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ASSESSMENT OF VIABILITY RETENTION OF LIQUID BACTERIAL INOCULANTS AND ITS IMPACT ON YIELD

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SUMMARY

Bacterial inoculants play a crucial role in enhancing agricultural productivity by facilitating nutrient uptake. However, the viability retention of these inoculants in liquid formulations remains a critical concern. The present study was carried out to investigate the viability retention of liquid bacterial inoculants (LBI) of *Azotobacter* sp., *Sphingobacterium* sp., *Stenotrophomonas maltophilia* and *Burkholderia seminalis* under different storage conditions. It was recorded that all the bacterial inoculants amended with 5mM trehalose remained viable upto a storage period of six months. A field experiment of forage pearl millet was conducted during *kharif* 2020 at Punjab Agricultural University, Ludhiana and Punjab Agricultural University, Regional Research Station, Bathinda involving combinations of LBI alongwith 100% recommended dose of fertilizer (RDF). It was observed that the treatment consisting of *B. seminalis* and *S. maltophilia* alongwith RDF yielded the highest green fodder and dry matter yield with percentage increase of 13.05 and 9.8 respectively, compared to the control. This research underscores the importance of optimizing storage conditions to maintain inoculant viability and highlights the pivotal role of viable bacterial populations in maximizing agricultural productivity and sustainability.

Key words: *Azotobacter* sp., *Burkholderia seminalis*, forage pearl millet, liquid bacterial inoculants, *Sphingobacterium* sp., *Stenotrophomonas maltophilia*

Bacterial inoculant technology has emerged as a potential tool to enhance the productivity of agricultural systems alongwith crop nutrition and protection. The major purpose of the bacterial inoculant formulation is to offer more suitable microhabitat for survival in the soil ecosystem (Meenakumari *et al.,* 2008). A suitable carrier is imperative in the bioformulation. It acts as a delivery vehicle employed for transferring live microorganism from industrial fermentor to rhizosphere of plant. Therefore, for the good-quality inoculant an outstanding carrier material should be used (Sahu and Brahmaprakash 2016). Some of the commonly used carrier materials in the production of good-quality bioformulations are neutralized peat soil/lignite, vermiculite, charcoal, press mud, farmyard manure, and soil mixture. However, there are certain drawbacks associated with these carriers such as lower shelf-life, temperature sensitivity, contamination prone, and low cell counts (Thomas and Singh, 2019). Liquid bacterial inoculants are attractive alternatives as the inoculants consist of desired microbes as well as its nutrients. These may also contain special cell protectants or substances that encourage the formation of resting spores or cysts for longer shelf life (Chandra *et al.,* 2005). The advantages of liquid bacterial inoculants over the powder based inoculants are improved shelf stability upto 2 years, resistant to high temperature, high bacterial count and better survival on seeds and soil.

In the present investigation, shelf stability of liquid bacterial inoculants of the liquid inoculants of *Azotobacter* sp., *Sphingobacterium* sp., *Stenotrophomonas maltophilia* and *Burkholderia seminalis* was studied at room and refrigerated temperature and its impact on productivity of forage pearl millet was evaluated.

MATERIALS AND METHODS

Liquid bacterial inoculants preparation

For the preparation of liquid bacterial inoculants of *Azotobacter* sp., *Sphingobacterium* sp., *S. maltophilia* and *B. seminalis,* each test culture was transferred at 5% to each two hundred fifty ml (250 ml) flask containing hundred ml (100 ml) of sterilized standardized growth medium ammended with 5 mM trehalose (Ramya 2019) respectively A two hundred fifty ml (250 ml) flask containing hundred ml (100 ml) of sterilized basal medium without any additive and inoculated with respective pure culture was used as control for each inoculant. The inoculated flasks were kept on shaker at 28 ± 1°C for 24 hours. They were then transferred into the sterilized hundred ml (100 ml) capacity plastic vials under aseptic conditions.

Viability retention of liquid bacterial inoculants

The plastic vials containing liquid bacterial inoculants of *Azotobacter* sp., *Sphingobacterium* sp., *S. maltophilia* and *B. seminalis* were kept at room temperature and in refrigerator respectively. One ml (1 ml) of each sample was drawn aseptically at 0th, 30th, 60th, 90th, 120th, 150th and 180th day for total viable count by serial dilution spread plate method and incubated at 28±2°C for three days.

Preparation of charcoal carrier based bacterial inoculants

The charcoal carrier was sterilized by the tyndalization process in an autoclave at 15 psi pressure and at temperature of 121 °C for 20 minutes, three times on three succeeding days. The charcoal carrier based formulations were prepared by mixing the broth cultures of respective bacterial culture with charcoal powder at 1: 2.5 ratio. Broth cultures were prepared by inoculating loopful inoculums of pure cultures of *Azotobacter* sp., *Sphingobacterium* sp., *S. maltophilia* and *B. seminalis* in sterilized two hundred ml (200 ml) of nutrient broth dispensed in five hundred ml (500 ml) flask respectively. The total viable count in each charcoal carrier based inoculants was determined by adding ten grams (10 g) of inoculant to ninety ml (90 ml) of sterilized distilled water and making a ten-fold dilution series. Then, one ml (1 ml) aliquots of the appropriate dilutions were spread plated on nutrient agar medium aseptically at 0th, 30th, 60th, 90th, 120th, 150th and 180th day and incubated at 28±2°C for three days.

Periodic assessment of PGP features of bacterial cultures isolated from liquid bacterial inoculants

The bacterial culture was re-isolated from each liquid bacterial inoculant (fresh as well as six month old) and analysed for the PGP (Plant Growth Promoting) traits at optimum temperature i.e 40°C at 0 and 180 days of storage. The PGP traits studied were indole acetic acid production (Gordon and Weber 1951), siderophore production (Arnow 1937 and Csaky 1948), phosphate solubilisation (Jackson 1973), ACC deaminase production (Govindasamy *et al* 2008), gibberellic acid production (Borrow *et al* 1965) and ammonia production (Dye 1962) were determined using the standardized procedures.

Experimental details

The field experiment was conducted at Punjab Agricultural University, Regional Research Station, Bathinda and Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during *kharif* 2020.The experiment was laid out in a randomised complete block design (RCBD) with 12 treatments and replicated thrice at both the locations i.e Bathinda and Ludhiana respectively. The different treatments were as follows: T_1: RDF; T_2: RDF + *Azotobacter* sp.; T_3: RDF + *Burkholderia seminalis*; T_4: RDF + *Stenotrophomonas maltophilia*; T_5: RDF + *Sphingobacterium* sp.; T_6: RDF + *Azotobacter* sp. + *Burkholderia seminalis*; T_7: RDF + *Azotobacter* sp. + *Stenotrophomonas maltophilia*; T_8: RDF + *Azotobacter* sp. + *Sphingobacterium* sp.; T_9: RDF + *Burkholderia seminalis* + *Stenotrophomonas maltophilia*; T_{10}: RDF + *Burkholderia seminalis* + *Sphingobacterium* sp.; T_{11}: RDF + *Stenotrophomonas maltophilia* + *Sphingobacterium* sp. and T_{12}: RDF + Consortium (Commercially available biofertilizer from the Department of Microbiology, Punjab Agricultural University, Ludhiana).

The land preparations were done mechanically with proper care to avoid mixing of soil from adjacent plots. The pearl millet cultivar 'FBC-16' was sown at the rate of 8 kg/acre. Pearl millet seeds were inoculated with liquid microbial inoculants of *Azotobacter* sp., *Sphingobacterium* sp., *S. maltophilia* and *B. seminalis* as per treatments @ 100 ml/acre. Inoculated seeds were air dried in shade and planted within 2 h. Weeding

and hoeing was done so as to avoid weeds and appropriate control measures were taken to prevent insects and pests. Other cultural operations and plant protection measures were followed as per the recommendations.

Crop Productivity

Green fodder yield was recorded by weighing fresh weight of the pearl millet fodder per net plot at harvest and the dry matter yield was calculated based on dry matter content obtained by drying known quantity of green forage from each plot in hot air oven at 70°C.

Statistical Analysis

The data was statistically analysed using IBM SPSS Statistics V22.0 (2013) by the Duncan's Multiple Range Test (DMRT) for comparison of mean values and student's t-test for comparison of PGP traits. The data related to the influence of liquid bacterial inoculants on yield was statistically analyzed using randomized complete block design (RCBD) and the significance of difference was tested at five per cent (5%) level of probability.

RESULTS

Periodic assessment of viability retention of liquid bacterial inoculants

The data regarding the survival of *Azotobacter* sp., Sphingobacterium sp., *Burkholderia seminalis* and *Stenotrophomonas maltophilia* in the liquid bacterial inoculant (LBI) amended with 5mM trehalose, charcoal carrier based inoculant and control without 5mM trehalose at room temperature for the period of 180 days has been represented in the Table 1. The maximum viable count of each bacterium during storage period of 180 days was observed in liquid bacterial inoculant amended with 5mM trehalose followed by charcoal carrier based and liquid inoculant without 5mM trehalose respectively. However, statistically significant reduction was observed during storage period of 180 days. Nevertheless, total bacterial inoculant in LBI amended with trehalose was according to BIS guidelines which state that the minimum CFU of $5x10^7$cells/gram of powder, granules or carrier material or $1x10^8$cells/ml of liquid should be maintained.

The initial viable count in the liquid inoculant amended with 5mM trehalose first increased and then gradually reduced during the storage period of 180 days. At the end of the study period, the maximum viable count was observed in LBI of *Sphingobacterium* sp. *i.e.* 8.97 log_{10} no. of cells, followed by LBI of *Azotobacter* sp. (8.86 log_{10} no. of cells), *S. maltophilia* (8.66 log_{10} no. of cells) and *B. seminalis* (8.57 log10 no. of cells), respectively. Statistically, the survivability of the cells at 0 and 180th day was significantly different from each other as well as from the rest of the storage days.

Under refrigerated conditions also, the maximum viability was recorded in the liquid bacterial inoculant amended with 5mM trehalose followed by charcoal carrier based and control without 5mM trehalose (Table 2). It was recorded that the survivability of the bacterial inoculants was higher in refrigerated conditions as compared to room temperature.

Periodic assessment of PGP features of bacterial cultures isolated from the liquid bacterial inoculants

The bacterial cultures were re-isolated from the liquid bacterial inoculants amended with trehalose at 0 day and 180 day to evaluate the retention of PGP traits of the bacterial cultures at the end of the storage period. These traits were studied at 40°C and the data regarding the same has been demonstrated in the Table 3.

Productivity

Green fodder yield

Data pertaining to the pooled analysis of green fodder yield at both the locations has been presented in the Table 4. The green fodder yield of forage pearl millet increased non-significantly through the application of liquid bacterial inoculants over control at both the locations, Bathinda and Ludhiana. The maximum yield was obtained by the treatment T_9 (489.31 q/ha) and the minimum by T_1 (432.82 q/ha). Elevation in the green fodder yield was observed in each treatment with the use of liquid microbial inoculants, irrespective of the treatment applied over T_1. The percentage increase of 13.05 percent was observed with the treatment T_9 over T_1. Thereupon, application of liquid microbial inoculants was found promising in improving the green fodder yield over control.

TABLE 1

Survival of *Azotobacter sp., Sphingobacterium sp., Stenotrophomonas maltophilia* and *Burkholderia seminalis* in the liquid bacterial inoculants at room temperature

Days	Survival of cells upto 6 months (log_{10} no. of cells/ml)											
	Treatments											
	AT_1	AT_2	AT_3	ST_1	ST_2	ST_3	SMT_1	SMT_2	SMT_3	BST_1	BST_2	BST_3
0	10.45±0.05^{b}	10.28±0.02^{a}	10.11±0.02^{a}	10.40±0.04^{b}	10.27±0.14^{a}	10.46±0.08^{a}	10.22±0.03^{ab}	10.18±0.07^{a}	9.90±0.17^{a}	10.54±0.05^{b}	9.28±0.11^{a}	10.29±0.01^{a}
30	10.75±0.09^{a}	10.63±0.03^{a}	10.04±0.01^{a}	10.88±0.04^{a}	10.58±0.01^{a}	9.42±0.22^{b}	10.41±0.02^{a}	10.22±0.04^{a}	9.57±0.19^{a}	10.86±0.05^{a}	9.25±0.04^{a}	10.06±0.03^{a}
60	9.84±0.04^{c}	9.40±0.21^{b}	9.55±0.03^{b}	10.34±0.05^{b}	9.59±0.12^{b}	8.26±0.09^{c}	9.99±0.05^{bc}	8.93±0.01^{b}	8.64±0.07^{b}	10.10±0.06^{c}	8.45±0.16^{b}	8.35±0.08^{b}
90	9.75±0.04^{c}	9.32±0.14^{b}	8.46±0.16^{c}	9.94±0.03^{c}	8.78±0.03^{c}	7.59±0.04^{d}	9.94±0.04^{bc}	8.62±0.06^{b}	7.31±0.13^{c}	9.97±0.02^{c}	8.31±0.07^{b}	7.37±0.11^{c}
120	9.32±0.05^{d}	8.37±0.09^{c}	7.56±0.06^{d}	9.63±0.05^{d}	8.37±0.09^{c}	6.48±0.03^{e}	9.90±0.17^{c}	7.43±0.11^{c}	6.49±0.16^{d}	9.73±0.03^{d}	8.15±0.05^{b}	6.48±0.16^{d}
150	8.96±0.07^{e}	8.20±0.06^{c}	5.40±0.11^{e}	9.06±0.05^{e}	7.66±0.20^{d}	5.67±0.12^{f}	9.05±0.04^{d}	7.36±0.09^{c}	6.23±0.03^{d}	8.96±0.04^{e}	7.70±0.10^{c}	5.31±0.13^{e}
180	8.86±0.03^{e}	7.48±0.13^{d}	3.27±0.10^{f}	8.97±0.07^{e}	7.41±0.11^{d}	4.24±0.09^{g}	8.66±0.05^{e}	7.31±0.13^{c}	3.24±0.07^{e}	8.57±0.04^{f}	7.33±0.14^{c}	4.25±0.04^{f}

The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a>b>c.

Note

AT_1: Liquid bacterial inoculant of *Azotobacter* sp. with 5 mM Trehalose

AT_2: Charcoal carrier based inoculant of *Azotobacter* sp.

AT_3: Liquid bacterial inoculant of *Azotobacter* sp. without 5 mM Trehalose

ST_1: Liquid bacterial inoculant of *Sphingobacterium* sp. with 5 mM Trehalose

ST_2: Charcoal carrier based inoculant of *Sphingobacterium* sp.

ST_3: Liquid bacterial inoculant of *Sphingobacterium* sp. without 5 mM Trehalose

SMT_1: Liquid bacterial inoculant of *S. maltophilia* with 5 mM Trehalose

SMT_2: Charcoal carrier based inoculant of *S. maltophilia*

SMT_3: Liquid bacterial inoculant of *S.maltophilia* without 5 mM Trehalose

BST_1: Liquid bacterial inoculant of *B. seminalis* with 5 mM Trehalose

BST_2: Charcoal carrier based inoculant of *B. seminalis*

BST_3: Liquid bacterial inoculant of *B. seminalis* without 5 mM Trehalose

TABLE 2

Survival of *Azotobacter sp., Sphingobacterium sp., Stenotrophomonas maltophilia* and *Burkholderia seminalis* in the liquid bacterial inoculants at refrigerated temperature

Days	Survival of cells upto 6 months ($\log_{10}$ no. of cells/ml)											
	Treatments											
	AT_1	AT_2	AT_3	ST_1	ST_2	ST_3	SMT_1	SMT_2	SMT_3	BST_1	BST_2	BST_3
0	10.28 ± 0.07^{b}	10.19 ± 0.07^{a}	10.24 ± 0.03^{a}	10.16 ± 0.06^{bc}	10.14 ± 0.50^{a}	10.12 ± 0.02^{a}	10.32 ± 0.14^{ab}	10.23 ± 0.03^{a}	10.19 ± 0.02^{a}	10.27 ± 0.14^{ab}	10.23 ± 0.13^{a}	10.14 ± 0.03^{a}
30	10.40 ± 0.09^{ab}	10.20 ± 0.02^{a}	10.11 ± 0.03^{a}	10.47 ± 0.07^{ab}	10.20 ± 0.02^{a}	10.10 ± 0.03^{a}	10.48 ± 0.06^{ab}	10.24 ± 0.04^{a}	9.67 ± 0.11^{b}	10.51 ± 0.07^{a}	10.33 ± 0.10^{a}	10.10 ± 0.03^{a}
60	10.52 ± 0.04^{a}	10.29 ± 0.01^{a}	9.65 ± 0.13^{b}	10.54 ± 0.11^{a}	10.26 ± 0.03^{a}	10.07 ± 0.03^{a}	10.58 ± 0.02^{a}	10.32 ± 0.06^{a}	8.54 ± 0.04^{c}	10.55 ± 0.04^{a}	10.34 ± 0.10^{a}	9.52 ± 0.05^{b}
90	10.24 ± 0.04^{b}	9.74 ± 0.11^{b}	9.42 ± 0.11^{b}	10.41 ± 0.09^{ab}	10.05 ± 0.03^{a}	9.54 ± 0.04^{b}	10.27 ± 0.15^{ab}	9.73 ± 0.13^{b}	8.25 ± 0.05^{c}	10.15 ± 0.05^{b}	10.20 ± 0.16^{a}	9.24 ± 0.04^{c}
120	10.21 ± 0.02^{b}	9.69 ± 0.08^{b}	7.75 ± 0.11^{c}	10.35 ± 0.12^{ab}	9.79 ± 0.12^{b}	8.63 ± 0.10^{c}	10.16 ± 0.04^{bc}	9.65 ± 0.08^{b}	7.41 ± 0.12^{d}	9.83 ± 0.08^{c}	9.96 ± 0.10^{ab}	7.70 ± 0.03^{d}
150	9.85 ± 0.03^{c}	9.45 ± 0.08^{bc}	6.31 ± 0.07^{d}	9.85 ± 0.05^{cd}	9.75 ± 0.05^{b}	7.63 ± 0.06^{d}	9.85 ± 0.01^{cd}	9.48 ± 0.06^{b}	6.53 ± 0.06^{e}	9.77 ± 0.04^{c}	9.58 ± 0.05^{bc}	7.53 ± 0.04^{d}
180	9.54 ± 0.02^{d}	9.20 ± 0.06^{c}	5.21 ± 0.06^{e}	9.79 ± 0.02^{d}	9.51 ± 0.05^{c}	6.32 ± 0.07^{e}	9.67 ± 0.10^{d}	8.63 ± 0.10^{c}	4.31 ± 0.14^{f}	9.63 ± 0.02^{c}	9.41 ± 0.03^{c}	6.25 ± 0.09^{e}

The values of the experiment are means of three replications ± standard error. Means with the same letter down the column are not significantly different based on Duncan's multiple range test ($p \leq 0.05$) n=3, a>b>c.

Note

AT_1: Liquid bacterial inoculant of *Azotobacter* sp. with 5 mM Trehalose
AT_2: Charcoal carrier based inoculant of *Azotobacter* sp.
AT_3: Liquid bacterial inoculant of *Azotobacter* sp. without 5 mM Trehalose
ST_1: Liquid bacterial inoculant of *Sphingobacterium* sp. with 5 mM Trehalose
ST_2: Charcoal carrier based inoculant of *Sphingobacterium* sp.
ST_3: Liquid bacterial inoculant of *Sphingobacterium* sp. without 5 mM Trehalose
SMT_1: Liquid bacterial inoculant of *S. maltophilia* with 5 mM Trehalose
SMT_2: Charcoal carrier based inoculant of *S. maltophilia*
SMT_3: Liquid bacterial inoculant of *S. maltophilia* without 5 mM Trehalose
BST_1: Liquid bacterial inoculant of *B. seminalis* with 5 mM Trehalose
BST_2: Charcoal carrier based inoculant of *B. seminalis*
BST_3: Liquid bacterial inoculant of *B. seminalis* without 5 mM Trehalose

TABLE 3

Periodic assessment of PGP features of bacterial cultures isolated from the liquid bacterial inoculants

Potential Cultures	*Azotobacter* sp.			*Sphingobacterium* sp			*S. maltophilia*			*B. seminalis*		
PGP Traits	Fresh Liquid Inoculant	Six-Month Old Liquid Inoculant	t-test @5%	Fresh Liquid Inoculant	Six-Month Old Liquid Inoculant	t-test @5%	Fresh Liquid Inoculant	Six-Month Old Liquid Inoculant	t-test @5%	Fresh Liquid Inoculant	Six-Month Old Liquid Inoculant	t-test @5%
Indole Acetic Acid (µg/ml)	36.77±0.92	34.98±1.28	NS	40.16±1.28	36.96±1.45	NS	46.93±0.53	46.3±0.44	NS	44.19±1.27	42.67±1.30	NS
Phosphate Solubilisation (µg/ml)	26.20±0.40	24.83±0.70	NS	34.76±1.00	32.39±1.09	NS	23.36±1.49	22.01±0.98	NS	16.81±1.03	14.57±0.71	NS
Ammonia Production (µM/ml)	34.38±0.55	30.34±1.04	NS	43.16±1.68	35.77±2.04	NS	42.03±1.54	39.69±1.82	NS	38.25±1.18	34.63±0.47	NS
Gibberellic Acid Production (µg/ml)	88.24±1.68	82.54±1.23	NS	109.16±1.92	104.86±2.03	NS	111.01±1.42	107.88±1.11	NS	109.08±3.82	106.09±2.85	NS
Siderophore Production (µg/ml)	162.58±1.18	159.22±0.47	NS	233.93±2.48	230.75±2.02	NS	143.87±2.39	140.81±1.17	NS	160.77±1.68	158.11±1.58	NS
ACC Deaminase Production	+	+		+	+		+	+		+	+	

The values of the experiment are means of three replications ± standard error.

Dry Matter Yield

The pooled analysis of the data concerning the dry matter yield at both the locations has been revealed in the Table 4. It is evident from the table that the influence of inoculation of seeds with the liquid microbial inoculants on dry matter yield was statistically non-significant. However, numeric enhancement in the dry matter yield of forage pearl millet at both the locations was observed. The maximum dry matter yield was obtained by the application of T_9 (119.55 q/ha) and minimum by T_1 (108.86 q/ha), respectively.

DISCUSSION

Periodic assessment of viability retention of liquid bacterial inoculants

Similar results were reported by Manimekalai and Kannahi (2018) who studied the effect of four different cell protective substances and selected trehalose (1%) as the potential additive because it could maintain a relatively high population and conferred greater bacterial vitality.

Gopal and Baby (2016) also standardized liquid formulation for *Azospirillum* (KAU isolate) and phosphate solubilizing bacteria (KAU isolate) along with chemical amendments. The highest population of *Azospirillum* (1.77×10^8 cfu ml^{-1}) was recorded in the case of trehalose (15 mM) amended medium. Studies at University of Agricultural Sciences, Bangalore showed that the liquid inoculants of *Rhizobium*, *Azotobacter*, *Azospirillum* and PSB may be stored at ambient temperature without significant loss in viability for more than one year (Dayamani 2010). Increased survival of cells in the liquid inoculant amended with trehalose might be due to the enhanced cell tolerance of trehalose to desiccation, osmotic pressure and temperature stress (Gopi *et al.,* 2019).

It is evident from the Table 3 that there was non-significant difference in the plant growth promoting features between the bacterial cultures isolated from freshly prepared liquid bacterial inoculant and the six-month old liquid bacterial inoculant. Thus, suggesting that these bio-inoculants can be used efficiently even after the storage period of six months without any compromise on the viability and growth promoting traits.

Productivity

The increase in the green fodder yield of forage pearl millet could be attributed to the integrated

TABLE 4
Influence of liquid bacterial inoculants on yield of forage pearl millet

Treatments		Green fodder yield (q/ha)	Dry Matter yield (q/ha)
T_1	RDF	432.82	108.86
T_2	RDF + *Azotobacter* sp.	442.43	111.21
T_3	RDF + *Burkholderia seminalis*	449.7	112.43
T_4	RDF + *Stenotrophomonas maltophilia*	454.63	114.39
T_5	RDF + *Sphingobacterium* sp.	444.37	111.52
T_6	RDF + *Azotobacter* sp.+*Burkholderia seminalis*	472.25	116.67
T_7	RDF + *Azotobacter* sp.+*Stenotrophomonas maltophilia*	477.62	117.20
T_8	RDF + *Azotobacter* sp.+*Sphingobacterium* sp.	460.43	115.61
T_9	RDF + *Burkholderia seminalis*+*Stenotrophomonas maltophilia*	489.31	119.55
T_{10}	RDF + *Burkholderia seminalis*+*Sphingobacterium* sp.	479.78	118.55
T_{11}	RDF + *Stenotrophomonas maltophilia*+*Sphingobacterium* sp.	484.09	119.02
T_{12}	RDF + Consortium	438.92	109.85
	C. D. (5%)	NS	NS

influence of nitrogen fixation, phosphate solubilisation and production of phytohormones by the inoculated liquid bacterial inoculants on all the growth parameters as forage yield is a cumulative function of the yield components and agronomic characteristics. In addition, nutrient cycling, decomposition of organic matter and improvement of soil health are some of the major activities of bio-inoculants that stimulate plant growth. Vyas *et al.* (2015) conducted an experiment in a factorial randomized block design with three replications, comprising of three levels of Bio-fertilizer; B1: *Azotobacter*, B2: PSB and B3: *Azotobacter* + PSB. The data on seed and fodder yields of pearl millet revealed numerically higher seed (891 kg/ha) and fodder (3197 kg/ha) yields with the application of *Azotobacter* + PSB.

Escalation in the dry matter yield might be due to the fact that phytohormones produced by the liquid bacterial inoculants might have stimulated the root growth and induced better changes in the root morphology, which in turn affected the assimilation of the nutrients from the soil. Further, improvement in the uptake of plant nutrients might have empowered the inoculated plants to manufacture more quantity of photosynthates and thus resulted in the elevation of the dry matter yield. The results obtained were in accordance with Saifullah *et al.* (2011) who observed similar values of dry matter yield in pearl millet.

CONCLUSION

The knowledge gained from this study can help in developing strategies to optimize the storage and application of liquid bacterial inoculants, thereby improving their efficacy in enhancing crop yield.

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