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Seed treatment with liquid microbial consortia for germination and vigour improvement in tomato (*Solanum lycopersicum* L.)

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Abstract

Use of effective microorganisms as a pre-sowing seed treating agent is considered to be ecologically sound and beneficial to both seed and environment. Therefore to ensure the benefits, studies were conducted in tomato seeds with different liquid microbial cultures. The results revealed that the tomato seeds treated with liquid cultures *viz.*, *Azospirillum*, phosphobacteria and Pink Pigmented Facultative Methylotroph (PPFM) have showed significant increase in germination and vigour. The seeds soaked in equal volume of *Azospirillum* @ 1:50 dilution for 24 h or phosphobacteria @ 1:50 dilution for 12 h or PPFM liquid culture @ 1:100 dilution for 18 h have registered the higher germination and vigour. Among these microbial cultures, PPFM has performed well in enhancing the seed germination and seedling vigour. Also, the viability and vigour of the inoculants infused seed were not much affected in three months storage. However, consortia of these microbial cultures showed antagonistic effect in seed germination and seedling vigour. In addition, the seeds infused with PPFM @1:100 dilution for 18 h followed by polymer coating @ 5 mL and carbendazim fungicide treatment @ 2 g kg^{-1} recorded significant improvement in seed germination and vigour with minimal reduction in the microbial population. Therefore, it would be possible to infuse the beneficial microbes into the seed through liquid cultures and also storing such seeds without much reduction in the microbial population. Therefore, it is beneficial if the seeds treated with the effective microorganisms which favour the better seed germination and seedling growth. Also, the microbes can easily be added into the soil along with the seed which may reflect on the better coloization of the microbes in plant root zone.

Key words: Liquid biofertilizers, *Azospirillum*, phosphobacteria, PPFM, tomato seeds.

Introduction

Seed is a prime input in agriculture and thus, the quality of the seed used for sowing should be in high order. The quality can be achieved in many ways of which seed treatment is an important method to get the higher productivity and plant population. Among the seed treatments, pre-sowing management with organic products have proved very effective in controlling the pathogens and increasing the seedling vigour. Nevertheless, use of effective microorganisms as a pre-sowing seed treating agent is considered to be ecologically sound and beneficial to both seed and environment. In general, about 5 to 30 per cent yield increase has been recorded from various crops by such bacterial inoculation (Datta *et al.*, 1982). The application of inoculum to the seeds is 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 was found to be time consuming and difficult when used for large quantities of seed. Hence, alternate 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). The bacterial cultures *viz.*, *Azospirillum*, *Pseudomonas, Azotobacter* (Shaukat *et al.*, 2006) and *Methylobacterium* (Nkpwatt *et al.*, 2006) promotes seed germination and seedling growth. Qureshi *et al.* (2012) found that the co-inoculation of *Rhizobium* and *Bacillus* sp. increased the root length, root mass, number of nodule and mass as compared to control in blackgram. Similarly, PPFM inoculated with a diazotroph as individual and combined inoculant treatments has resulted in increased seedling vigor, dry matter production and yield and this might be due to the increased rhizosphere population of the inoculants (Raja and Sundaram, 2006). Based on the background, it was decided to introduce the microbes into the seed rather through surface coating which enables transfer of the microbial population to the soil for better seedling growth. Therefore, the present study was contemplated to find out the suitable liquid microbial culture for effective seed treatment and the effect of seed treating chemicals on the survival of the inoculants in the tomato seed.

Materials and methods

Standardization of concentration and duration: Tomato seeds cultivar PKM 1 were collected from Vegetable Research Station, Palur (India) and dried well for the purpose of microbial treatments. The bacterial strains *viz.*, *Azospirillum*,

phosphobacteria, *Methylobacterium* (Pink pigmented Facultative Methylotroph, PPFM) 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 % methanol. The liquid based bio-inoculant formulations were prepared by diluting at various concentrations *viz.*, 1:1, 1:10, 1:50 and 1:100 ratios along with undiluted one. Then, the tomato seeds were soaked in these concentrations at different time durations *viz.*, 6, 12, 18 and 24 h with equal seed to culture ratio *i.e.* 1:1 ratio (v/v). After that the seeds were shade dried to the original moisture. The germination test was conducted by placing 400 seeds in four replications as per the ISTA (1999) procedure and evaluated.

Microbial consortia on seed quality: The consortia of microbial cultures *viz.*, *Azospirillum*, phosphobacteria and PPFM were prepared by diluting the cultures as standardized in the earlier experiment like *Azospirillum* @ 1:50, phosphobacteria @ 1:50 and PPFM @ 1:100 concentrations. The consortia were prepared by mixing the different cultures at 1:1 or 1:1:1 ratio. Then, the medium vigour seeds were soaked for 18 h in the microbial consortia in the equal volume by following the treatment schedule *viz.*, 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 *Azospirillum* @1:50 dilution + phosphobacteria @1:50 dilution (1:1); T_7 - seed soaking in *Azospirillum* @1:50 dilution + PPFM @1:100 dilution (1:1); T_8 - seed soaking in *Azospirillum* @1:50 dilution + phosphobacteria @1:50 dilution + PPFM @1:100 dilution (1:1:1). The seeds were dried to the original moisture content. The germination test was conducted by following the ISTA norms. The speed of germination was also assessed during the germination test by following the formula, $X_1 / Y_1 + X_2\text{-}X_1 / Y_2 + \ldots\ldots + X_n (X_{n-1}) / Y_n$, where, X_n - number of seeds germinated at n^{th} count, Y_n - number of days from sowing on n^{th} count (Maguire, 1962). The microbial populations in the treated seeds were also 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.

Compatibility with chemicals: Similarly, the effect of seed treating chemicals on the survival of microbes in tomato seeds was 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} of seed; T_4 - seed soaking in *Azospirillum* @1:50 dilution + carbendazim seed treatment @ 2 g kg^{-1} of seed; T_5 - seed soaking in *Azospirillum* @1:50 dilution + polymer coating @ 5 mL kg^{-1} + carbendazim seed treatment @ 2 g kg^{-1} of seed; T_6 - seed soaking in phosphobacteria @1:50 dilution; T_7 - seed soaking in phosphobacteria @1:50 dilution + polymer coating @ 5 mL kg^{-1} of seed; T_8 - seed soaking in phosphobacteria @1:50 dilution + carbendazim seed treatment @ 2 g kg^{-1} of seed; T_9 - seed soaking in phosphobacteria @1:50 dilution + polymer coating @ 5 mL kg^{-1} + carbendazim seed treatment @ 2 g kg^{-1} of seed; T_{10} - seed soaking in PPFM @1:100 dilution; T_{11} - seed soaking in PPFM @1:100 dilution + polymer coating @ 5 mL kg^{-1} of seed; T_{12} - seed soaking in PPFM @1:100 dilution + carbendazim seed treatment @ 2 g kg^{-1} of seed; T_{13} - seed soaking in PPFM @1:100 dilution + polymer coating @ 5 mL kg^{-1} of seed + carbendazim seed treatment @ 2 g kg^{-1} of seed. The treated seeds were stored for a week and evaluated for the germination, vigour and microbial population.

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

Results and discussion

Standardization of concentration and duration: Tomato seeds treated with liquid *Azospirillum* culture showed significant differences in germination in which, the *Azospirillum* culture diluted at 1:50 ratio increased the seed germination (92 %) at 24 h soaking period when compared with untreated control (74 %) (Fig. 1a). The undiluted (100 %) and higher *Azospirillum* culture concentration (1:1 dilution) showed deleterious effect on germination irrespective of the soaking durations. Similarly, the phosphobacteria @ 1:50 dilution for 12 h soaking recorded higher germination (96 %) than the control (89 %). The germination was declined if the concentration of the culture was increased. Undiluted and 1:1 diluted phosphobacteria cultures severely affected the germination irrespective of the soaking periods. Lower germination of 36 per cent was recorded when the seeds soaked in undiluted phosphobacteria culture for 24 h (Fig. 1b). In addition, the seed infused with PPFM liquid culture at 1:100 dilution for 18 h soaking duration has recorded the highest germination (97 %) when compared with control (89 %) (Fig. 1c). The germination was much affected if the PPFM culture concentration is higher. Similarly, increase in soaking duration has shown the negative effect on the seed germination. Decline in mean germination in 24 h soaking (46.7 %) and undiluted higher concentration (60.5 %) has been recorded as the evidence of the antagonistic effect. Similar findings of seed inoculation with plant growth promoting bacteria for increased germination and seedling vigour were studied by many workers (Murty and Ladha, 1988; Sharma *et al.*, 2007; Meena *et al.*, 2012; Bakonyi *et al.*, 2013). Adesemoye *et al.* (2009) opined that microorganisms or plant growth promoting rhizobacteria can help to reduce the application of inorganic fertilizers and contribute to improving soil fertility and reducing a negative environmental impact. The positive effect of plant growth promoting bacteria on germination and growth might be due to excreting phytohormones and enhancing the nutrient mobilization from the seed (Murty and Ladha, 1988). Goes *et al.* (2012) found the growth-promoting activities particularly auxin synthesis in the plant growth-promoting bacteria. Among the cultures, PPFM performed better in increasing germination (87 %), speed of germination (2.6) and seedling length (17.2 cm) at 1:100 dilution (Table 1). Also, no significant differences were observed in the germination and seedling vigour during the three months storage of this treated seeds. But the reduction in microbial population was noticed irrespective of the inoculants during seed storage. Pink pigmented

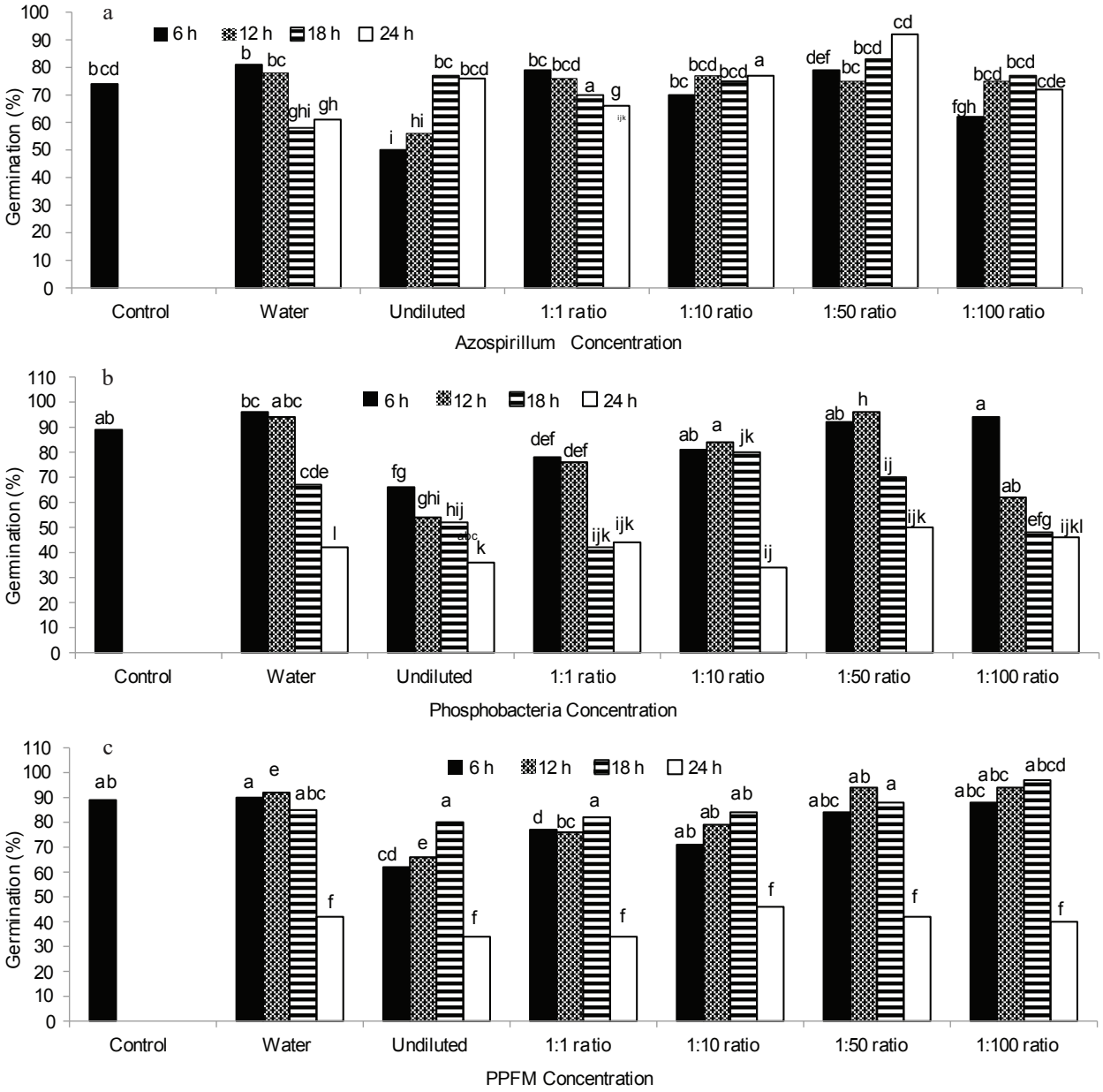

Fig. 1. Effect of seed infusion with a) *Azospirillum,* b) phosphobacteria and c) PPFM cultures on germination in tomato

facultative methylotroph recorded the highest population during initial (40 x 10^5 cfu g^{-1} of seed) as well as three months storage (22 x 10^5 cfu g^{-1} of seed). The available seed moisture might have supported the viability of the microorganisms in the seed.

Microbial consortia on seed quality: Based on the results obtained, the microbial consortia were prepared with 1:50 (*Azospirillum* and Phosphobacteria) or 1:100 (PPFM) dilutions and seeds were soaked for 18 h in the consortia. The consortia seed treatment resulted negative effect in germination and seedling vigour. In which, the consortia *Azospirillum* and phosphobacteria or *Azospirillum* and PPFM or *Azospirillum*, phosphobacteria and PPFM recorded 68, 68 and 59 per cent germination, respectively compated to untreated control (78 %). Nevertheless, monoculture treatments improved the germination of medium vigour seeds to certain extent like 85, 84 and 89 % in *Azospirillum*, phosphobacteria and PPFM, respectively (Table 2). Among the cultures, PPFM performed better in terms of enhancement in germination, speed of germination and seedling vigour. However, the combination of two or more microbial cultures showed the decrease in the germination and seedling vigour. In contrast, Qureshi *et al.* (2012) found that the co-inoculation of phosphate solubilizing bacteria and rhizobia increased the root length, root mass, number of nodule and mass in mash bean. Combined inoculation of phosphate-solubilising bacteria and *Azotobacter* exhibited beneficial effect on yield, as well as on nitrogen and phosphorous storage in different crops (Kundu and Gaur 1984; Monib *et al.*, 1984). Being small in size, tomato seeds was not able to tolerate the higher concentrations during consortia formulation, therefore, the soaking of tomato seeds in microbial consortia had negative impact on the seed quality. Also, the seed infusion treatment with different bacterial cultures showed positive response in penetration of the colonies into the seed. In this regard, the microbial population was higher in PPFM seed treatment in which a load of 41 x 10^5 cfu g^{-1} of seed was recorded. Similar findings of enhanced seed germination by seed coating or seed inoculum of methylotrophs were recorded earlier (Anitha, 2010 and Meena *et al.*, 2012). Corpe

Table 1. Seed infusion with liquid bioinoculants on seed viability and microbial population in tomato seed

Treatments	Seed germination (%)			Speed of germination			Seedling length (cm)			Microbial population in seed (cfu g^{-1} of seed)	
	Initial	3MAS*	Mean	Initial	3MAS	Mean	Initial	3MAS	Mean	Initial	3MAS
T_1 - Untreated Control	83	83	82.7	2.0	2.0	2.0	12.9	12.7	12.8	-	-
T_2 - Seed soaking in *Azospirillum* liquid culture @1:50 dilution for 18 h	86	84	85.0	1.9	2.1	2.0	16.4	15.9	16.2	22×10^6	9×10^5
T_3 - Seed soaking in Phosphobacteria liquid culture @1:50 dilution for 18 h	83	83	83.0	1.9	2.2	2.1	15.0	14.7	14.9	44×10^4	41×10^4
T_4 - Seed soaking in PPFM liquid culture @1:100 dilution for 18 h	87	86	86.5	2.6	2.6	2.6	17.2	16.9	17.1	40×10^5	22×10^5
Mean	84.8	84.0		2.1	2.2		15.4	15.1			
	T	S		T	S		T	S			
SEd	1.1	0.7		0.1	0.08		0.3	0.2			
CD (P=0.05)	2.2	NS		0.2	NS		0.6	NS			

* months after storage

Table 2. Seed infusion with liquid microbial consortia on germination, vigour and microbial population in tomato seed

Treatments	Seed germination and seedling vigour			Microbial population in seed (cfu g^{-1} of seed)		
	Germination (%)	Speed of germination	Seedling length (cm)	*Azospirillum*	Phosphobacteria	PPFM
T_1-Control	78	1.6	13.5	-	-	-
T_2-Seed soaking in water for 18 h	82	1.7	14.7	-	-	-
T_3-Seed soaking in *Azospirillum* liquid culture @1:50 dilution for 18 h	85	2.1	17.0	22×10^6	-	-
T_4-Seed soaking in Phosphobacteria liquid culture @1:50 dilution for 18 h	84	1.9	13.7	-	30×10^4	-
T_5-Seed soaking in PPFM liquid culture @1:100 dilution for 18 h	89	2.4	17.1	-	-	41×10^5
T_6-Seed soaking in *Azospirillum* @1:50 dilution + Phosphobacteria @1:50 dilution liquid cultures (1:1) for 18 h	68	2.1	15.9	12×10^5	20×10^4	-
T_7-Seed soaking in *Azospirillum* @1:50 dilution + PPFM @1:100 dilution liquid cultures (1:1) for 18 h	68	1.6	12.4	20×10^6	-	30×10^5
T_8-Seed soaking in *Azospirillum* @1:50 dilution + Phosphobacteria @1:50 dilution + PPFM @1:100 dilution liquid cultures (1:1:1) for 18 h	59	1.5	15.8	7×10^6	17×10^4	23×10^5
SEd	7.0	0.1	1.0			
CD (P=0.05)	15.3	0.2	2.2			

and Rheem (1989) reported that the PPFMs are present in the rhizosphere and phyllosphere regions of plants and even on the surface of the seeds of various plants. 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. Pink pigmented facultative methylotroph mediate the cytokinin or other plant growth promoting substances on germinating seeds (Holland and Polacco, 1994) and that might be the reason for enhanced germination in tomato seeds. The increased seedling vigour by the production of IAA by *Methylobacterium* was confirmed earlier in tomato (Subhaswaraj *et al.*, 2017). Agafonova *et al.* (2013) found the phosphate-solubilizing activity of methylobacteria in phytosymbiosis. In addition, methylotrophs play a major role in phosphorus acquisition, nitrogen fixation, phytohormone production, iron chelation and plant growth promotion and therefore, co-inoculation of these bacteria as biofertilizers can result in viable agriculture practices (Manish Kumar *et al.*, 2016).

Compatibility with chemicals: In another experiment, tomato seeds infused with PPFM liquid culture @1:100 dilution for 18 h followed by polymer coating @ 5 mL kg^{-1} and seed treatment with carbendazim @ 2 g kg^{-1} of seed recorded higher germination (96 %), speed of germination (3.0) and seedling length (17.8 cm) compared to control (Table 3). Other seed treatments with PPFM culture combination like polymer @ 5 mL kg^{-1} or carbendazim @ 2 g kg^{-1} of seed have also recorded the better germination and seedling vigour. Similarly, *Azospirillum* and phosphobacteria infused seeds treated with chemicals like polymer or carbendazim showed advantages in seed quality enhancement than untreated control. However, the advantage was higher in PPFM infused seeds than the others as discussed earlier. While, analyzing the microbial density in the seed, carbendazim fungicide treatment was found to have negative impact. Seeds treated with carbendazim recorded lowest

Table 3. Chemical treatment on germination, seedling vigour and microbial population in bioinoculants infused tomato seed

Treatments	Seed germination (%)	Speed of germination	Seedling length (cm)	Microbial population (cfu g^{-1} of seed)
T_1-Control	80	1.4	11.9	-
T_2-Seed soaking in *Azospirillum* liquid culture @1:50 dilution for 18 h	91	1.9	15.3	17 x 10^5
T_3-Seed soaking in *Azospirillum* liquid culture @1:50 dilution for 18 h + Polymer coating @ 5 mL kg^{-1} of seed	92	2.2	15.7	12 x 10^5
T_4-Seed soaking in *Azospirillum* liquid culture @1:50 dilution for 18 h + Carbendazim seed treatment @ 2 g kg^{-1} of seed	85	2.3	16.7	6 x 10^5
T_5-Seed soaking in *Azospirillum* liquid culture @1:50 dilution for 18 h + Polymer coating @ 5 mL kg^{-1} + Carbendazim seed treatment @ 2 g kg^{-1} of seed	81	2.6	16.3	8 x 10^5
T_6-Seed soaking in Phosphobacteria liquid culture @1:50 dilution for 18 h	90	1.9	12.8	52 x 10^4
T_7-Seed soaking in Phosphobacteria liquid culture @1:50 dilution for 18 h + Polymer coating @ 5 mL kg^{-1} of seed	91	1.8	14.9	56 x 10^4
T_8-Seed soaking in Phosphobacteria liquid culture @1:50 dilution for 18 h + Carbendazim seed treatment @ 2 g kg^{-1} of seed	92	2.0	16.3	48 x 10^4
T_9-Seed soaking in Phosphobacteria liquid culture @1:50 dilution for 18 h + Polymer coating @ 5 mL kg^{-1} + Carbendazim seed treatment @ 2 g kg^{-1} of seed	93	2.0	16.1	50 x 10^4
T_{10}-Seed soaking in PPFM liquid culture @1:100 dilution for 18 h	93	2.9	16.5	40 x 10^5
T_{11}-Seed soaking in PPFM liquid culture @1:100 dilution for 18 h + Polymer coating @ 5 mL kg^{-1} of seed	95	2.9	17.1	31 x 10^5
T_{12}-Seed soaking in PPFM liquid culture @1:100 dilution for 18 h + Carbendazim seed treatment @ 2 g kg^{-1} of seed	95	2.9	17.4	7 x 10^4
T_{13}-Seed soaking in PPFM liquid culture @1:100 dilution for 18 h + Polymer coating @ 5 mL kg^{-1} of seed + Carbendazim seed treatment @ 2 g kg^{-1} of seed	96	3.0	17.8	32 x 10^4
SEd	2.3	0.2	0.9	
CD (*P*=0.05)	4.8	0.4	1.8	

population (7 x 10^4 cfu g^{-1} seed). Likewise, population of *Azospirillum* and phosphobacteria was reduced drastically due to this fungicide (Table 2). Fortunately, the polymer coating followed by carbendazim treatment recorded the minimum reduction in the microbial population. This might be due to the barrier effect of polymer between the microbes and carbendazim. Similar findings on the survival of the bioinoculants in the chemical treated seeds were observed in many crops (Dunfield *et al.*, 2000; Bikrol *et al.*, 2005; Mehta *et al.*, 2011; Tariq *et al.*, 2016). Khalequzzaman (2008) opined that the inoculation of lentil and chickpea seeds with *Rhizobium* followed by bavistin treatment significantly decreased foot and root rot incidence and increased plant stand and grain yield. However, seed inoculation techniques used for research purposes are often not feasible at a commercial scale and there are significant technical challenges in maintaining viable microbial inocula on seed throughout commercial seed treatment processes and storage (Callaghan, 2016). Therefore, it is concluded that the tomato seed infused with the liquid cultures *viz.*, *Azospirillum*, phosphobacteria and pink pigmented facultative methylotroph (PPFM) showed significant improvement in germination and seed vigour in which the soaking duration and concentration played a major role. *Azospirillum* microbial culture diluted at 1:50 ratio increased the germination (92 %). Also, the seeds infused with phosphobacteria @ 1:50 dilution for 12 h have recorded the higher germination (96 %). Among the different cultures, PPFM performed better in terms of improvement in seedling vigour with highest microbial population in the seed. However, antagonistic effect was recorded when the seeds infused with more than one culture. Nevertheless, seed soaking in PPFM liquid culture @1:100 dilution for 18 h followed by polymer coating @ 5 mL and carbendazim treatment @ 2 g kg^{-1} of seed has recorded the higher germination and seedling vigour with minimum reduction in the microbial population. The study revealed that the beneficial microbial inoculants can be introduced into the seed and transferred to the field without coating with carrier based inoculants.

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