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Method and Quantity of Liquid Formulation of Phosphobacteria Required for Seed Inoculation

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Abstract: The survival of vegetative and sporulated cells of the *Bacillus* cultures on the seeds of the crop plants was tried in different combinations. One milliliter inoculum with 1 mL adhesive combination or sterile water showed better results followed by 1.5 mL inoculum with 0.5 mL adhesive or sterile water. The population of 5.5×10^5 cfu seed^{-1} on black gram, 10.5×10^5 cfu seed^{-1} on soybean and 6.5×10^5 cfu seed^{-1} on maize were observed after 12 h of incubation in 1 mL sporulated inoculum mixed with 1 mL of rice gruel. The sporulated inoculum along with rice gruel favoured the adherence of the regenerated cells as rice gruel is rich in nutrient content.

Key words: Liquid inoculum, *Bacillus*, maize, soy bean, black gram, seed inoculation

INTRODUCTION

Phosphorus (P) is one of the major essential nutrients for biological growth and development. Many soil microorganisms are able to solubilize the unavailable forms of calcium bound P through their metabolic activities. Obsessive and excessive use of chemical fertilizers coupled with irrigation has rendered the soils unfit for cultivation. It's replaced by environment friendly liquid biofertilizers. Application of inoculum to the seeds of host plants is still an 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; Jahuri, 2001). 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 microphos culture containing *Pseudomonas striata* and *Bacillus polymyxa* tested with wheat, rice, chickpea, soybean showed that inoculation of seed and seedlings increased the grain yield of crops. Mudalagiriyappa *et al*. (1995) showed that seed inoculation of groundnut with P solubilizers increased dry matter production and yield. The adherence and survival of sporulated *Bacillus* culture on the seeds of black gram, green gram, 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 24 h on the seeds (Poonguzhali, 2002). Usually for seed treatment carrier based formulation is practiced. But irrespective of the carrier most of the carrier based inoculant production and application procedure were found to be time consuming untidy and difficult when used for large quantities of seed. Hence alternate liquid inoculants were developed for seed treatment as it is easy to use, spread well, mixed easily and no need of additional supply of water (Nethery, 1991). Though the seed inoculation of carrier based inoculants is a known practice, information on the liquid formulation are not available. The method of application and the quantity of liquid formulation needed for seed treatment should be standardized as it is new to farmers. With this view the objective was framed to standardize the dose and method of inoculation of liquid inoculum of phosphobacteria required for seed treatment.

MATERIALS AND METHODS

A lab experiment was conducted at Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. In this experiment sporulated inoculum of *Bacillus* sp. (PB-1) and its log phase cells were used for the study. The seeds used were black gram

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(Co-5), soybean (Co-1) and maize (Co-1) seeds. The seeds were coated with the inoculum by preparing slurry. A sample size of 20 seeds was taken in each treatment for examining the survival and adherence at 0, 12, 24, 36, 48, 60 and 72 h 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^{-1}. Survival of vegetative cells and sporulated inoculum was observed on black gram (Co-5), soybean (Co-1) and maize (Co-1) seeds. Treatments details were given below.

Treatment details for black gram

T_1: 0.5 mL of vegetative cell + 0.5 mL of sterile water
T_2: 0.5 mL of vegetative cell + 0.5 mL of rice gruel
T_3: Direct seed application of vegetative cells as such (1%)
T_4: 0.5 mL of sporulated inoculum + 0.5 mL of sterile water
T_5: 0.5 mL of sporulated inoculum + 0.5 mL of rice gruel
T_6: Direct seed application of sporulated inoculum as such (1%)

Similarly, for soybean and maize seeds the quantity of liquid inoculum used was 2 mL with a combination of 0.5 mL liquid inoculum + 1.5 mL adhesive, 1 mL liquid inoculum + 1 mL adhesive and 1.5 mL liquid inoculum + 0.5 mL adhesive. In this study different combination of vegetative cells, sporulated cultures were tried on seeds of blackgram, maize and soybean, which are small, medium and large seeds respectively. The sporulated and vegetative cells of phosphobacteria (*Bacillus megaterium* var. *phosphaticum* PB-1) was mixed with seeds along with rice gruel or sterile water or applied as such in different combinations and the population was estimated at different hours of incubation.

RESULTS

Survival of the liquid inoculum on the seeds of black gram: The results of different seed treatment methods on the survival and adherence of sporulated and vegetative *Bacillus* cells on black gram seeds are shown in Fig. 1. In general, maximum number of cells adhered on the black gram seed was observed when treated with the rice gruel. Inoculation of 0.5 mL of sporulated cells along with 0.5 mL of rice gruel has shown a population of 10 cfu seed^{-1} even after 72 h of inoculation. Phosphobacterial survival rate with time showed significant difference among the treatments. When compared to vegetative inoculum, sporulated inoculum performed better. Use of rice gruel as adhesive instead of sterile water has enhanced the adherence of phosphobacteria population to the seed. There were significant differences among the treatments. In 0.5 mL sporulated inoculum with 0.5 mL rice gruel performed better than other treatments. When the vegetative cells were applied as such on seeds it performed poor, as it did not have any adhesive. In 0.5 mL vegetative cell and 0.5 mL sterile water the population was up to 48 h (2.0×10^3 cfu mL^{-1}).

Survival of the liquid inoculum on the seeds of soybean: Adherence of sporulated cell gave the maximum population (3.5×10^6 cfu seed^{-1}) for the seed where rice gruel was used as an adhesive agent. But the population drastically reduced after 24 h in all the treatments. The results of 1 mL inoculum and 1 mL adhesive combination are shown in Table 1. The survival and adherence of

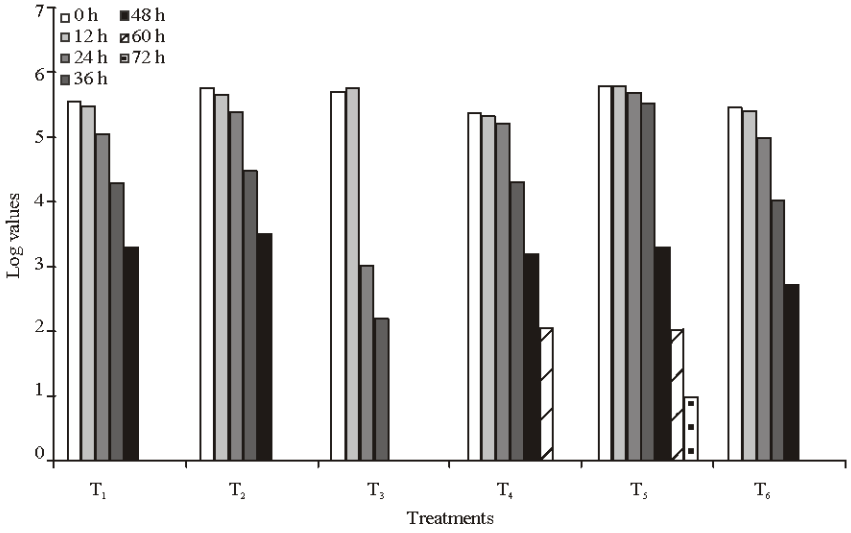

Fig. 1: Survival of liquid *Bacillus megaterium* var. *phosphaticum* (PB-1) on the seeds of black gram

Table 1: Survival of 1% liquid *Bacillus megaterium* var. *phosphaticum* (PB-1) on the seeds of soybean (Co-1)

	Population (cfu seed^{-1})						
	0	12	24	36	48	60	72
Treatments	(h)						
1 mL vegetative cell +1 mL sterile water	7.5×10^5	5.0×10^5	3.5×10^5	3.0×10^4	1.5×10^3	-	-
	(5.8751)	(5.6989)	(5.5441)	(4.4771)	(3.1760)		
1 mL vegetative cell + 1 mL rice gruel	9.5×10^5	8.5×10^5	7.0×10^5	3.5×10^4	3.5×10^3	1.5×10^2	-
	(5.9778)	(5.9294)	(5.8451)	(4.5441)	(3.5440)	(2.1761)	
2 mL Vegetative cell	5.0×10^5	5.5×10^4	4.5×10^3	2.5×10^2	-	-	-
	(5.6989)	(4.7403)	(3.6532)	(2.3979)			
1 mL sporulated inoculum + 1 mL sterile water	6.5×10^5	5.5×10^5	5.5×10^4	2.5×10^4	3.5×10^3	-	-
	(5.8129)	(5.7403)	(5.7403)	(4.3979)	(3.5441)		
1 mL sporulated inoculum +1 mL rice gruel	10.5×10^5	10.5×10^5	8.5×10^4	6.5×10^4	5.5×10^3	3.5×10^2	2.0×10^1
	(6.0211)	(6.0212)	(5.9294)	(4.8129)	(3.7404)	(2.5441)	(1.3010)
2 mL sporulated inoculum	5.0×10^5	4.5×10^5	4.0×10^4	3.5×10^3	-	-	-
	(5.6989)	(5.6532)	(4.6021)	(3.5441)			
	SED			CD (0.05)			
Period (P)	0.0270			0.0560			
Treatments (T)	0.0260			0.0520			
P×T	0.0680			0.1320			

Values in parenthesis indicate log transformed values, -: Population could not be estimated

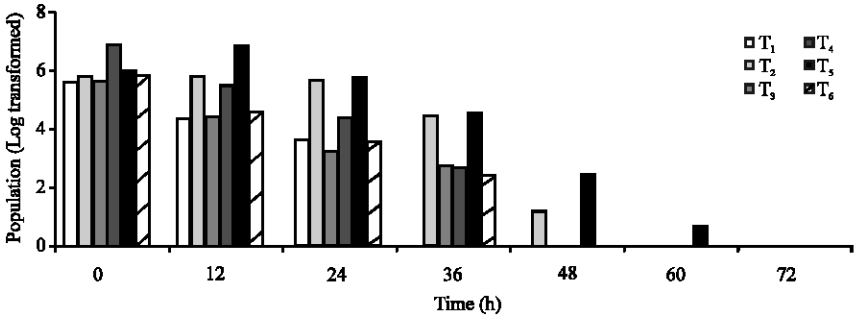

Fig. 2: Survival of 1.5% liquid *Bacillus megaterium* var. *phosphaticum* (PB-1) on the seeds of soybean

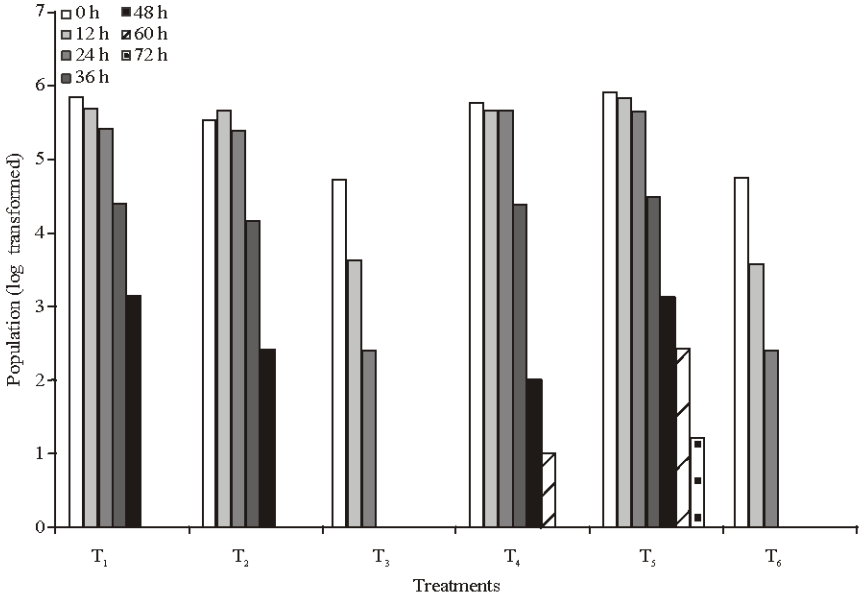

Fig. 3: Survival of 1% liquid *Bacillus megaterium* var. *phosphaticum* (PB-1) on the seeds of maize

sporulated phosphobacterial cells were maximum (10.5×10^5 cfu seed^{-1}) at 12th h for the seeds, which were added with 1 mL rice gruel as an adhesive agent. The results of 1.5 mL inoculum and 0.5 mL adhesive combination are shown in Fig. 2 and 5. As above, rice gruel was found to be effective where maximum number of

Table 2: Survival of 1.5% liquid *Bacillus megaterium* var. *phosphaticum* (PB-1) on the seeds of maize (CO-1)

	Population (cfu seed^{-1})						
	0	12	24	36	48	60	72
Treatments	(h)						
1.5 mL vegetative cell + 0.5 mL sterile water	3.5×10^5 (5.5441)	3.5×10^4 (4.5441)	2.5×10^3 (3.3979)	1.0×10^2 (2.0000)	-	-	-
1.5 mL vegetative cell + 0.5 mL rice gruel	6.0×10^5 (5.7781)	3.5×10^5 (5.5441)	2.5×10^5 (5.3979)	2.5×10^4 (4.3979)	2.0×10^1 (1.3010)	-	-
2 mL vegetative cell	5.5×10^4 (4.7404)	4.5×10^3 (3.6532)	2.5×10^2 (2.3979)	-	-	-	-
1.5 mL sporulated inoculum + 0.5 mL sterile water	5.0×10^5 (5.6989)	3.0×10^5 (5.4771)	2.5×10^4 (4.3979)	2.0×10^3 (3.3010)	1.0×10^1 (1.0000)	-	-
1.5 mL sporulated inoculum + 0.5 mL rice gruel	6.5×10^5 (5.9294)	4.05×10^5 (5.6021)	2.0×10^5 (5.3010)	2.5×10^4 (4.3979)	1.5×10^3 (3.1761)	0.5×10^2 (1.3689)	-
2 mL sporulated inoculum	5.5×10^4 (4.7404)	3.5×10^3 (3.5441)	2.5×10^2 (2.3979)	-	-	-	-
	SED			CD (0.05)			
Period (P)	0.0332			0.0671			
Treatments (T)	0.0307			0.0621			
P×T	0.0813			0.1651			

Values in parenthesis indicate Log transformed values, -: Population could not be estimated

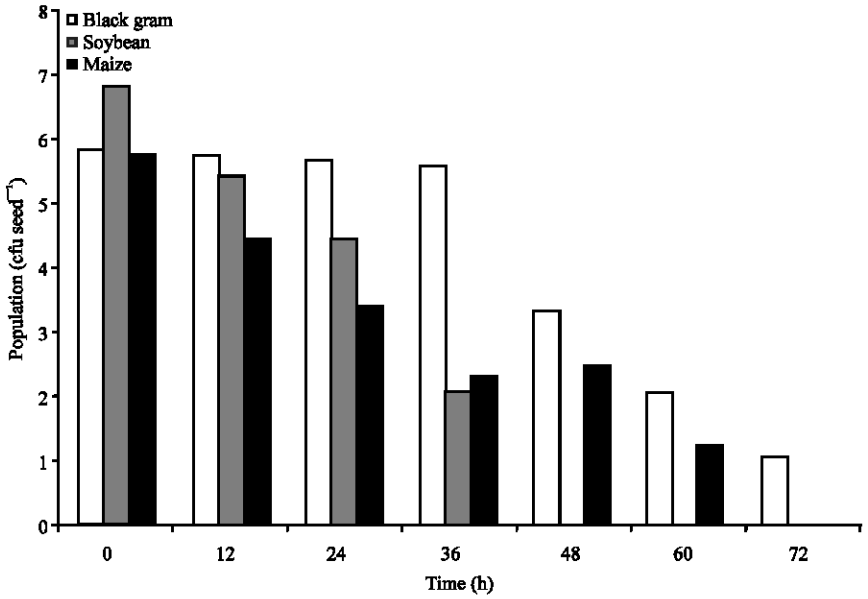

Fig. 4: Survival of 0.5% liquid *Bacillus megaterium* var. *phosphaticum* (PB-1) on the seeds of black gram, soybean and maize

cells was adhered (6.5×10^6 cfu seed^{-1}) at 0 h in the treatment receiving 1.5 mL of sporulated inoculum mixed with 0.5 mL of rice gruel followed by sterile water.

As such survival of vegetative cell was poor. When these seeds were inoculated with sporulated culture of *Bacillus* mixed with rice gruel the survival was maximum up to 60 h in combinations such as 1.0, 1.5 mL vegetative cells or sporulated inoculum with 1.0, 0.5 mL sterile water or adhesive.

Survival of the liquid inoculum on the seeds of maize: Sporulated inoculum with rice gruel showed maximum number of population (3.5×10^5 cfu seed^{-1}) and adherence were noticed even after 60 h whereas without adhesive the population drastically reduced after 24 h (Fig. 3). One milliliter inoculum and 1 mL adhesive combination results are shown in Table 1. Rice gruel was found to be a good adhesive agent. Maximum population of 8.5×10^5 cfu seed^{-1} was noticed in 1 mL sporulated medium plus 1 mL rice gruel followed by vegetative cells and rice gruel combination.

In case of 1.5 mL inoculum and 0.5 mL adhesive combination (Table 2) adherence and survival population of phosphobacterial cells were maximum

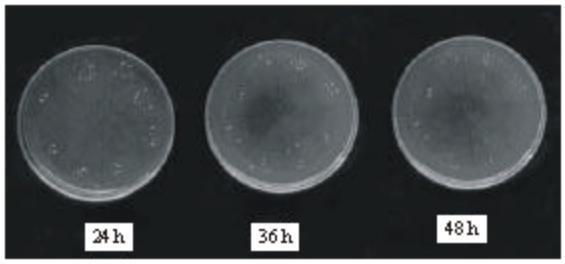

Fig. 5: Survival of 1.5 mL sporulated inoculum of *Bacillus megaterium* var. *phosphaticum* (PB-1) with 0.5 mL rice gruel on the seeds of soybean at different intervals

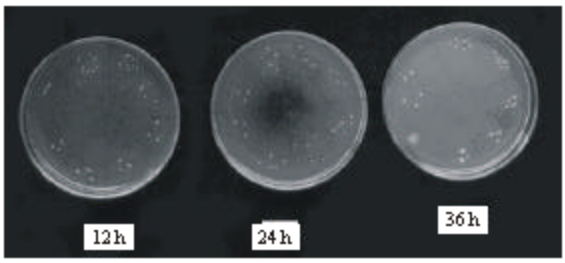

Fig. 6: Survival of 1.5 mL sporulated inoculum of *Bacillus megaterium* var. *phosphaticum* (PB-1) with 0.5 mL rice gruel on the seeds of maize at different intervals

(6.5×10^5 cfu seed^{-1}) for the treatment of sporulated inoculum with rice gruel (Fig. 6). Microbial population of 2.5×10^2 cfu seed^{-1} was observed up to 60th h in case of 1% inoculum. Survival of 0.5% liquid *Bacillus megaterium* var *phosphaticum* (PB-1) on the seeds of black gram, soybean and maize was compared and it was given in Fig. 4.

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; Jahuri, 2001).

In this study different combination of vegetative cells, sporulated cultures were tried on seeds of black gram, maize and soybean, which are small, medium and large seeds respectively. In case of black gram 0.5 mL sporulated inoculum with 0.5 mL rice gruel performed better than other treatments. When the vegetative cells were applied as such on seeds it performed poor, as it did not have any adhesive. In 0.5 mL vegetative cell and 0.5 mL sterile water the population was up to 48 h (2.0×10^3 cfu mL^{-1}).

These results were supported by Sumathy (2001) who reported that survival of sporulated cultures of *Bacillus* with rice gruel was optimum up to 12 h of inoculation. The inoculation of sporulated cultures through seedling root dipping also showed better establishment. Interestingly, she found that establishment of *Bacillus* was more when inoculated through carrier based spores than as vegetative cells. Rice and Olsen (1992) suggested liquid inoculation as a better method than seed treatments with carrier inoculant. The liquid rhizobial inoculant for pea and lentil resulted in yield equal to or better than those obtained for the peat inoculant (Hynes *et al*., 1995). Burton *et al*. (1965) reported that the liquid inoculum was as effective as peat-based inoculant the number of rhizobia per seed was increased 2.5 times.

In soybean and maize seeds, the vegetative cells as such survived poor. When these seeds were inoculated with sporulated culture of *Bacillus* mixed with rice gruel the survival was maximum up to 60 h in combinations such as 1.0, 1.5 mL vegetative cells or sporulated inoculum with 1.0, 0.5 mL sterile water or adhesive. The inoculation of sporulated inoculum along with rice gruel favoured the adherence of the regenerated cells on the seeds. The results also supported the earlier discussion that the spores could germinate at nutrients rich environments.

The rice gruel is rich in carbon, which favoured 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). Burton *et al*. (1965) reported that both solid and liquid based inoculants were effective when the seeds were planted one day after inoculation. Schiffman and Apler (1968) observed that liquid inoculants were particularly suited to large seeded grain legumes, which because of their bulk, made seed inoculation a formidable task. Boonkerd *et al*. (1978) reported that inoculation by peat or liquid formulation with any rate or strain did not affect inoculation or plant growth. However when the level of inoculum in the broth was increased by ten times and hundred times the initial rate, the recovery of applied strains increased. In this study, the sporulated inoculum mixed with rice gruel got significant results compared to other treatments

CONCLUSION

From this study, it has been concluded that liquid inoculum for seed treatment can be supplied as vegetative

cells or as spores. But however, sporulated inoculum had the population of cells up to 3 days when compared to vegetative cells. So the liquid inoculum which contains spores of phosphobacteria, can be used for seed treatment after mixing with the adhesive.

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