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 Methylo troph (PPFM) @ 1:100 dilution for 18 h have recorded higher germination and seedling vigour. Pink Pigmented Facultative Methylo troph 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 carbenzadim 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 5.5x10⁵ cfu seed⁻¹ on black gram, 10.5x10⁴ cfu seed⁻¹ on soybean and 6.5x10⁵ 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 Methylo troph (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...
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., T1 - control; T2 - seed soaking in water; T3 - seed soaking in Azospirillum @1:50 dilution; T4 - seed soaking in phosphobacteria @1:50 dilution; T5 - seed soaking in PPFM @1:100 dilution; T6 - seed soaking in Azospirillum @1:50 dilution + phosphobacteria @1:50 dilution (1:1); T7 - seed soaking in Azospirillum @1:50 dilution + PPFM @1:100 dilution (1:1); T8 - 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., T1 - control; T2 - seed soaking in Azospirillum @1:50 dilution; T3 - seed soaking in Azospirillum @1:50 dilution + polymer coating @ 5 ml kg\(^{-1}\) of seed; T4 - seed soaking in Azospirillum @1:50 dilution + carbendazim seed treatment @ 2 g kg\(^{-1}\) of seed; T5 - seed soaking in Azospirillum @1:50 dilution + polymer coating @ 5 ml kg\(^{-1}\) of seed + carbendazim seed treatment @ 2 g kg\(^{-1}\) of seed; T6 - seed soaking in phosphobacteria @1:50 dilution; T7 - seed soaking in phosphobacteria @1:50 dilution + polymer coating @ 5 ml kg\(^{-1}\) of seed; T8 - seed soaking in phosphobacteria @1:50 dilution + carbendazim seed treatment @ 2 g kg\(^{-1}\) of seed; T9 - seed soaking in phosphobacteria @1:50 dilution + polymer coating @ 5 ml kg\(^{-1}\) of seed + carbendazim seed treatment @ 2 g kg\(^{-1}\) of seed; T10 - seed soaking in PPFM @1:100 dilution; T11 - seed soaking in PPFM @1:100 dilution + carbendazim seed treatment @ 2 g kg\(^{-1}\) of seed; T12 - seed soaking in PPFM @1:100 dilution + polymer coating @ 5 ml kg\(^{-1}\) of seed + carbendazim seed treatment @ 2 g kg\(^{-1}\) of seed; T13 - seed soaking in PPFM @1:100 dilution + 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 methylbacteria 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 |
|----------------|------|------|------|------|------|------|------|------|------|------|------|------|
| SEd Treatment Duration | T x D | Treatment Duration | T x D | Treatment Duration | T x D | Treatment Duration | T x D |
| 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 |

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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   | 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 @ 1:10 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 | 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 |

| SEd                                      | Treatment | 2.4 | Duration | 1.8 | T x D | 4.9 | Treatment | 0.04 | Duration | 0.02 | T x D | 0.07 |
|                                         |           |     |          |     |       |     |           |      |          |      |       |      |
| CD (P=0.05)                              | Treatment | 5.0 | Duration | 3.8 | 10.0 | 0.07 | Treatment | 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 | 8.8 | 14.5| 14.5| 14.5 | 31.1| 31.1| 31.1| 31.1| 31.1 |
| Seed soaking in PPFM @ 100% concentration | 44   | 16   | 22   | 6    | 22.0 | 6.2 | 3.6 | 3.0 | 1.0 | 3.5 | 30.8| 26.8| 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 | 61.7 | 10.2| 10.0 | 9.3 | 7.4 | 30.4 | 30.6| 30.4| 29.0 |      |      |
| SEM                            | 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 | Mean |
| T₁ - Untreated Control                         | 86        | 86   | 86.0 | 11.9    | 12.2 | 12.1 | 30.6    | 31.1 | 30.8 | -       | -    |       |
| T₂ - 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 10⁴ | 4 x 10⁴ |
| T₃ - 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 10⁴ | 11 x 10⁴ |
| T₄ - 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 10⁴ | 3 x 10⁴ |
| Mean                                           | 93.5      | 93.0 | 15.1 | 14.9    | 33.8 | 33.4 |                      |       |       |                      |       |       |

| SEd Treatment | 1.0 | 0.7 | 0.3 | 0.2 | 0.4 | 0.3 |
| SEd Period    | 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 |
| T₁ - Control                                                              | 87             | 11.8        | 32.4               | -            | -              | -    |
| T₂ - Seed soaking in water for 18 h                                       | 94             | 15.2        | 32.0               | -            | -              | -    |
| T₃ - Seed soaking in Azospirillum @1:50 dilution for 18 h                  | 93             | 13.5        | 35.2               | 60 x 10⁴     | -              | -    |
| T₄ - Seed soaking in phosphobacteria @1:50 dilution for 18 h              | 94             | 16.1        | 34.5               | -            | 12 x 10⁴      | -    |
| T₅ - Seed soaking in PPFM @1:100 dilution for 18 h                        | 99             | 18.7        | 36.8               | -            | -              | 15 x 10⁶ |
| T₆ - Seed soaking in Azospirillum @1:50 dilution + phosphobacteria @1:50 dilution (1:1) for 18 h | 97 | 17.7 | 36.6 | 20 x 10⁴ | 15 x 10⁴ | - |
| T₇ - Seed soaking in Azospirillum @1:50 dilution + PPFM @1:100 dilution (1:1) for 18 h | 100 | 19.7 | 40.2 | 35 x 10⁶ | - | 6 x 10⁶ |
| T₈ - 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 10⁵ | 14 x 10⁵ | 2 x 10⁶ |
| SEd                                                                        | 2.3            | 0.3         | 1.7                |              |              |      |
| CD (P=0.05)                                                               | 5.1            | 0.7         | 3.8                |              |              |      |
3.2 Microbial Consortia on Seed Quality

Similarly, the microbial population in the seed to the higher concentrations of the consortia. It might be due cultures has showed decrease in speed of (Table 5). However, combination of three and seedling length (40.2 cm) that 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

### 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) |
|------------------------------------------------|----------------------|-------------|----------------------|----------------------------------------|
| T1 - Control                                    | 87                   | 11.9        | 33.7                 | -                                      |
| T2 - Seed soaking in Azospirillum @1:50 dilution for 18 h | 92                   | 15.2        | 35.4                 | 5 x 10^4                              |
| T3 - 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                              |
| T4 - Seed soaking in Azospirillum @1:50 dilution for 18 h + carbenzadim treatment @ 2 g kg^-1 of seed | 96                   | 16.1        | 36.2                 | 2 x 10^4                              |
| T5 - Seed soaking in Azospirillum @1:50 dilution for 18 h + polymer coating @ 5 ml + carbenzadim treatment @ 2 g kg^-1 of seed | 96                   | 16.1        | 36.1                 | 3 x 10^4                              |
| T6 - Seed soaking in phosphobacteria @1:50 dilution for 18 h | 91                   | 14.0        | 34.2                 | 22 x 10^5                             |
| T7 - 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                             |
| T8 - Seed soaking in phosphobacteria @1:50 dilution for 18 h + carbenzadim treatment @ 2 g kg^-1 of seed | 91                   | 14.2        | 35.4                 | 19 x 10^5                             |
| T9 - Seed soaking in phosphobacteria @1:50 dilution for 18 h + polymer coating @ 5 ml + carbenzadim treatment @ 2 g kg^-1 of seed | 92                   | 15.0        | 35.4                 | 20 x 10^5                             |
| T10 - Seed soaking in PPFM @1:100 dilution for 18 h | 95                   | 15.8        | 36.2                 | 15 x 10^4                             |
| T11 - 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                             |
| T12 - Seed soaking in PPFM @1:100 dilution for 18 h + carbenzadim treatment @ 2 g kg^-1 of seed | 96                   | 16.2        | 39.0                 | 1 x 10^4                              |
| T13 - Seed soaking in PPFM @1:100 dilution for 18 h + polymer coating @ 5 ml + carbenzadim 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

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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 vigour, 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 has recorded the higher germination (96%), speed of germination (17.4) and seedling length (40.4 cm) when compared with untreated control (Table 6).

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 pearl millet 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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