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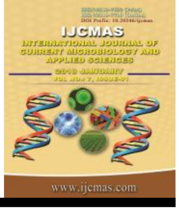

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Co-inoculation of Liquid Microbial Cultures and Compatibility with Chemicals for Improvement of Seed Germination and Vigour in Paddy

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ABSTRACT

Keywords

Paddy, Seed germination, Vigour, Biofertilizers, Co-Inoculation, Chemicals, Compatibility

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Seed treatment with bio-inoculants is widely followed as it is beneficial to the plants and environment. However, the carrier based inoculants has short shelf life and difficult to use for large quantities of seed. Therefore, in the present study the liquid microbial cultures were used for seed infusion in paddy and assessed their compatibility with seed treating chemicals. Results showed that the seed soaking in consortia of *Azospirillum* @1:50 and PPFM @1:100 diluted cultures (1:1) for 18 h has recorded highest germination and seedling vigour with the microbial population of 51 x 10^4 (*Azospirillum*) and 1 x 10^4 (PPFM) cfu g^{-1} of seed. In addition, seed soaking in PPFM liquid culture @1:100 dilution for 18 h followed by polymer coating @ 5 ml and carbendazim @ 2 g kg^{-1} of seed has performed better in improving germination and vigour with the PPFM population of 4 x 10^4 cfu g^{-1} of seed.

Introduction

The quality of the seed which is used for sowing decides the crop yield and the quality alone contributes 20 per cent yield increase. It can be improved by many ways, of which the pre-sowing seed management technique plays a vital role. Among the pre-sowing seed management techniques, the seed treatment with the biofertilizers is one of the important method by which the yield can be improved by 5 to 30 per cent (Datta *et al.*, 1982). Use of these effective microorganisms as a pre-sowing seed treating agent is considered to be ecologically sound and beneficial to both seed and environment. Such seed treatments deliver the microorganisms directly to the plant rhizosphere, the narrow zone of soil that surrounds the roots where plants interact directly with microorganisms (Philippot *et al.*, 2013). Application of inoculum to the seeds of host plants is still in vogue with carrier based bacterial inoculants (Graham *et al.*, 1987). Sometimes in order to improve stickiness on the seed, adhesive is added (Jahuri, 2001). However carrier-based inoculants have a short shelf life, poor quality and most of the carrier based inoculants production and application procedure were found to be time consuming and difficult when used for large quantities of seed. Hartley *et al.*, (2012) recommended that each seed treatment ingredient and stage in the seed-coating process be tested for compatibility to determine best practices to promote microbial survival on seed.

Alternatively, liquid inoculants were developed for seed treatment as it is easy to use, spreads well, mixes easily and needs no additional water supply (Nethery, 1991). 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).

Bacillus spp. have proven to be ideal candidates for development as stable and efficient biological products because their ability to produce heat-resistant endospores (Yanez-Mendizabal *et al.*, 2012) which survive the stresses of commercial seed treatment better than nonspore-forming species such as *Pseudomonas* spp. Mehta *et al.*, (2011) opined that the combined application of Zn, biofertilizers and pesticides is possible to increase yield due to early nourishment through Zn and biofertilizers as well as insecticides.

However, the chemicals are non-specific in their lethal action of the organisms. The response of seed treating chemicals such as captan, thiram, mancozeb, ridomil, benlate and vitavax etc. have been studied on the survival of *Rhizobium* and *Bradyrhizobium* inoculated seeds of some leguminous crops (Dunfield *et al.*, 2000; Bikrol *et al.*, 2005). Therefore, studies were conducted to infuse the co-inoculant liquid microbial cultures for enhancing seed germination and vigour in paddy and to assess the compatibility of the seed treating chemicals on the survival of the inoculants.

Materials and Methods

Paddy variety ADT 43 seeds were collected from the Vegetable Research Station, Palur (India) and dried well for the purpose of microbial inoculation. The bacterial strains *viz.*, *Azospirillum*, phosphobacteria, *Methylobacterium* (Pink pigmented Facultative Methylotroph (PPFM)), and *Bacillus subtilis* were obtained from the Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai (India). The strains were cultured in NFb nutrient broth and ammonium mineral salts medium supplemented with 0.5 per cent methanol. The liquid based bio-inoculant formulations were prepared in their respective medium. These inoculants were diluted *viz.*, *Azospirillum* and phosphobacteria @ 1:50 and PPFM and *Bacillus subtilis* @ 1:100 concentrations as per the standardization. Then, the microbial consortia were prepared by mixing the different cultures at 1:1 or 1:1:1 or 1:1:1:1 ratio. The paddy seeds were soaked in these liquid cultures with equal volume (v/v) for 18 h vide the treatment schedule: T_1 - Control, T_2 - Seed soaking in water, T_3 - Seed soaking in *Azospirillum* @1:50 dilution, T_4 - Seed soaking in Phosphobacteria @1:50 dilution, T_5 - Seed soaking in PPFM @1:100 dilution, T_6 - Seed soaking in *Bacillus subtilis* @1:100 dilution, T_7 - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 dilutions (1:1), T_8 - Seed soaking in *Azospirillum* @1:50 + PPFM @1:100 dilutions (1:1), T_9 - Seed soaking in *Azospirillum* @1:50 + *Bacillus subtilis* @1:100 dilutions (1:1), T_{10} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + PPFM @1:100 dilutions (1:1:1), T_{11} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + *Bacillus subtilis*

@1:100 dilutions (1:1:1), T_{12} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + PPFM @ 1:100 + *Bacillus subtilis* @1:100 dilutions (1:1:1:1). Then, the seeds were shade dried to the original moisture content and evaluated for its germination and vigour. The germination test was conducted as per the ISTA (1999) procedure and evaluated at final counting day. The speed of germination was assessed during the germination test (Maguire, 1962). Also, five randomly selected seedlings in each treatment were measured for its length and mean was arrived.

In addition, the effect of seed treating chemicals on the survival of microbes in paddy seeds were assessed by infusing them with different liquid microbial cultures for 18 h in equal volume. These bioinoculated seeds were shade dried to the original moisture content. Then, the seeds were treated with different chemicals as per the treatment details *viz*., T_1 - Control, T_2 - Seed soaking in *Azospirillum* @1:50 dilution, T_3 - Seed soaking in *Azospirillum* @1:50 dilution + Polymer coating @ 5 ml kg^{-1}, T_4 - Seed soaking in *Azospirillum* @1:50 dilution + Carbendazim treatment @ 2 g kg^{-1}, T_5 - Seed soaking in *Azospirillum* @1:50 dilution + Polymer coating @ 5 ml kg^{-1} + Carbendazim treatment @ 2 g kg^{-1}, T_6 - Seed soaking in Phosphobacteria @1:50 dilution, T_7 - Seed soaking in Phosphobacteria @1:50 dilution + Polymer coating @ 5 ml kg^{-1}, T_8 - Seed soaking in Phosphobacteria @1:50 dilution + Carbendazim treatment @ 2 g kg^{-1}, T_9 - Seed soaking in Phosphobacteria @1:50 dilution + Polymer coating @ 5 ml kg^{-1} + Carbendazim treatment @ 2 g kg^{-1}, T_{10} - Seed soaking in PPFM @1:100 dilution, T_{11} - Seed soaking in PPFM @1:100 dilution + Polymer coating @ 5 ml kg^{-1}, T_{12} - Seed soaking in PPFM @1:100 dilution + Carbendazim treatment @ 2 g kg^{-1}, T_{13} - Seed soaking in PPFM @1:100 dilution + Polymer coating @ 5 ml kg^{-1} + Carbendazim treatment @ 2 g kg^{-1}, T_{14} - Seed soaking in *Bacillus subtilis* @1:100 dilution, T_{15} - Seed soaking in *Bacillus subtilis* @1:100 dilution + Polymer coating @ 5 ml kg^{-1}, T_{16} - Seed soaking in *Bacillus subtilis* @1:100 dilution + Carbendazim treatment @ 2 g kg^{-1}, T_{17} - Seed soaking in *Bacillus subtilis* @1:100 dilution + Polymer coating @ 5 ml kg^{-1} + Carbendazim treatment @ 2 g kg^{-1}. The treated seeds were stored for a week and evaluated for the germination and vigour. Also, the microbial populations in the treated seeds were assessed. In this regard, the treated seeds were first washed with sterile water for about four to five times to remove the chemicals adhering on the surface of the seeds. Then, the seeds were soaked in the sterile water and allowed in arbitrary shaker for about one hour. The serial dilutions were prepared and inoculated in the respective medium.

The data collected were subjected to statistical analysis (Panse and Sukhatme, 1967) and the critical difference values were calculated at 5 per cent probability level.

Results and Discussion

Results of the co-inoculation of microbial liquid cultures indicated that the paddy seeds soaked in PPFM @1:100 dilution for 18 h (T_5) have recorded the highest germination (94 %) followed by *Azospirillum* @1:50 dilution + PPFM @1:100 dilution (1:1) for 18 h (T_8) (90 %) (Table 1). However the speed of germination (15.8) and seedling vigour (33.1 cm) were higher in the seed soaking treatment with microbial consortia, *Azospirillum* @1:50 dilution + phosphobacteria @1:50 dilution + PPFM @ 1:100 dilution + *Bacillus* @1:100 dilution (1:1:1:1) for 18 h (T_{12}) followed by T_8 in which the speed of germination and seedling vigour were 15.0 and 32.7 cm, respectively. Nevertheless, the germination was reduced in T_{12} (82 %). This antagonistic effect might be due to the combination of four cultures that would have increased the soaking culture concentration. Therefore, the seeds soaked in *Azospirillum* @1:50 dilution + PPFM @1:100 dilution (1:1) for 18 h (T_8) can be considered as better consortia for paddy seeds. The microbial population in the seed showed a range between 1 x 10^4 and 51 x 10^4 cfu g^{-1} of seed. However, highest population in *Azospirillum* (51 x 10^4 cfu g^{-1} of seed) was observed along with PPFM (1 x 10^4 cfu g^{-1} of seed) in the seed soaking treatment *viz*., *Azospirillum* @1:50 dilution + PPFM @1:100 dilution cultures (1:1) (Table 2). Such inoculation of rice seeds with *Azospirillum lipoferum* increased the phosphate ion content and ultimately resulted in seedling vigour improvement (Murty and Ladha, 1998). Similarly, PPFM inoculated with a diazotroph as individual and combined inoculant treatments has resulted in increased seedling vigour and this might be due to the increased rhizosphere population of the inoculants (Raja and Sundaram, 2006).

Therefore, co-inoculation of methylotrophs with a phosphate-solubilizing bacterium (*Burkholderia pyrrocinia*) or nitrogen-fixing bacterium (*Azospirillum brasilense*) was found to enhance plant growth due to the enhancement of soil nitrogenase, urease and phosphatase activity (Madhaiyan *et al*., 2010).

Table.1 Effect of seed infusion with liquid microbial consortia on germination and Vigour in paddy

Treatments	Germination (%)	Speed of germination	Seedling length (cm)
T_1 - Control	79	5.6	29.7
T_2 - Seed soaking in water	84	15.4	32.0
T_3 - Seed soaking in *Azospirillum* @1:50 dilution	82	12.1	33.9
T_4 - Seed soaking in Phosphobacteria @1:50 dilution	84	8.0	30.1
T_5 - Seed soaking in PPFM @1:100 dilution	94	12.7	32.4
T_6 - Seed soaking in *Bacillus subtilis* @1:100 dilution	84	12.3	29.9
T_7 - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 dilutions (1:1)	87	13.3	30.4
T_8 - Seed soaking in *Azospirillum* @1:50 + PPFM @1:100 dilutions (1:1)	90	15.0	32.7
T_9 - Seed soaking in *Azospirillum* @1:50 + *Bacillus subtilis* @1:100 dilutions (1:1)	87	14.5	29.1
T_{10} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + PPFM @1:100 dilutions (1:1:1)	87	11.0	32.0
T_{11} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + *Bacillus subtilis* @1:100 dilutions (1:1:1)	86	14.5	32.7
T_{12} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + PPFM @ 1:100 + *Bacillus subtilis* @1:100 dilutions (1:1:1:1)	82	15.8	33.1
SEd	3.3	0.4	1.4
CD (P=0.05)	7.2	0.9	3.1

Table.2 Effect of seed infusion with liquid microbial consortia on microbial population in paddy

Treatments	**Microbial population (cfu g^{-1} of seed)**			
	Azospirillum	Phosphobacteria	PPFM	*Bacillus*
T_1 - Control	-	-	-	-
T_2 - Seed soaking in water	-	-	-	-
T_3 - Seed soaking in *Azospirillum* @1:50 dilution	10 x10^4	-	-	-
T_4 - Seed soaking in Phosphobacteria @1:50 dilution	-	2 x 10^4	-	-
T_5 - Seed soaking in PPFM @1:100 dilution	-	-	2 x 10^4	-
T_6 - Seed soaking in *Bacillus subtilis* @1:100 dilution	-	-	-	11 x 10^4
T_7 - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 dilutions (1:1)	1 x 10^4	2 x 10^4	-	-
T_8 - Seed soaking in *Azospirillum* @1:50 + PPFM @1:100 dilutions (1:1)	51 x 10^4	-	1 x 10^4	-
T_9 - Seed soaking in *Azospirillum* @1:50 + *Bacillus subtilis* @1:100 dilutions (1:1)	11 x 10^4	-	-	24 x 10^4
T_{10} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + PPFM @1:100 dilutions (1:1:1)	8 x 10^4	4 x 10^4	2 x 10^4	-
T_{11} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + *Bacillus subtilis* @1:100 dilutions (1:1:1)	3 x 10^4	1 x 10^4	-	15 x 10^4
T_{12} - Seed soaking in *Azospirillum* @1:50 + Phosphobacteria @1:50 + PPFM @ 1:100 + *Bacillus subtilis* @1:100 dilutions (1:1:1:1)	5 x 10^4	1 x 10^4	1 x 10^4	14 x 10^4

Table.3 Effect of chemical treatment on germination and microbial population in liquid bioinoculants infused paddy seed

Treatments	Seed germination (%)	Speed of germination	Seedling length (cm)	Microbial population (cfu g^{-1} of seed)
T_1-Control	84	6.0	29.9	-
T_2-Seed soaking in *Azospirillum* @1:50 dilution	89	13.8	33.7	32 x 10^5
T_3-Seed soaking in *Azospirillum* @1:50 dilution + Polymer coating @ 5 ml kg^{-1}	92	14.8	34.2	20 x 10^5
T_4-Seed soaking in *Azospirillum* @1:50 dilution + Carbendazim treatment @ 2 g kg^{-1}	93	14.8	34.3	54 x 10^4
T_5-Seed soaking in *Azospirillum* @1:50 dilution + Polymer coating @ 5 ml kg^{-1} + Carbendazim treatment @ 2 g kg^{-1}	95	15.4	35.2	11 x 10^5
T_6-Seed soaking in Phosphobacteria @1:50 dilution	89	13.9	33.9	5 x 10^5
T_7-Seed soaking in Phosphobacteria @1:50 dilution + Polymer coating @ 5 ml kg^{-1}	90	14.8	33.3	84 x 10^5
T_8-Seed soaking in Phosphobacteria @1:50 dilution + Carbendazim treatment @ 2 g kg^{-1}	91	14.0	33.4	7 x 10^4
T_9-Seed soaking in Phosphobacteria @1:50 dilution + Polymer coating @ 5 ml kg^{-1} + Carbendazim treatment @ 2 g kg^{-1}	94	14.8	33.0	12 x 10^5
T_{10}-Seed soaking in PPFM @1:100 dilution	94	15.2	35.9	8 x 10^4
T_{11}-Seed soaking in PPFM @1:100 dilution + Polymer coating @ 5 ml kg^{-1}	95	15.6	35.3	9 x 10^4
T_{12}-Seed soaking in PPFM @1:100 dilution + Carbendazim treatment @ 2 g kg^{-1}	97	15.2	36.0	2 x 10^4
T_{13}-Seed soaking in PPFM @1:100 dilution + Polymer coating @ 5 ml kg^{-1} + Carbendazim treatment @ 2 g kg^{-1}	98	15.5	37.0	4 x 10^4
T_{14}-Seed soaking in *Bacillus subtilis* @1:100 dilution	91	14.1	34.7	23 x 10^5
T_{15}-Seed soaking in *Bacillus subtilis* @1:100 dilution + Polymer coating @ 5 ml kg^{-1}	93	13.9	34.0	18 x 10^4
T_{16}-Seed soaking in *Bacillus subtilis* @1:100 dilution + Carbendazim treatment @ 2 g kg^{-1}	93	13.6	35.6	2 x 10^4
T_{17}-Seed soaking in *Bacillus subtilis* @1:100 dilution + Polymer coating @ 5 ml kg^{-1} + Carbendazim treatment @ 2 g kg^{-1}	93	13.8	35.9	11 x 10^4
SEd	1.5	0.5	0.7	
CD (P=0.05)	3.2	1.0	1.5	

Another study investigated that co-inoculation of *Pseudomonas striata* and *Bacillus polymyxa* strains with a strain of *Azospirillum brasilense*, resulted in a significant improvement in yield with a concomitant increase in N and P uptake compared with separate inoculations with each strain (Alagawadi and Gaur, 1992). Nkpwatt *et al.*, (2006) found that the cell-free supernatant of the *Methylobacterium* bacterial culture stimulated germination, suggesting the production of a growth-promoting agent by the methylotroph. Methylotrophs mediate the cytokinin on germinating seeds (Holland and Polacco, 1994) and IAA on increased seedling vigour (Subhaswaraj *et al.*, 2017) has been considered the most probable means of enhanced germination and vigour. Bakonyi *et al.*, (2013) opined that there is a positive effect of plant growth promoting bacteria (PGPB) on germination and growth by reason of excreting phytohormones and enhancing the nutrient mobilization from the seed.

Generally, the seed treatment with microbial cultures and chemicals has recorded the enhanced germination and vigour. In which, the highest germination (98 %), speed of germination (15.5) and seedling length (37.0 cm) were recorded in the seeds infused with PPFM liquid culture @1:100 dilution for 18 h followed by polymer coating @ 5 ml kg^{-1} and carbendazim @ 2 g kg^{-1} of seed (T_{13}) (Table 3). Generally, the polymer coating has not much affected the microbial population in the seed. However, the population was affected in the carbendazim treated seeds. Fortunately, the polymer coating followed by carbendazim treatment has recorded the minimum reduction in the microbial population. It shows that the polymer coating acts as a barrier between the microbes and carbendazim (Table 3). Among the different cultures, Phosphobacteria and *Bacillus subtilis* have found to sensitive to the chemicals in which the population declined drastically. The best performing treatment viz., seed soaking in PPFM liquid culture @1:100 dilution for 18 h + polymer coating @ 5 ml kg^{-1} + carbendazim @ 2 g kg^{-1} has recorded the PPFM population of 4 x 10^{4} cfu g^{-1} of seed. Similar findings on the survival of the bioinoculants in the chemical treated seeds were studied in many crops (Dunfield *et al.*, 2000; Bikrol *et al.*, 2005; Mehta *et al.*, 2011; Tariq *et al.*, 2016). Sunita *et al.*, (2007) opined that diazotrophs and phosphate solubilizing bacteria showed decline in their viable population on prolonged contact with fungicides during seed treatment. Nevertheless, Khalequzzaman (2008) found that the inoculation of lentil and chickpea seeds with *Rhizobium* followed by bavistin treatment gave significant decrease in foot and root rot incidence and increase in plant stand and grain yield.

It is concluded that the co-inoculation of *Azospirillum* and PPFM has performed well in increasing the seed germination and seedling vigour in paddy. The pre-inoculated seed treatment with polymer coating has not affected the microbial population in the seed. However, the fungicidal treatment has affected the inoculants population.

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