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### DEVELOPMENT OF LIQUID INOCULANTS: AN INNOVATIVE AGRONOMIC PRACTICE FOR SUSTAINABLE AGRICULTURE

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#### KEYWORDS

*Azotobacter* sp.

Liquid inoculants

PEG

*Streptomyces badius*

Wheat

#### ABSTRACT

The Present investigation was undertaken to develop liquid inoculants of *Azotobacter* sp. and *Streptomyces badius* and to evaluate its affect on yield attributes of wheat (*Triticum aestivum* L.) under field conditions. Both the cultures of *Azotobacter* sp. and *Streptomyces badius* were procured from the Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab and evaluated for their plant growth promoting traits. The quantity of IAA, phosphate solubilization, gibberellic acid, ammonia production by *Azotobacter* sp. was 10.75 µg/ml, 21.74 µg/100ml, 77.86 µg/ml and 27.68 µmole/ml respectively and by *Streptomyces badius* was 13.39 µg/ml, 28.73 µg/100ml, 108.44 µg/ml and 32.48 µmole/ml respectively. Further, siderophore production was exhibited by *Streptomyces badius* only. It produced 104.89 µg/ml of catechol type and 62.84 µg/ml of hydroxamate type siderophores. Both the cultures were also found positive for ACC-deaminase. Liquid inoculants of *Azotobacter* sp. and *Streptomyces badius* were formulated using 2% PEG in basal medium and showed maximum viability even after 180 days as compared to charcoal carrier based formulation both at room and refrigerated temperature. Further, the field experiment was conducted during winter (*rabi*) season of 2016-17 at Punjab Agricultural University, Regional Research Station, Bathinda, Punjab with three treatments viz. RDF, RDF + Liquid inoculants of *Azotobacter* sp. and *Streptomyces badius* and; RDF + Charcoal carrier based inoculants of *Azotobacter* sp. and *Streptomyces badius*. This experiment was laid out in randomized complete block design and replicated thrice. The liquid inoculants as well as charcoal based

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inoculants of *Azotobacter* sp. and *Streptomyces badius* were found superior in yield attributing characters over control. However, percentage increase in grain yield and biological yield was highest with liquid inoculants. This might be due to higher survival of *Azotobacter* sp. and *Streptomyces badius* in liquid formulation. Thus, it can be concluded that liquid inoculants of *Azotobacter* sp. and *Streptomyces badius* can enhance yield of wheat. This combination may be used as an efficient PGPR for wheat production.

## 1 Introduction

Microbial inoculants (bio-inoculants) are the mean of inoculation of one or more beneficial microbial strains or species in an economical and easy to use carrier based formulation. Bio-inoculants produces felicitous effects on plant growth by direct and indirect mechanism such as by phytohormone production (auxin, cytokinin or gibberallin), by enzymatic lowering of plant ethylene levels, nitrogen fixation, phosphate solubilization or by siderophores production. Bio-inoculants reintegrate the natural nutrient cycle by augmenting organic matter content and further maintain optimum nutrient level, thus results in healthy plant growth, while preserving fertility and sustainability of the soil (Shelat et al., 2017).

Despite good potentiality of microbial inoculants, the actual utilization is very low at about 2% of its potential. Meager adoption among countryman is ascribed mostly to their unpredictable response, low quality in terms of total viable counts at the time of use, short shelf life and temperature sensitiveness (Yadav & Chandra, 2014). The possible scope of contamination is very huge in the case of carrier based bio-inoculants as massive sterilization does not provide the desired outcomes (Bhavya et al., 2017)

Liquid inoculants formulations could be a possible solution to the aforementioned tribulations as it contains cell protectants/ additives for promotion of lengthy shelf life and tolerance to unpropitious conditions of the desired microorganisms in addition to their growth nutrients (Hegde, 2008). The contamination can be managed by means of proper sterilization techniques and maintenance of rigorous hygiene conditions by appropriate quality control measures in the case of liquid based biofertilizer (Bhavya et al., 2017). Depending upon the ability to heat transfer, high water activities and rheological properties of different polymers like polyethylene glycol (Temprano et al., 2002), polyvinyl alcohol (Deaker et al., 2004), gum Arabic (Mugnier & Jung, 1985), polyvinyl pyrrolidone (Singleton, 2002) and sodium alginate (Bashan et al., 2004) have been used for inoculants production.

Liquid inoculants can be comfortably adjusted to modish seeding equipments as it can be sprayed onto the seed as it passes through the seed auger and dries before it travels into the seed bin on the planter. The mean life of the microbes in liquid inoculants is higher without ample loss in cell counts than carrier based

inoculants. They are further resistant to ultra violet radiations and high temperatures (Santhosh, 2015).

Further, the global market for microbial inoculants/ biofertilizer is expected to exceed a market worth of USD 10.2 billion by 2018 (Raja, 2013). Keeping foresaid points in view present investigation was carried out with the object to develop liquid inoculants containing *Azotobacter* sp. and *Streptomyces badius* for wheat using poly ethylene glycol @ 2% in basal medium respectively and its comparison with charcoal carrier based inoculants under field condition.

## 2 Materials and Methods

### 2.1 Procurement of standard cultures

Standard cultures of *Azotobacter* sp. and *Streptomyces badius* were procured from Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India.

### 2.2 Maintenance of cultures

*Azotobacter* sp. and *Streptomyces badius* were maintained at 4°C in Jensen's medium and Starch casein agar medium respectively and subcultured once in a month throughout the period of investigation.

### 2.3 Characterization of *Azotobacter* sp. and *Streptomyces badius* for plant growth promoting traits

Characterization of cultures for the PGP traits were executed by the following method

PGP traits	Methods used
IAA ( $\mu\text{g/ml}$ )	Gordon & Werber (1951)
Phosphate solubilisation ( $\mu\text{g}/100\text{ ml}$ )	Barrow et al. (1964)
Siderophore production ( $\mu\text{g/ml}$ )	Jackson (1973)
Gibberellic acid ( $\mu\text{g/ml}$ )	Arnou (1937), Csaky (1948)
Ammonia production ( $\mu\text{mole/ ml}$ )	Cappuccino & Sherman (1992)
ACC- deaminase activity	Govindasamy et al. (2009)

## 2.4 Preparation of mother culture

A loopful inoculum of pure culture of *Azotobacter* sp. and *Streptomyces badius* was inoculated in sterilized 200 ml of nutrient broth dispensed in 500 ml flask respectively. Flasks were incubated at  $28 \pm 1^\circ\text{C}$  for 24 hrs. The growing cells of log phase were used for the further inoculation.

## 2.5 Preparation of medium for liquid inoculants

A modified nutrient broth medium composed of 5.0 gram of NaCl, 3.0 gram of yeast extract, 5.0 gram of peptone per litre and poly ethylene glycol @ 2% was used as basal medium for liquid inoculants formulation.

## 2.6 Vial preparation

New plastic vials of 100 ml were filled with small volume of water and autoclaved at  $121^\circ\text{C}$  (15 psi) for about 15 min.

## 2.7 Preparation of liquid inoculants

For the preparation of liquid inoculants of *Azotobacter* sp. and *Streptomyces badius*, mother culture of *Azotobacter* sp. and *Streptomyces badius* was transferred @ 1% to each 250 ml flask containing 100 ml of sterilized basal medium amended with 2% PEG and each 250 ml flask containing 100 ml of sterilized basal medium without 2% PEG (control). All the flasks were kept on shaker at  $28 \pm 1^\circ\text{C}$  for 24 hrs. These log phase cells were transferred into the sterilized 100 ml capacity plastic vials under aseptic conditions after removing the water from the vials. The plastic vials containing liquid inoculants were stored in two sets viz. first set was stored at room temperature while second was stored in a refrigerator (Figure 1).



Figure 1 Plastic vials with liquid inoculants

## 2.8 Preparation of charcoal carrier based inoculants

Charcoal carrier was obtained from the Department of Microbiology, PAU, Ludhiana, India. The charcoal based formulation was prepared by mixing the broth culture of respective culture with charcoal powder at 1: 2.5 ratio. (Figure 2)



Figure 2 Charcoal based carrier inoculants

Broth culture of *Azotobacter* sp. and *Streptomyces badius* was prepared by inoculating loopful inoculums of pure culture of *Azotobacter* sp. and *Streptomyces badius* in sterilized 200 ml of nutrient broth dispensed in 500 ml flask respectively. The total viable count in charcoal carrier based microbial inoculants was determined by adding 10 grams of inoculant to 90 ml of sterilized distilled water and making a 10-fold dilution series. Then, 0.1ml aliquots of the appropriate dilutions were pour plate on jensen's medium and starch casein agar medium respectively at 0, 30, 60, 90, 120, 150 and 180<sup>th</sup> day. Colonies were counted after incubation of three days at  $28 \pm 2^\circ\text{C}$ .

## 2.9 Shelf life studies of liquid inoculants at room temperature and refrigerated temperature

The plastic vials containing liquid inoculants of *Azotobacter* sp. and *Streptomyces badius* were stored in two sets. First set was stored at room temperature while other one was stored in a refrigerator. One ml of sample was drawn aseptically at 0, 30, 60, 90, 120, 150 and 180<sup>th</sup> day for taking total viable count by dilution pour plate method and plates were incubated at  $28 \pm 2^\circ\text{C}$  for 48-72 hrs.

## 2.10 Effect of liquid inoculants on wheat under field conditions

To evaluate the efficacy of inoculants of *Azotobacter* sp. and *Streptomyces badius* on wheat, a field experiment was conducted

with three treatments i.e. RDF, RDF + Liquid inoculants of *Azotobacter* sp. and *Streptomyces badius* and RDF + Charcoal carrier based inoculants of *Azotobacter* sp. and *Streptomyces badius*

The field experiment was conducted during winter (*rabi*) season of 2016-17 at Punjab Agricultural University, Regional Research Station, Bathinda, Punjab ( 30° 09' 36" N latitude, 74° 55' 28" E longitude; 211m above sea level). The field experiment was laid out in RCBD and replicated thrice.

Wheat cultivar 'HD 3086' seeds were sown manually as per treatment schedule. The sowing was made with row to row spacing of 22.0cm with the plot dimensions of 5m X 2.60m. Seed bacterization was done with liquid inoculants of *Azotobacter* sp. and *Streptomyces badius* @ 100ml/40kg respectively for liquid inoculants and 250 gram of charcoal carrier based inoculants of *Azotobacter* sp. and *Streptomyces badius* respectively per 40kg of wheat seeds. Inoculated seeds were air dried in shade at room temperature and planted within 2 hrs. The recommended dose of N: P: K was applied through urea, DAP and MOP respectively. The whole of the phosphorus and potassium was applied at the time of sowing along with half of nitrogen and remaining nitrogen was applied at crown root initiation (CRI) and ear initiation (EI) stages of the crop in two splits respectively. Weeding and hoeing was done to avoid weeds and suitable control measures were taken to prevent insects and pests. A total of 3 to 4 irrigations were applied i.e first irrigation was given at 21 DAS and thereafter the experiment plots were irrigated every 25 to 30 days until the end of the season,

The net plot was harvested manually at the physiological maturity by cutting plants near ground surface after leaving the border rows. The plant were dried and weighed for biomass as well as for grain yield. The experimental data collected on various aspects of investigation were statistically analyzed with the procedure as described by Cochran & Cox (1967). The comparisons were made at 5% level of significance.

### 3 Results and Discussion

#### 3.1 Characterization of *Azotobacter* sp. and *Streptomyces badius* for plant growth promoting attributes

Plant growth promoting rhizobacteria (PGPR) are heterogenous group of bacteria that can be found in rhizosphere and capable of improving the extent or quality of plant growth directly or indirectly (Shahzadi et al., 2012). Rhizobacteria are now being used worldwide as bio-inoculants to promote plant growth and development under various stress conditions. Plant growth promoting attributes are important characteristic of PGPR, which influence plant growth.

In present study, the quantity of IAA, phosphate solubilization, gibberellic acid and ammonia production by *Azotobacter* sp. was 10.75 µg/ml, 21.74 µg/100ml, 77.86 µg/ml and 27.68 µmole/ml respectively and by *Streptomyces badius* was 13.39 µg/ml, 28.73 µg/100ml, 108.44 µg/ml and 32.48 µmole/ml respectively. The siderophore production was exhibited by *Streptomyces badius* only. It produced 104.89 µg/ml of catechol type and 62.84 µg/ml of hydroxamate type siderophores. Both the cultures were also found positive for ACC-deaminase activity (Table 1). Thus, both *Azotobacter* sp. and *Streptomyces badius* could play important role in crop plant growth and development. Doran & Zeiss (2000) also reported that *Azotobacter* can boost crop production under different stress circumstances for agriculture sustainability. Therefore there is an indispensable demand to characterize such bacterial inoculants. Similarly *Streptomyces* and other actinobacteria are surprisingly under explored for plant growth promotion as compared to *Pseudomonas* or *Bacillus* spp. (Doubou et al., 2001).

Table 1 Evaluation of plant growth promoting traits of *Azotobacter* sp. and *Streptomyces badius*

Microbial cultures		<i>Azotobacter</i> sp.	<i>Streptomyces badius</i>
IAA production (µg/ml)	With tryptophan	30.27 ± 1.7	42.09 ± 2.8
	Without tryptophan	10.75 ± 0.36	13.39 ± 0.83
Phosphate solubilization (µg/100ml)		21.74 ± 0.4	28.73 ± 0.4
Siderophore production (µg/ml)	Catechol type	-	104.89 ± 2.3
	Hydroxamate type	-	62.84 ± 0.5
Gibberellic acid production (µg/ml)		77.86 ± 1.4	108.44 ± 1.5
Ammonia production (µmole/ml)		27.68 ± 0.7	32.48 ± 1.6
ACC deaminase		+	+

Values are the mean of three replications ±SE

#### 3.2 Survival of liquid inoculants

In present study the liquid inoculants were prepared by inoculating *Azotobacter* sp. and *Streptomyces badius* in basal broth medium amended with 2% poly ethylene glycol respectively. The liquid inoculants were analyzed for their survival at room temperature and refrigeration temperature over a period of six months through serial dilution pour plate method on jensen's and starch casein agar respectively.

##### 3.2.1 Shelf life studies of liquid inoculant of *Azotobacter* sp.

The data pertaining to survivability of *Azotobacter* sp. in liquid inoculants without PEG (AT<sub>1</sub>), liquid inoculants with 2% of PEG

Table 2 Survival of *Azotobacter* sp. in liquid inoculants at room and refrigerated temperature and its comparison with charcoal based inoculants

Treatments	Population density Log <sub>10</sub> cfu/ml							CD (p=0.05)
	Total viable count days after storage							
	0	30	60	90	120	150	180	
AT <sub>1</sub>	11.86 ± 0.9	10.61 ± 1.0	9.89 ± 0.8	8.29 ± 0.6	7.28 ± 0.6	5.17 ± 0.4	3.09 ± 0.2	2.26
AT <sub>2</sub>	11.78 ± 0.9	11.88 ± 0.9	11.73 ± 0.9	10.89 ± 0.8	10.77 ± 0.8	10.66 ± 0.8	10.36 ± 0.8	NS
*AT <sub>3</sub>	10.93 ± 1.0	11.30 ± 0.9	9.88 ± 0.9	9.75 ± 0.9	9.59 ± 0.9	9.11 ± 0.8	7.90 ± 0.7	NS
LAT <sub>1</sub>	11.48 ± 1.0	11.38 ± 0.9	10.83 ± 1.0	10.28 ± 0.8	7.47 ± 0.6	6.25 ± 0.5	5.72 ± 0.5	2.47
LAT <sub>2</sub>	12.38 ± 1.1	12.14 ± 1.0	11.82 ± 1.0	11.57 ± 0.9	11.20 ± 0.9	11.39 ± 1.0	11.30 ± 1.0	NS
*LAT <sub>3</sub>	12.25 ± 1.1	11.32 ± 0.9	11.25 ± 1.0	11.06 ± 0.9	10.17 ± 0.8	10.12 ± 0.1	9.89 ± 0.9	NS

\* Log<sub>10</sub> no. of cell/g, Values are the mean of three replications ±SE; NS = Non significant at 5% level of significance

AT<sub>1</sub>: Liquid Inoculants of *Azotobacter* sp. without PEG 2% at room temperature;

AT<sub>2</sub>: Liquid Inoculants of *Azotobacter* sp. with 2% Polyethylene glycol at room temperature;

AT<sub>3</sub>: Charcoal carrier based inoculants of *Azotobacter* sp. at room temperature;

LAT<sub>1</sub>: Liquid Inoculants of *Azotobacter* sp. without PEG 2% at refrigerated temperature;

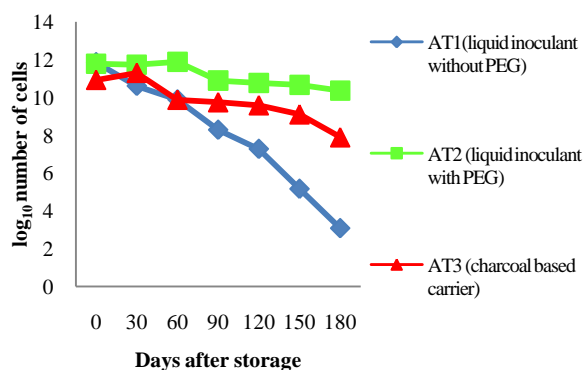
LAT<sub>2</sub>: Liquid Inoculants of *Azotobacter* sp. with 2% Polyethylene glycol at refrigerated temperature;

LAT<sub>3</sub>: Charcoal carrier based inoculants of *Azotobacter* sp. at refrigerated temperature

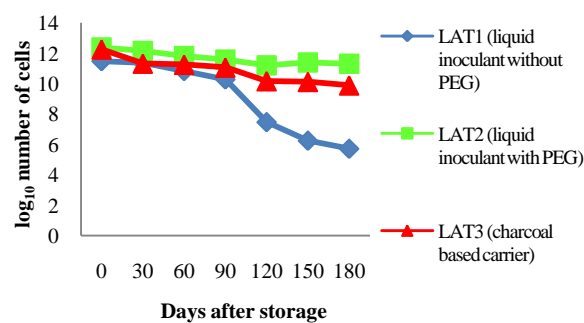
(AT<sub>2</sub>) and in charcoal carrier based inoculants (AT<sub>3</sub>) at room temperature has been presented in Table 2 and Figure 3a. The perusal of data indicated that viable count of liquid inoculants of *Azotobacter* sp. without PEG decreased significantly at 5% level where as viable count of liquid inoculants with 2% of PEG (AT<sub>2</sub>) and in charcoal carrier based inoculants (AT<sub>3</sub>) at room temperature decreased non significantly at 5% level of significance.

Initial viable count of *Azotobacter* sp. was 11.86 log<sub>10</sub> viable cells which reduced to 3.09 log<sub>10</sub> viable cells at 180<sup>th</sup> day (with decrease of 73.94 per cent) in liquid inoculants without PEG (AT<sub>1</sub>). In liquid inoculants amended with 2% of PEG (AT<sub>2</sub>) initial count was 11.78 log<sub>10</sub> viable cells which further reduced to 10.36 log<sub>10</sub> viable cells at 180<sup>th</sup> day (with decrease of 12.05 per cent). In charcoal carrier based inoculants, 10.93 log<sub>10</sub> no. of viable cells were observed initially, the count declined to 7.90 log<sub>10</sub> viable cells at 180<sup>th</sup> day after storage at room temperature (with decrease of 27.72 per cent).

The data pertaining to survivability of *Azotobacter* sp. in liquid inoculants without PEG (LAT<sub>1</sub>), liquid inoculants with 2% of PEG (LAT<sub>2</sub>) and in charcoal carrier based inoculants (LAT<sub>3</sub>) at refrigerated temperature has been presented in Table 2 and Figure 3b. There was a significant effect (p=0.05) of storage time on viable count of liquid inoculants of *Azotobacter* sp. without PEG where as non significant effect on viable count of liquid inoculants with 2% of PEG (LAT<sub>2</sub>) and charcoal carrier based inoculants (LAT<sub>3</sub>) was observed at refrigerated temperature. Initially 12.38 log<sub>10</sub> viable cells in liquid inoculants amended with



3(a)



3(b)

Figure 3 Survival of *Azotobacter* sp. inoculants at room temperature (a) and refrigerated temperature (b)

Table 3 Survival of *Streptomyces badius* in liquid inoculants at room and refrigeration temperature and its comparison with charcoal based inoculants

Treatments	Population density (Log <sub>10</sub> cfu/ml)							CD (p=0.05)
	Total viable count days after storage							
	0	30	60	90	120	150	180	
ST <sub>1</sub>	11.89 ± 1.1	10.19 ± 0.8	8.86 ± 0.8	8.1 ± 0.7	7.28 ± 0.6	4.20 ± 0.4	2.17 ± 0.2	2.10
ST <sub>2</sub>	11.76 ± 0.95	11.81 ± 1.1	11.67 ± 0.9	10.25 ± 0.8	10.18 ± 0.8	10.12 ± 0.8	10.35 ± 0.8	NS
*ST <sub>3</sub>	11.82 ± 1.1	11.30 ± 0.9	11.25 ± 0.9	9.19 ± 0.8	8.46 ± 0.7	9.33 ± 0.8	6.86 ± 0.6	2.63
LST <sub>1</sub>	12.09 ± 1.0	11.99 ± 1.1	10.79 ± 0.8	8.07 ± 0.7	7.89 ± 0.6	6.41 ± 0.5	5.29 ± 0.5	2.40
LST <sub>2</sub>	11.83 ± 1.1	12.21 ± 1.0	12.14 ± 1.1	11.07 ± 0.9	10.98 ± 0.9	10.61 ± 1.0	10.57 ± 1.0	NS
*LST <sub>3</sub>	10.88 ± 1.0	12.14 ± 1.0	12.06 ± 1.1	10.83 ± 0.8	10.23 ± 0.8	8.35 ± 0.7	8.21 ± 0.7	2.77

\* Log<sub>10</sub> no. of cell/g. Values are the mean of three replications ±SE; NS = Non significant at 5% level of significance

ST<sub>1</sub> : Liquid Inoculants of *Streptomyces badius* without PEG 2% at room temperature

ST<sub>2</sub> : Liquid Inoculants of *Streptomyces badius* with 2% Polyethylene glycol at room temperature

ST<sub>3</sub> : Charcoal carrier based inoculants of *Streptomyces badius* at room temperature

LST<sub>1</sub> : Liquid Inoculants of *Streptomyces badius* without PEG 2% at refrigerated temperature

LST<sub>2</sub> : Liquid Inoculants of *Streptomyces badius* with 2% Polyethylene glycol at refrigerated temperature

LST<sub>3</sub> : Charcoal carrier based inoculants of *Streptomyces badius* at refrigerated temperature

PEG 2% (LAT<sub>2</sub>) which further reduced to 11.30 log<sub>10</sub> viable cells at 180<sup>th</sup> day. In charcoal carrier based inoculants (LAT<sub>3</sub>), initial count was 12.25 log<sub>10</sub> viable cells that declined to 9.89 log<sub>10</sub> viable cells at 180<sup>th</sup> day (with reduction of 19.26 percent). The minimum log<sub>10</sub> viable cells were recorded in liquid inoculants without PEG (LAT<sub>1</sub>). The initial count (11.48 log<sub>10</sub> viable cells) reduced to 5.72 log<sub>10</sub> viable cells at 180<sup>th</sup> day (with reduction of 50.17 percent). Higher survival of respective inoculants at refrigerated conditions may be due to the fact that freezing temperature slows down the decomposition of nutrients by turning residual moisture into ice thus inhibiting the growth of most of the bacteria.

Overall findings in present study were in line with Dayamani & Brahmprakash (2014) who demonstrated that media amended with PEG 400 at all concentrations, PEG 600 and PEG 6000 at the 2 % level and glycerol at the 0.5 % level increased population density substantially. Similar findings were reported by Kumaresan & Reetha, (2011) who concluded that liquid *Azospirillum* bio-inoculants with poly ethylene glycol (1%), poly vinyl alcohol (0.5%) and control (lignite carrier) recorded the same population of 10<sup>8</sup> cells ml<sup>-1</sup> upto 8 months, 6 months and 5 months of storage respectively under ambient temperature (28°C to 32°C).

Thus amendment of liquid inoculants with poly ethylene glycol (PEG) enhances shelf life of liquid inoculants because of its viscous nature that may slow down the drying process and its sticky consistency which further reinforce culture attachment to seed (Temprano et al., 2002).

### 3.2.2 Shelf life studies of liquid inoculant of *Streptomyces badius*

The perusal of data in Table 3 indicated that at room temperature, the liquid inoculants amended with PEG 2% (ST<sub>2</sub>) supported better viability as compared to liquid broth without PEG 2% (ST<sub>1</sub>) and charcoal based carrier (ST<sub>3</sub>). There was non significant (p=0.05) effect of storage time on viable count of liquid inoculants amended with PEG 2% (ST<sub>2</sub>) at room temperature. Initially 11.89 log<sub>10</sub> viable cells were observed in liquid inoculants without PEG (ST<sub>1</sub>) which decreased to 2.17 log<sub>10</sub> viable cells at 180<sup>th</sup> day at room temperature. This may be due the achievement of stationary phase and desiccation of cells. The liquid inoculants amended with 2% PEG, initially showed 11.76 log<sub>10</sub> viable cells which decreased to 10.35 log<sub>10</sub> viable cells at 180<sup>th</sup> day (with decrease of 11.98 per cent) followed by charcoal based carrier microbial inoculants of *Streptomyces badius* (ST<sub>3</sub>) with 11.82 log<sub>10</sub> viable cells initially which decreased to 6.86 log<sub>10</sub> viable cells (with decrease of 41.96 per cent) at 180<sup>th</sup> day (Figure 4a).

Similarly, perusal of data at refrigerated temperature indicated that liquid inoculants of *Streptomyces badius* with PEG 2% (LST<sub>2</sub>) showed the highest count (10.57 log<sub>10</sub> viable cells) at 180<sup>th</sup> days which was followed by charcoal carrier based inoculants (8.21 log<sub>10</sub> viable cells) at 180<sup>th</sup> days and least count was observed in liquid inoculants without PEG 2% (5.29 log<sub>10</sub> viable cells) at refrigerated temperature. The liquid inoculants of *Streptomyces badius* with PEG 2% (LST<sub>2</sub>) showed non significant (p=0.05) decrease in viable count (Figure 4b). Higher survival under refrigerated conditions might be due to little growth with little

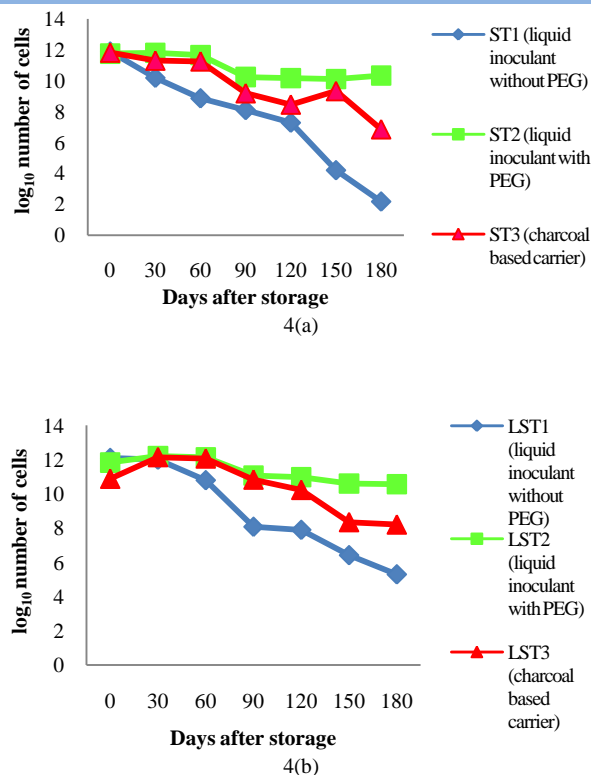


Figure 4 Survival of *Streptomyces badius* inoculants at room temperature (a) and refrigerated temperature (b)

utilization of nutrients during storage make the availability of nutrients to the organism in optimum concentration for longer period. Low temperature in refrigerator also protects the cell death in inoculums. In contrast, during storage at room conditions where temperature reached beyond 30°C, growth of organism results in creating depletion of nutrients and accumulation of toxic metabolites (Maurice et al., 2001). Desiccation and high temperature also influence the survival of cells on the seeds (Tittabutr et al., 2007; Daniel et al., 2013).

Thus overall highest viable count of *Streptomyces badius* was observed in liquid inoculants amended with 2% of PEG followed by charcoal carrier based inoculants and lowest count was observed in liquid inoculants without 2% PEG both at room temperature as well as at refrigerated temperature. The results are supported by the findings of Kumar et al. (2014) who reported a frequent decrease in viable count from 10<sup>9</sup> to 10<sup>7</sup> colony-forming units (cfu/g) per g after 90 days of storage. Temprano et al. (2002) further reported that poly ethylene glycol prevents the microbial inoculants from desiccation due to its viscosity which slow down the process of drying process. Moreover PEG is a synthetic polymer approved by the FDA for internal consumption and

injection in a variety of foods, cosmetics and drug delivery systems (Cavalla, 2001).

### 3.3 Effect of liquid inoculants on wheat under field conditions

The second most predominant cereal crop in India after rice is wheat. Wheat provides more than 50 per cent of calories to the people who mainly dependent on it and contributing considerably to the national food security. To fulfill the food supply requirements for projected population by 2050 significant increase (an estimated 50%) in grain yield of major crop plants like rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) is mandatory (Godfray et al., 2010).

Diverse PGPRs have been used worldwide as microbial inoculants, contributing to increasing crop yields and soil fertility and hence with potential to contribute to more sustainable agriculture and forestry (García-Fraile et al., 2015). During the past two decades the practice of microbial inoculants in agriculture has increased incredibly (Hayat et al., 2010) due to public and private sector agricultural research and development communities which are working for solutions to tribulations associated with contemporary agriculture. There are considerable advantages of liquid inoculants such as no contamination, huge cell count, lengthy shelf life, higher stability against environmental stress and increased field efficacy (Tittabutr et al., 2007; Liu et al., 2009). For evaluating the effect of liquid formulation of inoculants viz. *Azotobacter* sp. and *Streptomyces badius* on yield attributes of wheat and comparison with charcoal carrier based inoculants of *Azotobacter* sp. and *Streptomyces badius* an experiment was conducted at PAU, RRS, Bathinda during rabi 2016-17. The results of the study were discussed under following headings.

#### 3.3.1 Grain Yield

Grain yield in wheat is primarily a function of effective tillers number of spikes, number of grains per spike, spike length and thousand grain weight. The data pertaining to grain yield of wheat as influenced by liquid inoculants and charcoal carrier based inoculants of *Azotobacter* sp. and *Streptomyces badius* has been presented in Table 4. The perusal of data indicated that application of microbial inoculants of *Azotobacter* sp. and *Streptomyces badius* had not any significant effect on grain yield of wheat at 5% level of significance. However, highest grain yield was observed with liquid inoculants followed by charcoal carrier based inoculants. Son et al. (2006) also observed enhanced grain yield, nutrient availability and uptake in soybean crop (*Glycine max*) by the application of liquid PSB inoculants. Similar results were observed by Nezarat & Gholami (2009) who found increase in the yield of wheat upto 30% by the use of combined inoculation of *Azotobacter* and PSB over control. Poly ethylene glycol

Table 4 Grain yield and biological yield of wheat crop as affected by inoculants

S.No	Treatments	Grain Yield (t/ha)	Biological Yield (t/ha)
1	RDF	5.01	14.55
2	RDF + Liquid inoculants of <i>Azotobacter</i> sp. + <i>Streptomyces badius</i>	5.14	15.70
3	RDF + Charcoal carrier based inoculants of <i>Azotobacter</i> sp. + <i>Streptomyces badius</i>	5.13	15.29
CD (p=0.05)		NS	NS

NS = Non significant at 5% level of significance

prevents the microbial inoculants from desiccation due to its viscosity which slow down the drying process (Temprano et al., 2002). These might be the mechanisms that improves the survival of cells on the seeds and give rise to more plant growth.

### 3.3.2 Biological Yield

Biological yield represents the total biomass of plant organs and effective absorption of nutrient elements. The data pertaining to biological yield has been presented Table 4. The highest biological yield was produced with liquid inoculants (15.70 t/ha), followed by carrier based inoculants (15.29 t/ha) whereas lowest biological yield were recorded with control (14.55 t/ha). This might be because of nitrogen fixing, phosphate solubilization and ACC deaminase activity of inoculated culture i.e *Azotobacter* sp. and *Streptomyces badius* in the form of microbial inoculants. Similarly, enhancement in grain yield (16.3 %) was reported by Lakshminarayana et al. (2000) with inoculation of *Azotobacter chroococcum* strain A103 in wheat variety WH291. The Plant growth promoting potential of *Streptomyces* was also reported on wheat by Sadeghi et al. (2012).

However, application of microbial inoculants of *Azotobacter* sp. and *Streptomyces badius* had not any significant effect on biological yield of wheat at 5% level of significance but better respond was observed with liquid inoculants. The percentage increase in biological yield with application of liquid inoculants was 7.87 percent. This might be due to higher survival of *Azotobacter* sp. and *Streptomyces badius* in liquid formulation. BrahmaPrakash & Sahu (2012) also reported that the population density of *Acinetobacter* was highest in the presence of PEG.

### Conclusion

Liquid inoculants as well as charcoal carrier based inoculants of *Azotobacter* sp. and *Streptomyces badius* were found to enhance yield attributing characters of wheat under field conditions by direct and indirect mechanisms such as biological nitrogen fixation, phosphate solubilization, phytohormones production and ACC deaminase activity, siderophore production. However, liquid

inoculants of *Azotobacter* sp. and *Streptomyces badius* were found to be superior over charcoal carrier based inoculants. This might be due to higher survival of *Azotobacter* sp. and *Streptomyces badius* in liquid formulation.

### Conflict of Interest

Authors declare that there is no conflict of interests arising from this study.

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