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Infusing microbial consortia for enhancing seed germination and vigour in pigeonpea (*Cajanus cajan* (L.) Millsp.)

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Use of plant growth promoting bacteria for seed treatment is in trend nowadays as it is beneficial to the plants and environment. But, carrier-based inoculants have short shelf life and difficult to use for large quantities of seed. Therefore, in the present study we used liquid microbial cultures for seed infusion in pigeonpea and assessed their compatibility with seed treating chemicals. The results showed that the soaking of pigeonpea seeds in liquid cultures like pink pigmented facultative methylotroph (PPFM) @ 1 : 100 dilution for 3 h or *Rhizobium* or phosphobacteria @ 1 : 50 dilution for 4 h have showed increased germination and vigour. In the microbial infused seeds, *Rhizobium* (13×10^4 cfu g^{-1} of seed) and phosphobacteria (20×10^4 cfu g^{-1} of seed) populations observed, were slightly reduced during three months storage. Nevertheless, the population was drastically reduced in PPFM (11×10^4 to 2×10^4 cfu g^{-1} of seed). Conversely, PPFM has performed better in seed quality enhancement amongst cultures. Also, consortia of *Rhizobium* @ 1 : 50 dilution + PPFM @ 1 : 100 dilution (1 : 1) for 3 h increased seed vigour with better microbial populations (14×10^4 and 2×10^4 cfu g^{-1} of seed). Also, seed infusion with PPFM liquid culture @ 1 : 100 dilution for 3 h followed by polymer coating @ 5 ml kg^{-1} + carbendazim treatment @ 2 g kg^{-1} of seed recorded increased germination and vigour with the PPFM population of 1×10^4 cfu g^{-1} of seed.

Keywords: Pigeonpea, PPFM, phosphobacteria, *Rhizobium*, seed germination, vigour.

SEED is an important input in agriculture and the quality of the seed alone contributes 20% yield increase. Quality of the seed can be improved by pre-sowing seed management techniques. Among the pre-sowing seed management techniques, seed treatment with the plant growth promoting bacteria (PGPB), viz. biofertilizers or biocontrol agents, is one of the important methods by which the yield can be improved by 5% to 30% (ref. 1). 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. Application of inoculum to the seeds of host plants is still in vogue with carrier-based bacterial inoculants[2]. Sometimes, in order to improve stickiness on the seed, adhesive is added[3]. However, carrier-based inoculants have a short shelf life, poor quality and the production and application procedure for most of these inoculants were found to be time consuming and difficult when used for large quantities of seed.

Alternatively, liquid inoculants were developed for seed treatment as they are easy to use, spread well, mix easily and need no additional water supply[4]. The liquid rhizobial inoculant for pea and lentils resulted in yield equal to or better than those obtained for the peat inoculant[5]. However, treating the pulses seed in liquid culture will lead to cracking injury which ultimately affects the storability. Therefore, care should be taken to treat the seed with liquid inoculants. Also, the fungicides are non-specific in their lethal action against the organisms. The responses of seed treating chemicals such as captan, thiram, mancozeb, ridomil, benlate and vitavax, etc. have been studied on the survival of *Rhizobium* and

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Bradyrhizobium inoculated seeds of some leguminous crops[6,7]. Therefore, our study was conducted to infuse the liquid microbial cultures for enhancing seed germination and vigour in pigeonpea and also to know the effect of seed treating chemicals on the survival of the inoculants.

Pigeonpea variety, CO(RG)9 seeds were collected from the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, India and dried well for the purpose of microbial treatments. The bacterial strains, viz. *Methylobacterium* (pink pigmented facultative methylotroph (PPFM)), *Rhizobium* and phosphobacteria were obtained from the Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai, India. The strains were cultured in NFb (nitrogen fixing bacteria) nutrient broth and ammonium mineral salts medium supplemented with 0.5% methanol. The liquid based bio-inoculant formulations were prepared for treating the seeds.

The liquid cultures were diluted at various concentrations, viz. 1 : 1, 1 : 10, 1 : 50 and 1 : 100 dilutions along with undiluted one. Then, the pigeonpea seeds were soaked in these culture concentrations at different time durations, viz. 2, 3, 4 and 5 h with half of the culture volume i.e. 1 : 1.5 ratio (v/v) to avoid soaking injury to the seeds caused by excess water. Later, the seeds were shade dried to the original moisture content. The germination test was conducted as per the ISTA[8] procedure and evaluated. The speed of germination was also assessed during the germination test[9]. Five randomly selected seedlings in each treatment were measured for their lengths and mean was arrived. After standardization of the concentrations, the freshly collected pigeonpea seeds were inoculated with liquid microbial cultures to assess the storability of the seeds as per the treatments, such as T_1 – control; T_2 – seed soaking in *Rhizobium* @ 1 : 50 dilution for 3 h; T_3 – seed soaking in phosphobacteria @ 1 : 50 dilution for 3 h and T_4 – seed soaking in PPFM @ 1 : 100 dilution for 3 h. The seeds were shade dried to the original moisture content and evaluated for their initial germination. The seeds were then packed in polythene bags and stored under ambient condition for three months. After the storage period, the seeds were evaluated for their viability and vigour. Also, microbial populations in the seeds during initial and after three months storage were assessed. For this, 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.

In another experiment, different microbial cultures, namely *Rhizobium*, phosphobacteria and PPFM were prepared and diluted as standardized in the earlier experiment like *Rhizobium* @ 1 : 50, phosphobacteria @ 1 : 50 and PPFM @ 1 : 100 concentrations. The microbial consortia were then prepared by mixing the different cultures at 1 : 1 or 1 : 1 : 1 ratio. The seeds were soaked in the microbial consortia uniformly for 3 h in half of the volume by following the treatment schedule, which was: T_1 – control; T_2 – seed soaking in water; T_3 – seed soaking in *Rhizobium* @ 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 *Rhizobium* @ 1 : 50 dilution + phosphobacteria @ 1 : 50 dilution (1 : 1); T_7 – seed soaking in *Rhizobium* @ 1 : 50 dilution + PPFM @ 1 : 100 dilution (1 : 1) and T_8 – seed soaking in *Rhizobium* @ 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, vigour and microbial populations were assessed.

In addition, the effect of seed treating chemicals on the survival of microbes in pigeonpea seeds were assessed by infusing them with different liquid microbial cultures for 3 h in half of the volume. These bio-inoculated seeds were shade dried to the original moisture content. Later, they were treated with different chemicals as per the following treatment schedule: T_1 – control; T_2 – seed soaking in *Rhizobium* @ 1 : 50 dilution; T_3 – seed soaking in *Rhizobium* @ 1 : 50 dilution + polymer coating @ 5 ml kg^{-1} of seed; T_4 – seed soaking in *Rhizobium* @ 1 : 50 dilution + carbendazim seed treatment @ 2 g kg^{-1} of seed; T_5 – seed soaking in *Rhizobium* @ 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 and 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 germination and vigour. 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. Later, they 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[10] and the critical difference values were calculated at 5% probability level.

The results obtained showed that the pigeonpea seeds soaked in liquid cultures displayed significant increase in germination and vigour. In case of PPFM, highest germination (98%) was recorded in the seed soaking treatment with 1 : 100 dilution for 3 h. However, seeds soaked in

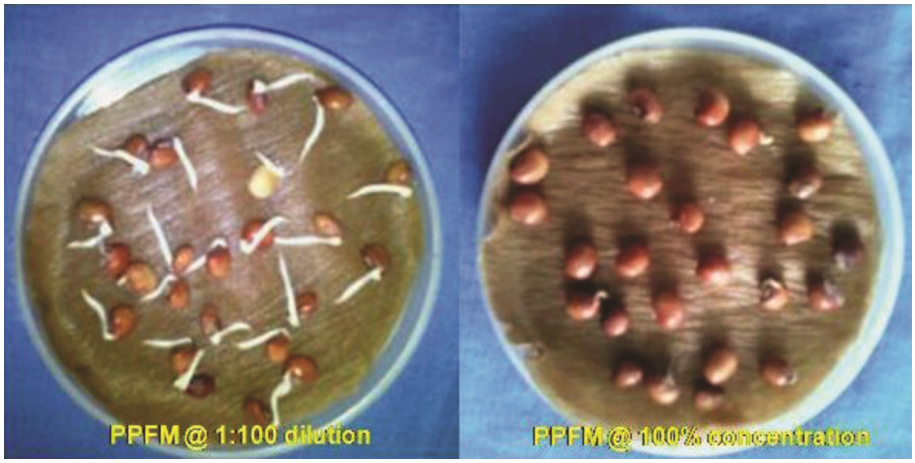

Figure 1. Effect of pink pigmented facultative methylotroph liquid culture on speed of seed germination in pigeonpea.

Table 1. Effect of seed infusion with pink pigmented facultative methylotroph (PPFM) liquid culture on germination and vigour in pigeonpea

	Seed germination (%)					Speed of germination					Seedling length (cm)				
Treatments	2 h	3 h	4 h	5 h	Mean	2 h	3 h	4 h	5 h	Mean	2 h	3 h	4 h	5 h	Mean
Untreated control	91	91	91	91	91.0	7.6	7.6	7.6	7.6	7.6	24.6	24.6	24.6	24.6	24.6
Seed soaking in water	86	92	90	88	89.0	5.6	7.2	6.8	8.8	8.1	22.9	24.7	26.0	24.7	24.6
Seed soaking in PPFM @ 100% concentration	62	76	76	74	72.0	8.2	7.3	5.5	6.9	6.9	31.3	27.6	24.8	22.8	26.6
Seed soaking in PPFM @ 1 : 1 dilution	80	82	72	72	76.5	8.2	7.7	6.7	7.5	7.5	32.0	30.7	33.8	30.3	31.7
Seed soaking in PPFM @ 1 : 10 dilution	88	84	79	72	80.8	8.1	9.8	9.8	9.0	9.2	32.5	31.4	33.6	30.2	31.9
Seed soaking in PPFM @ 1 : 50 dilution	97	86	84	86	88.3	9.6	10.9	10.2	10.2	10.2	32.5	33.5	32.3	30.8	32.2
Seed soaking in PPFM @ 1 : 100 dilution	97	98	94	90	94.6	12.3	13.3	13.3	10.2	12.3	32.1	35.3	32.6	31.0	32.7
Mean	85.9	86.9	83.7	81.7	84.6	8.5	9.1	8.5	8.6	8.7	29.7	29.7	29.7	28.0	29.2
	Treatment	Duration	T × D			Treatment	Duration	T × D			Treatment	Duration	T × D		
Standard error deviation (SEd)	1.9	1.5	3.9			0.03	0.02	0.05			1.2	0.9	2.4		
Critical difference (CD, $P = 0.05$)	3.9	2.9	7.9			0.05	0.04	0.10			2.5	NS	NS		

*NS, Nonsignificant.

undiluted (100% concentration) and the other diluted (1 : 1 and 1 : 10 dilutions) cultures showed drastic reduction in germination and even lesser than the control (Table 1). The extended period of soaking, viz. 4 and 5 h, too affected the germination in pigeonpea seeds. Speed of germination (13.3) and seedling length (35.3 cm) were also higher when the seeds were soaked in PPFM culture @ 1 : 100 dilution for 3 h when compared with control or undiluted culture (Figures 1 and 2). However, the higher culture concentrations and long soaking durations affected the seed vigour considerably. Similarly highest germination (100%), speed of germination (10.9) and seedling length (28.0 cm) were recorded in the treatment with 1 : 50 diluted *Rhizobium* liquid culture for 4 h (Table 2). The germination and vigour declined, whereas neither the culture concentration nor soaking period increased. Nevertheless, seeds soaked in undiluted culture for 5 h showed antagonistic effect on germination (67%). Likewise, the phosphobacteria treated seeds showed highest germination (100%), speed of germination (14.6) and seedling length (29.3 cm) at 1 : 50 dilution for 4 h (Table 3). The germination and vigour were affected at higher concentrations, i.e. undiluted and 1 : 1 diluted liquid cultures.

Among the cultures, PPFM performed better in increasing germination (98%), speed of germination (8.8) and seedling length (28.5 cm) at 1 : 100 dilution for 3 h (Table 4). Also, no significant differences were observed in the germination and seedling vigour during the three months storage of these treated seeds. But the reduction in microbial population was noticed irrespective of the inoculants during seed storage. Phosphobacteria recorded the highest population during initial (24×10^4 cfu g^{-1} of seed) as well as three months storage (20×10^4 cfu g^{-1} of seed). PPFM recorded the population between 11×10^4 and 2×10^4 cfu g^{-1} of seed during initial and three months storage respectively. The available seed moisture might have supported the viability of the microorganisms in the seed.

Table 2. Effect of seed infusion with *Rhizobium* liquid culture on germination and vigour in pigeonpea

Treatments	Seed germination (%)					Speed of germination					Seedling length (cm)				
	2 h	3 h	4 h	5 h	Mean	2 h	3 h	4 h	5 h	Mean	2 h	3 h	4 h	5 h	Mean
Untreated control	96	96	96	96	96.0	7.7	7.7	7.7	7.7	7.7	27.9	27.9	27.9	27.9	27.9
Seed soaking in water	94	86	86	88	88.5	7.8	8.1	8.5	8.5	8.2	26.5	22.0	23.7	19.4	22.9
Seed soaking in *Rhizobium* @ 100% concentration	92	84	84	67	81.8	8.5	8.1	7.9	8.1	8.1	22.4	25.8	26.3	23.7	24.5
Seed soaking in *Rhizobium* @ 1 : 1 dilution	88	90	86	80	86.0	8.0	8.3	8.4	8.6	8.3	24.3	25.2	23.0	23.3	23.9
Seed soaking in *Rhizobium* @ 1 : 10 dilution	94	92	94	92	93.0	8.7	8.5	8.2	7.2	8.2	25.8	23.2	26.0	26.5	25.3
Seed soaking in *Rhizobium* @ 1 : 50 dilution	86	86	100	98	92.5	8.7	9.7	10.9	10.2	9.8	27.5	24.8	28.0	27.0	26.8
Seed soaking in *Rhizobium* @ 1 : 100 dilution	92	94	98	98	95.5	9.2	8.8	9.8	10.4	9.5	26.2	26.9	23.2	22.8	24.7
Mean	91.7	89.7	92.0	88.4	90.8	8.3	8.5	8.8	8.7	8.5	25.8	25.1	25.4	24.3	25.1
	Treatment	Duration	T × D			Treatment	Duration	T × D			Treatment	Duration	T × D		
SEd	1.9	1.5	3.9			0.07	0.05	0.14			1.2	0.9	2.4		
CD ($P = 0.05$)	4.0	NS	8.0			0.14	0.10	0.28			2.4	1.8	4.9		

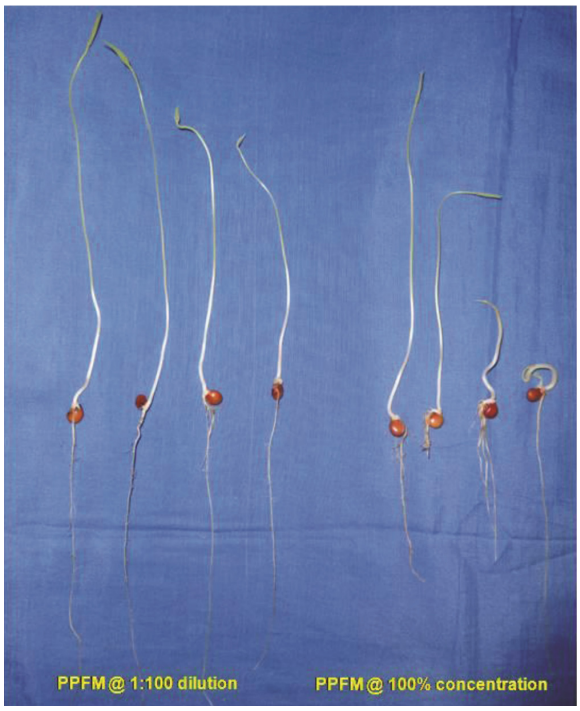

Figure 2. Effect of PPFM liquid culture on seedling vigour in pigeonpea.

Similarly, pigeonpea seeds showed positive response for the infusion of microbes as single or consortium cultures. Highest germination (98%) was found in seed soaking treatment with PPFM liquid culture @ 1 : 100 dilution for 3 h, which was on par with *Rhizobium* @ 1 : 50 dilution + PPFM @ 1 : 100 dilution (1 : 1) liquid cultures consortia (97%). Speed of germination (11.0) and seedling length (32.3 cm) were also higher in these consortia treated seeds (Table 5). Microbial populations, i.e. 14×10^4 and 2×10^4 cfu g^{-1} of seed were observed in *Rhizobium* and PPFM treated seeds which might have contributed for the enhanced germination and vigour.

In addition, pigeonpea seed treatment with microbial cultures and chemicals showed that the PPFM liquid culture @ 1 : 100 dilution for 3 h + polymer coating @ 5 ml kg^{-1} of seed + carbendazim seed treatment @ 2 g kg^{-1} of seed recorded increased germination (97%), speed of germination (10.2) and seedling length (29.7 cm) than the control (Table 6). With respect to microbial population, the above treatment recorded PPFM population of 2×10^4 cfu g^{-1} of seed. Among the treatments, seed treatment with phosphobacteria (24×10^4 cfu g^{-1} of seed) recorded the highest microbial population. Generally, polymer coating will not affect much the microbial population in the seed. However, the population was affected in the carbendazim treated seeds. Fortunately, polymer coating followed by carbendazim treatment recorded only a minimum reduction in the microbial population. This shows that the polymer coating acts as a barrier between the microbes and carbendazim.

Seed soaking in liquid microbial culture provides the benefit of penetration and survival in the seed. However, culture concentration and soaking duration are very important for getting the potential benefits. It was found that the PPFM had better synergistic effect on pigeonpea seed germination and seedling vigour among the cultures. The enhanced seed germination by seed coating or seed inoculum of methylotrophs has been recorded earlier[11,12]. Nkpwatt *et al.*[13] found that the cell-free supernatant of the *Methylobacterium* bacterial culture stimulated germination, suggesting the production of a growth-promoting agent by the methylotroph. PPFM mediate cytokinin on germinating seeds[14] and indole acetic acid (IAA) on increased seedling vigour[15]. Bakonyi *et al.*[16] opined that there was a positive effect of PGPB on germination and growth through reasons of excreting phytohormones and enhancement of nutrient mobilization from the seed.

Consortia can also be considered to deliver the seeds with different kinds of microbes into the fields. Qureshi *et al.*[17] found that the co-inoculation of *Rhizobium* and

Table 3. Effect of seed infusion with phosphobacteria liquid culture on germination and vigour in pigeonpea

Treatments	Seed germination (%)					Speed of germination					Seedling length (cm)				
	2 h	3 h	4 h	5 h	Mean	2 h	3 h	4 h	5 h	Mean	2 h	3 h	4 h	5 h	Mean
Untreated control	89	89	89	89	89.0	7.2	7.2	7.2	7.2	7.2	22.7	22.7	22.7	22.7	22.7
Seed soaking in water	90	88	88	93	89.8	12.0	12.0	12.5	10.8	11.8	22.0	25.1	22.9	18.8	22.2
Seed soaking in phosphobacteria @ 100% concentration	86	92	84	78	85.0	11.0	12.0	10.2	9.5	10.6	25.7	22.9	23.2	23.8	23.9
Seed soaking in phosphobacteria @ 1 : 1 dilution	80	79	81	78	80.0	11.6	11.1	11.6	8.4	10.6	28.1	24.1	26.5	25.1	25.9
Seed soaking in phosphobacteria @ 1 : 10 dilution	98	82	86	84	87.5	9.9	10.7	12.5	11.6	11.2	25.4	23.7	24.5	31.4	26.3
Seed soaking in phosphobacteria @ 1 : 50 dilution	90	96	100	94	95.0	12.5	12.4	14.6	10.6	12.5	22.8	27.3	29.3	27.7	26.8
Seed soaking in phosphobacteria @ 1 : 100 dilution	98	96	92	92	94.5	12.3	12.9	13.6	12.6	12.8	25.1	26.4	26.9	27.0	26.3
Mean	90.1	88.8	88.6	86.9	88.6	10.9	11.2	11.7	10.1	10.8	24.5	24.6	25.0	25.2	24.9
	Treatment		Duration		T × D	Treatment		Duration		T × D	Treatment		Duration		T × D
SEd	1.9		1.4		3.8	0.04		0.03		0.08	1.3		1.0		2.7
CD ($P = 0.05$)	3.9		NS		7.7	0.08		0.06		0.18	2.7		NS		NS

Table 4. Effect of seed infusion with liquid bio-inoculants on seed viability and microbial population in pigeonpea

Treatments	Seed germination (%)			Speed of germination			Seedling length (cm)			Microbial population (cfu g^{-1} of seed)	
	Initial	3 MAS	Mean	Initial	3 MAS	Mean	Initial	3 MAS	Mean	Initial	3 MAS
T_1 – Untreated control	85	84	84.5	6.8	7.7	7.3	23.9	23.5	23.7	–	–
T_2 – Seed soaking in *Rhizobium* liquid culture @ 1 : 50 dilution for 3 h	95	94	94.5	8.3	8.4	8.4	26.8	26.9	26.9	13×10^4	12×10^4
T_3 – Seed soaking in phosphobacteria liquid culture @ 1 : 50 dilution for 3 h	89	88	88.5	7.9	8.2	8.1	26.1	25.0	25.6	24×10^4	20×10^4
T_4 – Seed soaking in PPFM liquid culture @ 1 : 100 dilution for 3 h	98	96	97.0	8.9	8.7	8.8	28.7	28.3	28.5	11×10^4	2×10^4
Mean	91.8	90.5	91.1	7.9	8.3	8.2	26.4	25.9	26.2		
	Treatment		Period	Treatment		Period	Treatment		Period		
SEd	1.5		0.9	0.3		0.2	0.4		0.2		
CD ($P = 0.05$)	3.1		NS	0.6		NS	0.8		NS		

*MAS, Months after storage.

Bacillus sp. increased root length, root mass, number of nodules 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 vigour, dry matter production and yield which might be due to the increased rhizosphere population of the inoculants[18]. *Rhizobium* species besides N2-fixation, synthesizing growth hormones[19,20] and methylotroph mediating cytokinin[14] and IAA in the germinating seed[15], have been considered the most probable means of enhanced germination and vigour. Therefore, the present study offers a pathway to combine *Rhizobium* and PPFM for pigeonpea seeds in which PPFM plays a role of induction of growth hormones and *Rhizobium* in turn helpful for nodulation and N-fixation. However, compatibility of these microbes with seed protectants should also be considered during the seed delivery system. In this regard, the beneficial fungicide has showed antagonistic effect on the microbial populations in the pigeonpea seeds. Similarly, the survival of bio-inoculants in the chemical treated seeds was studied in many crops[6,7,21,22]. Khalequzzaman[23] opined that the inoculation of lentil and chickpea seeds with *Rhizobium* followed by bavistin treatment showed significant decrease in foot and root rot incidence and increase in plant stand and grain yield.

Table 5. Effect of seed infusion with microbial consortia on germination, vigour and microbial population in pigeonpea

Treatments	Seed germination and vigour			Microbial population (cfu g^{-1} of seed)		
	Germination (%)	Speed of germination	Seedling length (cm)	*Rhizobium*	Phosphobacteria	PPFM
T_1 – Control	85	6.7	25.2	–	–	–
T_2 – Seed soaking in water for 3 h	90	7.1	24.9	–	–	–
T_3 – Seed soaking in *Rhizobium* @ 1 : 50 dilution for 3 h	94	7.5	24.9	35×10^4	–	–
T_4 – Seed soaking in phosphobacteria @ 1 : 50 dilution for 3 h	86	6.9	27.0	–	11×10^4	–
T_5 – Seed soaking in PPFM @ 1 : 100 dilution for 3 h	98	8.7	26.5	–	–	2×10^5
T_6 – Seed soaking in *Rhizobium* @ 1 : 50 dilution + phosphobacteria @ 1 : 50 dilution (1 : 1) for 3 h	90	6.9	29.3	5×10^4	22×10^4	–
T_7 – Seed soaking in *Rhizobium* @ 1 : 50 dilution + PPFM @ 1 : 100 dilution (1 : 1) for 3 h	97	11.0	32.3	14×10^4	–	2×10^4
T_8 – Seed soaking in *Rhizobium* @ 1 : 50 dilution + phosphobacteria @ 1 : 50 dilution + PPFM @ 1 : 100 dilution (1 : 1 : 1) for 3 h	92	8.1	27.3	6×10^4	8×10^4	2×10^4
SEd	3.1	0.1	1.9			
CD ($P = 0.05$)	6.7	0.2	4.2			

Table 6. Effect of chemical treatment on germination and microbial population in bio-inoculants-infused pigeonpea seeds

Treatments	Seed germination (%)	Speed of germination	Seedling length (cm)	Microbial population (cfu g^{-1} of seed)
T_1 – Control	87	6.7	23.8	–
T_2 – Seed soaking in *Rhizobium* @ 1 : 50 dilution for 3 h	90	9.1	26.9	13×10^4
T_3 – Seed soaking in *Rhizobium* @ 1 : 50 dilution for 3 h + polymer coating @ 5 ml kg^{-1} of seed	92	9.0	26.8	10×10^4
T_4 – Seed soaking in *Rhizobium* @ 1 : 50 dilution for 3 h + carbendazim treatment @ 2 g kg^{-1} of seed	93	8.6	26.9	1×10^4
T_5 – Seed soaking in *Rhizobium* @ 1 : 50 dilution for 3 h + polymer coating @ 5 ml + carbendazim treatment @ 2 g kg^{-1} of seed	93	8.8	27.9	6×10^4
T_6 – Seed soaking in phosphobacteria @ 1 : 50 dilution for 3 h	91	6.9	25.8	24×10^4
T_7 – Seed soaking in phosphobacteria @ 1 : 50 dilution for 3 h + polymer coating @ 5 ml kg^{-1} of seed	90	8.3	27.1	18×10^4
T_8 – Seed soaking in phosphobacteria @ 1 : 50 dilution for 3 h + carbendazim treatment @ 2 g kg^{-1} of seed	92	8.2	27.8	5×10^3
T_9 – Seed soaking in phosphobacteria @ 1 : 50 dilution for 3 h + polymer coating @ 5 ml + carbendazim treatment @ 2 g kg^{-1} of seed	94	8.4	28.2	15×10^4
T_{10} – Seed soaking in PPFM @ 1 : 100 dilution for 3 h	92	9.1	27.9	11×10^4
T_{11} – Seed soaking in PPFM @ 1 : 100 dilution for 3 h + polymer coating @ 5 ml kg^{-1} of seed	95	9.6	29.1	9×10^4
T_{12} – Seed soaking in PPFM @ 1 : 100 dilution for 3 h + carbendazim treatment @ 2 g kg^{-1} of seed	94	9.8	29.1	1×10^4
T_{13} – Seed soaking in PPFM @ 1 : 100 dilution for 3 h + polymer coating @ 5 ml + carbendazim treatment @ 2 g kg^{-1} of seed	97	10.2	29.7	2×10^4
SEd	2.8	0.6	0.8	
CD ($P = 0.05$)	5.9	1.3	1.7	

Therefore, it is concluded that the seed germination and vigour in pigeonpea seeds can be increased through infusion of liquid microbial inoculants, viz. PPFM, *Rhizobium* and phosphobacteria, provided, the concentration and soaking duration are taken care of. Among the bio-inoculants, PPFM performed better in the germination and vigour improvement as single or co-inoculant with *Rhizobium*. Carbendazim treatment on the bio-inoculant-infused seeds showed reduction in the microbial population than the polymer treated seeds.

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