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Infusing Microbial Consortia on Seed Quality and Seedling Vigour in Pearlmillet [*Pennisetum glaucum* (L.) R. Br.]

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Authors' contributions

This work was carried out in collaboration between both authors. Author KR designed the study, performed the analysis, wrote the protocol and wrote the manuscript. Author RA analyzed the microbial population in the seed. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Seed treatment with Plant Growth Promoting Bacteria (PGPB) is considered to be safe for seed and environment and ecologically sound. To ensure the benefits of the PGPB, studies were conducted in pearlmillet by treating with liquid microbial cultures for enhancing seed germination and seedling vigour.
Methods: The pearlmillet seeds were infused with different concentrations of liquid microbial cultures and their consortia. Also, the bioinoculant infused seeds were treated with chemicals to assess their effect on the microbial population.
Results: The pearlmillet seeds soaked in equal volume of *Azospirillum* or phosphobacteria @ 1:50 dilution or Pink Pigmented Facultative Methylotroph (PPFM) @ 1:100 dilution for 18 h have recorded higher germination and seedling vigour. Pink Pigmented Facultative Methylotroph treated seeds have showed higher germination and seedling vigour amid the microbial cultures. In addition, the bioinoculant treated seed viability and vigour were not affected during three months storage.

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However, the microbial populations in the seed get declined during storage. The microbial consortia comprising of *Azospirillum*, phosphobacteria and PPFM did not affect the germination rather seedling vigour. Besides, seeds infused with *Azospirillum* @ 1:50 dilution and PPFM @1:100 dilution (1:1) for 18 h have recorded higher seedling vigour. In addition, seeds soaked in PPFM @1:100 dilution for 18 h followed by polymer coating @ 5 ml kg^{-1} and carbendazim seed treatment @ 2 g kg^{-1} of seed had higher germination and seedling vigour. But, the microbial population in the seed was reduced considerably due to the chemical treatment.

Conclusion: The liquid bioinoculants *viz*., *Azospirillum*, phosphobacteria and PPFM can be used to pre-inoculate the pearlmillet seeds with required population to be carried over to the field.

Keywords: Liquid microbial cultures; seed treatment; seed quality; pearlmillet.

1. INTRODUCTION

Usually, any agriculture starts with seed and therefore, the quality of the seed is essential for better plant population and yield. The quality of seed alone can contribute about 20 per cent increased yield. The quality of seed can be enhanced by several ways including seed crop management practices, post harvest operations, processing, seed treatment etc. Of which, the seed treatment plays a prime role in improving the quality of the seed. Amongst, pre-sowing treatment is one of the important methods by which the quality of the seed that is being used for sowing can be improved by eliminating the pest and disease, addition of nutrients and beneficial microorganisms etc.

Seed is also considered to be a cheap carrier for delivering other inputs. Thus, the bioinoculants and biocontrol agents can be delivered through seed, since they are all considered as safe to the environment. In general, yield increase of about 5 to 30 per cent has been reported in various crops through bacterial inoculation [1]. Usually, the application of carrier based bioinoculants to the seeds is in trend [2]. In this regard, adhesive is added along with the bioinoculants in order to improve stickiness on the seed [3]. However, carrier-based bioinoculants 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 seeds.

Alternatively, liquid inoculants were developed for seed treatment as it is easy to use, spreads well, mixes easily and needs no additional water supply [4]. Gomathy et al. [5] reported a population density of $5.5x10^5$ cfu $seed^{-1}$ on black gram, $10.5x10^5$ cfu $seed^{-1}$ on soybean and $6.5x10^5$ cfu $seed^{-1}$ on maize after 12 h of incubation in 1 ml sporulated inoculum mixed with 1 ml of rice gruel. Nevertheless, liquid bacterial cultures *viz*., *Azospirillum*, *Pseudomonas, Azotobacter* [6] and *Methylobacterium* [7] promote seed germination and seedling growth. Co-inoculation of *Rhizobium* and *Bacillus* sp. increased the root length, root mass, number of nodule and mass as compared to control in blackgram [8]. Likewise, PPFM inoculated with a diazotroph as individual and combined inoculant treatments have resulted in increased seedling vigor, dry matter production and yield and this might be due to the increased rhizosphere population of the inoculants [9]. Also, it is possible to infuse the beneficial microbes into the seed through liquid cultures and storing such seeds without much reduction in the microbial population [10]. Therefore, studies were conducted to find out the effect of liquid microbial cultures for enhancing seed germination and seedling vigour and the effect of seed treating chemicals on the survival of the bioinoculants in the pearlmillet seed.

2. MATERIALS AND METHODS

2.1 Standardization of Bioinoculants Concentration

Pearlmillet cultivar CO (CU) 9 seeds were collected from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore (India) and cleaned thoroughly for conducting the experiment. The bioinoculant bacterial strains *viz*., *Azospirillum*, phosphobacteria, 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 cultures were diluted into different concentrations *viz*., 1:1, 1:10, 1:50 and 1:100 ratios along with undiluted one. Then, the pearlmillet seeds were soaked in these diluted liquid cultures at various time

durations *viz*., 6, 12, 18 and 24 h with equal seed to culture ratio i.e. 1:1 ratio (v/v). Later, the inoculated seeds were shade dried to the original moisture. The germination test of this seeds was conducted by placing 400 seeds in four replications [11] and evaluated on final count day i.e. seventh day. The speed of germination (sprouts / day) was also assessed during the germination test by following the formula, $X_1 / Y_1 + X_2-X_1/ Y_2 + \ldots\ldots + X_n (X_{n-1}) / Y_n$, where, X_n - number of seeds germinated at n^{th} count, Y_n - number of days from sowing on n^{th} count [12]. The seedling length was recorded by measuring ten randomly selected normal seedlings at a distance between the tips of the primary shoot and root and the mean value was calculated. In addition, the treated seeds were stored at ambient condition (30±2°C) for about three months as to assess the storability of the bioinoculant treated seeds and survival of microbes in the seeds.

2.2 Microbial Consortia on Seed Quality

Microbial consortia was prepared by using the standardized dilutions of *Azospirillum*, phosphobacteria and PPFM as per 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 pearlmillet 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). After that, the treated seeds were dried to the original moisture content. The germination test was conducted by following the ISTA [11] protocol. Additionally, microbial population in the bioinoculated seeds was assessed by washing the seeds with sterile water for about four to five times to remove the chemicals remaining on the surface of the seeds. Subsequently, 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 for growth of colonies [13].

2.3 Compatibility of Bioinoculants with Chemicals

Influence of chemical treatment on the survival of microbes in pearlmillet seeds was assessed by introducing them into the seeds with different liquid microbial cultures for 18 h in equal volume. These treated 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. Finally, the treated seeds were stored for a week and evaluated for the germination, vigour and microbial population as per the procedures described earlier [11,13].

The data collected were analyzed statistically [14] and the critical difference values were calculated at 5% probability level.

3. RESULTS AND DISCUSSION

3.1 Standardization of Bioinoculants Concentration

The treatment of pearlmillet seeds with *Azospirillum* has resulted significant influence in germination, speed of germination and seedling vigour. In which, the seeds soaked in *Azospirillum* liquid culture at 1:50 dilution for 18 h have recorded higher germination (92%) than the control (86%) (Table 1). The concentrated cultures like undiluted and 1:1 diluted *Azospirillum* have showed deleterious effect on seed germination. Nevertheless, the germination

was declined when the soaking duration was increased more than 18 h. Similarly, the speed of germination was higher (25.0) in the seeds soaked with the *Azospirillum* @ 1:50 dilution for 18 h compared with control (20.9). Likewise, seedling length was more (32.2 cm) in the seeds treated with 1:50 diluted *Azospirillum* culture for 18 h. However, undiluted microbial culture has affected the seedling length (Table 1).

Significant improvement in germination and seedling length were noticed in the seed infusion treatment with phosphobacteria liquid culture. Maximum germination (100%) was recorded in the phosphobacteria at 1:50 diluted liquid culture for 18 h when compared to control (92%) (Table 2). The seeds treated with higher concentrations of microbial culture *viz*., undiluted and 1:1 diluted cultures have showed decrease in the germination per cent than the untreated control irrespective of the soaking duration. Also, the germination was drastically reduced to 36 per cent in the seed soaking treatment with undiluted culture at 24 h. Even in the diluted culture concentrations *viz*., 1:50 and 1:100 ratio, the pearlmillet seed germination was affected when the seeds soaked for 24 h. Higher speed of germination (24.6) and lengthier seedlings (33.6 cm) were recorded in the seed soaking treatment with phosphobacteria @ 1:50 dilution for 18 h. The deleterious effect on these parameters was recorded in the highly concentrated liquid cultures like undiluted and 1:1 diluted ones. The soaking duration was also affects the germination speed in which the 24 h soaking has recorded decreased rate.

Equally, the pearlmillet seeds soaked in higher PPFM concentrations *viz*., 100 per cent and 1:1 dilution have affected the germination when compared with lower concentrations *viz*., 1:50 and 1:100 dilutions. The soaking duration has also influenced the germination percentage. Undiluted higher concentration with more soaking period (24 h) has affected the germination (6%) when compared with control (74%). However, the seeds soaked in PPFM culture @ 1:100 dilution have recorded the higher germination (94%) at 18 h soaking followed by 12 h (82%) (Table 3). Similarly, the seeds soaked in PPFM @ 1:100 diluted culture have recorded the higher speed of germination at 12 h (17.0) and 18 h (16.7) soaking periods. When the concentration or soaking period is increased the speed of germination was reduced. However, the seeds soaked in undiluted culture have recorded lower speed of germination when compared with control. Similar trend was recorded in seedling length also. The higher concentration and prolonged soaking period have showed the hindrance in seedling vigour. Among the concentrations and durations, the pearlmillet seed soaked in PPFM culture @ 1:100 dilution for 18 h have recorded the lengthier seedlings (33.3 cm) when compared to others. Germination and seedling vigour enhancement through plant growth promoting bacteria were studied in rice [15,16], maize [17], gram [18] and black gram [19]. Murty and Ladha [15] found the positive effect of plant growth promoting bacteria on germination and growth by reason of excreting phytohormones and enhancing the nutrient mobilization from the seed. The growth-promoting activities particularly auxin synthesis in the plant growth-promoting bacteria has been found to occur in sunflower [20].

Among the different liquid microbial cultures, pearlmillet seeds soaked in PPFM @1:100 dilution for 18 h has showed the enhanced germination (98.5%), speed of germination (18.7) and seedling vigour (36.6 cm) than the other cultures (Table 4). The seed viability and vigour were not much affected in three months storage period. However, the microbial population in the seed gets declined during the seed storage. In this regard, initial populations in *Azospirillum* (42 x 10^4 cfu g^{-1} of seed), phosphobacteria (12 x 10^4 cfu g^{-1} of seed) and PPFM (15 x 10^4 cfu g^{-1} of seed) were decreased to 4 x 10^4, 11 x 10^4 and 3 x 10^4 cfu g^{-1} of seed at three months storage. But, the population counted after three months storage might be sufficient for the betterment of the seed quality. Similar findings of enhanced seed germination by inoculation of methylotrophs were recorded earlier [21,22]. Corpe and Rheem [23] revealed 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. [7] found that the cell-free supernatant of the *Methylobacterium* bacterial culture stimulated germination, suggesting the production of a growth-promoting agent by the methylotroph. Synthesis of cytokinin [24] and indole acetic acid [25] by PPFM in germinating seed might be the reason for enhanced vigour in pearlmillet seeds. Agafonova et al. [26] found the phosphate-solubilizing activity of methylobacteria in phytosymbiosis. Manish Kumar et al. [27] opined that the methylotrophs plays a major role in phosphorus acquisition, nitrogen fixation, phytohormone production, iron chelation and plant growth

Table 1. Seed treatment with *Azospirillum* liquid culture on seed germination and seedling vigour in pearlmillet

Treatments	Seed germination (%)					Sprouts/day					Seedling length (cm)				
	6 h	12 h	18 h	24 h	Mean	6 h	12 h	18 h	24 h	Mean	6 h	12 h	18 h	24 h	Mean
Untreated control	86	86	86	86	86.0	20.9	20.9	20.9	20.9	20.9	30.8	30.8	30.8	30.8	30.8
Seed soaking in water	74	77	80	64	73.7	24.2	21.5	20.8	21.6	22.0	26.2	29.4	30.0	32.0	29.4
Seed soaking in *Azospirillum* @ 100% concentration	62	74	80	86	75.5	22.4	23.7	21.9	22.0	22.5	29.2	30.0	30.3	29.5	29.7
Seed soaking in *Azospirillum* @ 1:1 dilution	74	78	84	72	77.0	21.4	23.8	24.6	23.6	23.4	30.5	31.4	29.9	29.7	30.4
Seed soaking in *Azospirillum* @ 1:10 dilution	72	84	92	72	80.0	24.0	23.3	23.5	23.7	23.6	26.7	30.0	32.2	29.4	29.6
Seed soaking in *Azospirillum* @ 1:50 dilution	84	86	92	72	83.5	23.0	24.6	25.0	24.2	24.2	28.4	32.2	32.1	26.9	29.9
Seed soaking in *Azospirillum* @ 1:100 dilution	78	90	80	70	79.5	21.5	24.0	22.8	21.2	22.4	27.7	28.3	28.9	23.5	27.1
Mean	75.7	82.1	84.8	74.5		22.5	23.1	22.8	22.4		28.5	30.3	30.7	28.8	
	Treatment		Duration	T x D		Treatment		Duration	T x D		Treatment		Duration	T x D	
SEd	1.7		1.3	3.3		0.3		0.2	0.7		0.9		0.7	1.9	
CD (P=0.05)	3.4		2.6	6.8		0.7		NS	1.4		2.0		1.5	NS	

Table 2. Seed treatment with phosphobacteria liquid culture on seed germination and seedling vigour in pearlmillet

Treatments	Seed germination (%)					Sprouts / day					Seedling length (cm)				
	6 h	12 h	18 h	24 h	Mean	6 h	12 h	18 h	24 h	Mean	6 h	12 h	18 h	24 h	Mean
Untreated control	92	92	92	92	92.0	15.2	15.2	15.2	15.2	15.2	29.3	29.3	29.3	29.3	29.3
Seed soaking in water	76	90	84	90	85.0	21.6	21.3	22.0	20.7	21.4	32.2	29.5	25.1	21.2	27.0
Seed soaking in phosphobacteria @ 100% concentration	77	77	26	36	54.0	7.4	6.8	7.6	1.9	5.9	31.2	27.4	31.1	31.9	30.4
Seed soaking in phosphobacteria @ 1:1 dilution	78	70	79	61	72.0	14.4	3.9	4.8	7.4	7.6	31.3	29.0	29.5	31.6	30.3
Seed soaking in phosphobacteria @ 1:10 dilution	86	94	86	81	86.8	20.2	12.5	10.8	22.2	16.4	32.4	28.9	32.5	31.2	31.2
Seed soaking in phosphobacteria @ 1:50 dilution	98	96	100	73	91.8	24.3	22.7	24.6	19.9	22.9	27.0	32.2	33.6	31.3	31.0
Seed soaking in phosphobacteria @ 1:100 dilution	96	90	92	77	88.8	22.2	22.2	23.2	19.4	21.7	31.4	32.6	29.2	27.9	30.2
Mean	92.0	87.0	76.0	72.8		17.8	14.9	15.4	15.2		30.7	29.8	30.0	29.2	
	Treatment		Duration	T x D		Treatment		Duration	T x D		Treatment		Duration	T x D	
SEd	2.4		1.8	4.9		0.04		0.02	0.07		1.4		1.1	2.9	
CD (P=0.05)	5.0		3.8	10.0		0.07		0.06	0.14		2.8		NS	NS	

Table 3. Seed treatment with Pink Pigmented Facultative Methylotroph (PPFM) liquid culture on seed germination and seedling vigour in pearlmillet

Treatments	Seed germination (%)					Sprouts/day					Seedling length (cm)				
	6 h	**12 h**	**18 h**	**24 h**	**Mean**	**6 h**	**12 h**	**18 h**	**24 h**	**Mean**	**6 h**	**12 h**	**18 h**	**24 h**	**Mean**
Untreated control	74	74	74	74	74.0	14.5	14.5	14.5	14.5	14.5	31.1	31.1	31.1	31.1	31.1
Seed soaking in water	74	54	55	59	60.5	7.5	6.8	5.6	5.8	6.4	27.8	32.6	31.0	31.9	30.8
Seed soaking in PPFM @ 100% concentration	44	16	22	6	22.0	6.2	3.6	3.0	1.0	3.5	30.8	26.6	24.6	23.3	26.3
Seed soaking in PPFM @ 1:1 dilution	75	65	59	39	69.5	6.7	6.1	5.5	1.6	5.0	29.9	29.2	27.6	22.8	27.4
Seed soaking in PPFM @ 1:10 dilution	81	80	85	75	80.3	10.3	9.6	8.1	5.5	8.4	29.5	29.9	32.3	31.8	30.9
Seed soaking in PPFM @ 1:50 dilution	86	85	85	76	82.0	13.3	12.1	11.4	10.8	11.9	30.9	31.9	32.9	30.7	31.5
Seed soaking in PPFM @ 1:100 dilution	78	82	94	72	81.5	12.8	17.0	16.7	12.3	14.7	32.7	32.9	33.3	31.6	32.6
Mean	73.1	64.6	67.7	57.3		10.2	10.0	9.3	7.4		30.4	30.6	30.4	29.0	
	Treatment	Duration	T x D			Treatment	Duration	T x D			Treatment	Duration	T x D		
SEd	1.8	1.3	3.6			0.03	0.02	0.06			0.8	0.6	1.7		
CD (P=0.05)	3.7	2.7	7.4			0.07	0.04	0.13			1.7	NS	3.4		

Table 4. Seed treatment with different microbial cultures on seed viability and microbial population in pearlmillet at initial and three months after sowing (3 MAS)

Treatments	Seed germination (%)			Sprouts/day			Seedling length (cm)			Microbial population (cfu g^{-1} of seed)	
	Initial	3MAS	Mean	Initial	3MAS	Mean	Initial	3MAS	Mean	Initial	3MAS
T_1 - Untreated Control	86	86	86.0	11.9	12.2	12.1	30.6	31.1	30.8	-	-
T_2 - Seed soaking in *Azospirillum* @1:50 dilution for 18 h	94	94	94.0	13.6	13.1	13.4	33.7	33.0	33.3	42 x 10^4	4 x 10^4
T_3 - Seed soaking in Phosphobacteria @1:50 dilution for 18 h	95	94	94.5	15.9	15.8	15.9	33.8	31.9	32.9	12 x 10^4	11 x 10^4
T_4 - Seed soaking in PPFM @1:100 dilution for 18h	99	98	98.5	18.9	18.6	18.7	37.1	36.0	36.6	15 x 10^4	3 x 10^4
Mean	93.5	93.0		15.1	14.9		33.8	33.4			
	Treatment	Period		Treatment	Period		Treatment	Period			
SEd	1.0	0.7		0.3	0.2		0.4	0.3			
CD (P=0.05)	2.1	NS		0.6	NS		0.8	NS			

Table 5. Seed treatment with microbial consortia on seed germination, seedling vigour and microbial population in pearlmillet

Treatments	Seed germination and vigour			Microbial population (cfu g^{-1} of seed)		
	Germination (%)	Sprouts /day	Seedling length (cm)	*Azospirillum*	Phosphobacteria	PPFM
T_1 - Control	87	11.8	32.4	-	-	-
T_2 - Seed soaking in water for 18 h	94	15.2	32.0	-	-	-
T_3 - Seed soaking in *Azospirillum* @1:50 dilution for 18 h	93	13.5	35.2	60 $x10^4$	-	-
T_4 - Seed soaking in phosphobacteria @1:50 dilution for 18 h	94	16.1	34.5	-	12 x 10^4	-
T_5 - Seed soaking in PPFM @1:100 dilution for 18 h	99	18.7	36.8	-	-	15 x 10^4
T_6 - Seed soaking in *Azospirillum* @1:50 dilution + phosphobacteria @1:50 dilution (1:1) for 18 h	97	17.7	36.6	20 x 10^4	15 x 10^4	-
T_7 - Seed soaking in *Azospirillum* @1:50 dilution + PPFM @1:100 dilution (1:1) for 18 h	100	19.7	40.2	35 x 10^4	-	6 x 10^4
T_8 - Seed soaking in *Azospirillum* @1:50 dilution + phosphobacteria @1:50 dilution + PPFM @1:100 dilution (1:1:1) for 18 h	99	16.4	34.8	15 x 10^5	14 x 10^5	2 x 10^4
SEd	2.3	0.3	1.7			
CD (P=0.05)	5.1	0.7	3.8			

Table 6. Chemical treatment on seed germination and microbial population in bioinoculants infused pearlmillet seed

Treatments	Seed germination (%)	Sprouts/ day	Seedling length (cm)	Microbial population (cfu g^{-1} of seed)
T_1 - Control	87	11.9	33.7	-
T_2 - Seed soaking in *Azospirillum* @1:50 dilution for 18 h	92	15.2	35.4	5 x 10^4
T_3 - Seed soaking in *Azospirillum* @1:50 dilution for 18 h + polymer coating @ 5 ml kg^{-1} of seed	95	15.6	35.0	5 x 10^4
T_4 - Seed soaking in *Azospirillum* @1:50 dilution for 18 h + carbendazim treatment @ 2 g kg^{-1} of seed	96	16.1	36.2	2 x 10^4
T_5 - Seed soaking in *Azospirillum* @1:50 dilution for 18 h + polymer coating @ 5 ml + carbendazim treatment @ 2 g kg^{-1} of seed	96	16.1	36.1	3 x 10^4
T_6 - Seed soaking in phosphobacteria @1:50 dilution for 18 h	91	14.0	34.2	22 x 10^5
T_7 - Seed soaking in phosphobacteria @1:50 dilution for 18 h + polymer coating @ 5 ml kg^{-1} of seed	91	14.5	34.5	20 x 10^5
T_8 - Seed soaking in phosphobacteria @1:50 dilution for 18 h + carbendazim treatment @ 2 g kg^{-1} of seed	91	14.2	35.4	19 x 10^5
T_9 - Seed soaking in phosphobacteria @1:50 dilution for 18 h + polymer coating @ 5 ml + carbendazim treatment @ 2 g kg^{-1} of seed	92	15.0	35.4	20 x 10^5
T_{10} - Seed soaking in PPFM @1:100 dilution for 18 h	95	15.8	36.2	15 x 10^4
T_{11} - Seed soaking in PPFM @1:100 dilution for 18 h + polymer coating @ 5 ml kg^{-1} of seed	95	16.5	36.2	15 x 10^4
T_{12} - Seed soaking in PPFM @1:100 dilution for 18 h + carbendazim treatment @ 2 g kg^{-1} of seed	96	16.2	39.0	1 x 10^4
T_{13} - Seed soaking in PPFM @1:100 dilution for 18 h + polymer coating @ 5 ml + carbendazim treatment @ 2 g kg^{-1} of seed	96	17.4	40.4	8 x 10^4
SEd	2.3	0.7	1.1	
CD (P=0.05)	4.8	1.4	2.2	

promotion and therefore, co-inoculation of these bacteria as biofertilizers can result in viable agriculture practices.

3.2 Microbial Consortia on Seed Quality

Pearlmillet seeds soaked in microbial consortia have recorded the significant differences in germination and seedling vigour. Microbial culture combinations *viz*., *Azospirillum* @1:50 dilution and PPFM @1:100 dilution (1:1) for 18 h soaking treatment have registered the maximum germination (100%), speed of germination (19.7) and seedling length (40.2 cm) than the control (Table 5). However, combination of three cultures has showed decrease in speed of germination and seedling length. It might be due to the higher concentrations of the consortia. Similarly, the microbial population in the seed showed that the pearlmillet seeds were responded well for the introduction of the microbes. In this regard, all the microbes got through the seed in which *Azospirillum* and phosphobacteria population were more in the pearlmillet seed. Particularly, seed soaking in *Azospirillum* @1:50 and PPFM @1:100 dilutions (1:1) for 18 h has recorded the population of 35 x 10^4 and 6 x 10^4 cfu g^{-1} of seed respectively. Similar findings of co-inoculation of bacterial cultures on improvement in seed germination and seedling vigour were studied by many workers [8,19,28,29]. Qureshi et al. [8] 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. Also, combined inoculation of phosphate-solubilizing bacteria and *Azotobacter* exhibited beneficial effect on yield, as well as on nitrogen

and phosphorous storage in different crops [30, 31]. Raja and Sundaram [9] found that the PPFM inoculated with a diazotroph as individual and combined inoculant treatments has resulted in increased seedling vigor, dry matter production and yield.

3.3 Compatibility of Bioinoculants with Chemicals

Microbial infusion followed by chemicals seed treatment on seed quality showed that the seed soaking in PPFM liquid culture @1:100 dilution for 18 h followed by polymer coating @ 5 ml kg^{-1} along with carbendazim @ 2 g kg^{-1} of seed has recorded the higher germination (96%), speed of germination (17.4) and seedling length (40.4 cm) when compared with untreated control (Table 6). Likewise, the effect of seed treating chemicals on viability of microbes showed that the population of the different microbes was reduced drastically in the seeds treated with carbendazim fungicide. However, the polymer coating alone did not affect much on the microbes. Also, the combination of polymer and carbendazim coating has not affected greatly in the microbial population. Among the different microbes, PPFM population was much reduced (1×10^4 cfu g^{-1} of seed) when it contacted with carbendazim. Similar findings of the survival of the bioinoculants in the chemical treated seeds were studied earlier in many crops [29,32-35]. Inoculation of lentil and chickpea seeds with *Rhizobium* followed by carbendazim treatment gave significant decrease in foot and root rot incidence and increase in plant stand and grain yield [36]. Callaghan [37] opined that the 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.

4. CONCLUSION

It is concluded that the pearlmillet seeds treated with the liquid cultures *viz*., *Azospirillum*, phosphobacteria and PPFM has showed considerable enhancement in germination and seedling vigour. Among the cultures, PPFM has performed better compared with other cultures. In addition, microbial consortia consisting of *Azospirillum* and PPFM have recorded significant improvement in seed germination seedling vigour. Similarly, carbendazim treatment affected the microbial population in the seed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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