(4.3979)	(3.1761)	(2.3979)	(1.5441)		
2 ml vegetative cell	5.5 x104	4.5 x103	2.5 x102	-	-	-	-
	(4.7404)	(3.6532)	(2.3979)				
0.5 ml sporulated inoculum	2.5 x105	3.5 x104	2.0 x103	1.5 x102	0.5 x101	-	-
+ 1.5 ml sterile water	(5.3979)	(4.5441)	(2.3010)	(2.1761)	(0.6989)		
0.5 ml sporulated inoculum	3.5 x105	2.5 x104	2.0 x103	1.5 x102	25 x101	1.5 x101	-
+ 1.5 ml rice gruel	(5.7403)	(4.3979)	(3.3979)	(2.3010)	(2.3979)	(1.1761)	
2 ml sporulated inoculum	5.5 x104	3.5 x103	2.5 x102	-	-	-	-
	(4.7404)	(3.5441)	(2.3979)				
		S.E.±				C.D. (P=0.05)	
Period (P)		0.0220				0.0441	
Treatments (T)		0.0203				0.0401	
PxT		0.0530				0.1071	

Figures in parenthesis indicate Log transformed values; - Population could not be estimated

Table 2 : Survival of 1 per cent liquid *Bacillus megaterium* var *phosphaticum* (PB-1) on the seeds of maize (CO-1)

Treatments	Population (cfu/seed)						
	0hr	12 hr	24hr	36hr	48 hr	60 hr	72 hr
1ml vegetative cell + 1ml	6.0×10^5	4.5×10^5	2.5×10^5	2.5×10^4	1.5×10^3	-	-
sterile water	(5.7781)	(5.6532)	(5.3979)	(4.3979)	(3.1761)		
1ml vegetative cell + 1ml	6.5×10^5	4.5×10^5	2.5×10^5	1.5×10^4	2.5×10^2	-	-
rice gruel	(5.5294)	(5.6532)	(5.3979)	(4.1761)	(2.3979)		
2 ml Vegetative cell	5.5×10^4	4.5×10^3	2.5×10^2	-	-	-	-
	(4.7404)	(3.6532)	(2.3979)				
1ml sporulated inoculum	5.5×10^5	4.5×10^5	4.5×10^5	2.5×10^4	1.0×10^3	1.0×10^1	-
+ 1 ml sterile water	(5.7404)	(5.6532)	(5.6532)	(4.3979)	(2.0000)	(1.0000)	
1 ml sporulated inoculum	8.5×10^5	6.5×10^5	4.5×10^5	3.0×10^4	1.5×10^3	2.5 x102	1.5×10^1
+ 1 ml rice gruel	(5.9294)	(5.8129)	(5.6532)	(4.4771)	(3.1761)	(2.3979)	(1.1761)
2 ml sporulated inoculum	5.5×10^4	3.5×10^3	2.5×10^2	-	-	-	-
	(4.7404)	(3.5441)	(2.3979)				
		S.E.±				C.D (P=0.05)	
Period (P)		0.0365				0.7222	
Treatments (T)		0.0330				0.0660	
PxT		0.0880				0.1770	

Figures in parenthesis indicate Log transformed values; - Population could not be estimated

Table 3 : Survival of 1.5 per cent liquid *Bacillus megaterium* var *phosphaticum* (PB-1) on the seeds of maize (CO-1)

Treatments	Population (cfu/seed)						
	0hr	12 hr	24hr	36hr	48hr	60 hr	72 hr
l.5ml vegetative cell +	3.5 x105	3.5 x104	2.5 x103	1.0 x102	-	-	-
0.5ml sterile water	(5.5441)	(4.5441)	(3.3979)	(2.0000)			
1.5 ml vegetative cell +	6.0 x105	3.5 x105	2.5 x105	2.5 x104	2.0x101	-	-
0.5ml rice gruel	(5.7781)	(5.5441)	(5.3979)	(4.3979)	(1.3010)		
2 ml vegetative cell	5.5 x104	4.5 x103	2.5 x102	-	-	-	-
	(4.7404)	(3.6532)	(2.3979)				
1.5 ml sporulated inoculum	5.0 x105	3.0 x105	2.5 x104	2.0 x103	1.0 x101	-	-
+ 0.5 ml sterile water	(5.6989)	(5.4771)	(4.3979)	(3.3010)	(1.0000)		
1.5 ml sporulated inoculum	6.5 x105	4.05 x105	2.0x105	2.5 x104	1.5 x103	0.5 x102	-
+ 0.5 ml rice gruel	(5.9294)	(5.6021)	(5.3010)	(4.3979)	(3.1761)	(1.3689)	
2 ml sporulated inoculum	5.5 x104	3.5 x103	2.5 x102	-	-	-	-
	(4.7404)	(3.5441)	(2.3979)				
		S.E.±				C.D (P=0.05)	
Period (P)		0.0332				0.0671	
Treatments (T)		0.0307				0.0621	
PxT		0.0813				0.1651	

Figures in parenthesis indicate Log transformed values; - Population could not be estimated

environments.

The rice gruel is rich in carbon, which favored the germination of spores and establishment on the treated seeds (Kandasamy and Prasad, 1971). Rice gruel is usually employed as an adhesive agent in seed treatment because of its sticky nature and nutrition contents (Kundu and Gaur, 1981).

In this study, one ml inoculum and one ml sterile water or adhesive combination was the best. Here, the rice gruel combination has population of 15 cfu/seed upto 72 h, followed by 1.5 ml inoculum and 0.5 ml sterile water or adhesive combination. Here, the sporulated inoculum mixed with rice gruel survived in the seed upto 60 h. There was poor performance when the vegetative cells were applied as such.

In general one per cent inoculum showed better results followed by 1.5 per cent inoculum. The population of one per cent inoculum was higher than the 1.5 per cent and or 0.5 per cent inoculum. Moreover, population of 2.5 x 102 cfu/seed was observed up to 60th h in case of one per cent inoculum.

REFERENCES

Datta, M., Banik Sand and Gupta, R.K. (1982). Studies on the efficacy of a phytohormone producing phosphate solubilizing Bacillus firm us in augmenting paddy yield in acid soils of Nagaland. *Plant Soil*, **69** : 365-373.

Fages, J. (1994). Azospirillum inoculants and field experiments. In: *Azospirillum / Plant Associations*, (ed.) y. okon. CRC Press, Florida, USA. p. 175.

Graham-Weiss, L., Bennett, M.L. and Paau, A.S. (1987). Production of bacterial inoculants by direct fermentation on nutrient supplemented vermiculite. *Appl. Environ. Microbiol.*, **53** : 2138-2140.

Jauhri, K.S. (2001). Preparation. of legume inoculants and bacterization of seed. In: training manual on biofertilizers. Iari, India, PP. 54-62.

Kandasamy, R. and, Prasad, N.N. (1971). Lignite as a carrier of *Rhizobia*. *Curr. Sci*., **40** : 496.

Kundu, B.S. and Gaur, A.C. (1980). Establishment of nitrogen fixing and phosphate solubilizing bacteria in rhizosphere and their effect on yield and nutrient uptake of wheat crop. *Plant Soil*, **57**: 223-230.

Poonguzhali, S. (2002). Standardization gf media for co culturing of Diazotroph (*Azospirillum / Rhizobium*) and phosphobacteria (*B. megaterium*). P.G. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore. P. 48.

Sumathy, S. (2001). Enhancement of shelf life of phosphobacteria biofertilizers (*Bacillus* sp.) through induction of sporulation. P.G. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore. P. 38.