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Effect of Differerent Concentrations of Trehalose on Survival of Native Strain of *Azotobacter chroococcum* isolated from rhizosphere of wild Grass *Iseilema prostratum* L.

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Abstract: The plant growth promoting rhizobacterias inoculants used in various formulations in Agriculture. In addition to rhizobacterias, the formulation may also contain certain additives. Furthermore, it is very important to understand the interaction between bacteria and formulation additives. The formulation additives serve as cell protectants which help to increase the shelf- life of bacteria. It is experimentally proved that, the Rhizobium is the effective inoculants along with specific nutrient media for survival (Deaker et. al., 2004). Many researchers have shown that, liquid rhizobial inoculants are more beneficial than solid rhizobial inoculants. In the present work, for survival study, a native strain of *Azotobacter chroococcum* isolated from rhizosphere of wild grass *Iseilema prostratum* L and identified from Agarkhar Research Institute pune (MH) India was used. Liquid formulations were evaluated by using Trehalose at different concentration in Jenson's broth. It was noted that survival of Azotobacter was concentration correlated. Lowest number of colonies in 5mM in the medium containing trehalose (28.66 colonies at zero days and 3 colonies on 360th day) and highest number of colonies in25mM in the medium containing trehalose (40.66 colonies on zero day and 14 colonies on 360th day) (10^{-9} CFU/ml).

Key Words- Rhizobium, Azotobacter, Trehalose, Survival.

I. Introduction

In India, the bio-fertilizers are mostly lignite, coal, peat based. The microbial inoculants are prepared with the above carried based. Generally it called solid formulations. But this has many disadvantages such as shorter shelf life, poor quality, high contamination and poor performance. In addition to this the carrier based inoculants production is tedious, energy consuming activity. It involves milling, sieving and correcting pH (Somasengaran and hoben 1994). The liquid inoculants formulation is one solution to the problem associated within processing of solid carriers. The use of various broths cultures amended with substances that promote the cell survival in the package and after applications for seed or soil. Additives to liquid inoculants formulations should have a role in protecting Azotobacter cells on seeds at high temperature and during desiccation. Many kinds of polymer have been used for inoculants production because of their ability to limit heat transfer, their good rheological properties and high water activities (Mugnier and Jung 1985). The application of nitrogen fixing bacteria, soil rhizosphere microorganisms is a sustainable aspect for improving crop growth. However, the inoculation of beneficial microorganism in agriculture field is sometimes very critical to prolonged self life due to soil climatic factors. The environmental conditions may have negatively impact on the viability and ultimately limit efficacy in the field. Therefore, some protective additives are requiring protecting the microorganisms. There are various factors which influence the survival of microbial cells. Thus, certain techniques have to develop against the desiccation stress on beneficial bacteria which will be for successful

formulation development. The present work has the aim of increasing the self life and high cell viability of Nitrogen fixing bacteria to be applied as microbial inoculants in agriculture. We have elaborated the suitability of commonly applied trehalose as potential measures to prevent cell damage from desiccation.
In the present work the survival of Azotobacter in liquid formulations were evaluated by using trehalose The trehalose were tried in formulation to understand the survival life of Azotobacter and interaction between Azotobacter and formulation materials.

II. MATERIALS AND METHODS

Preparation of formulations with Trehalose.

Jensen's Basal Medium- The N_2 free Jensen's medium containing 20 gm sucrose, 1.0 gm K_2HPO_4, 0.5 gm $MgSO_4.7H_2O$, 0.5 gm NaCl, 2gm $CaCO_3$, 0.005 gm Na_2MoO_4, and 0.1 gm FeSO4 dissolved in 1 lit Distilled water. pH was adjusted to 7.1

Liquid State Formulation with Trehalose: For the standardization of proper quantity of amendments and to find out the survival time of *Azotobacter chroococcum* and Trehalose added with Jensen's basal medium. These various formulation were as follows

1. Basal medium (Control)

2. Basal medium + Trehalose with 5, 10, 15, 20 and 25 mM.

10 ml culture of *Azotobacter chroococcum* isolate was inoculated in each flask of above 5 types of liquid formulation and in basal medium as control under aseptic condition called broth. The broths were kept for continuous shaking on rotary shaker for 72 hours at 121 rpm, and then the cultures were transferred in sterilized glass bottle, plugged with cotton and stored at room temperature. These broth cultures were tested for total viable count at 30 days interval up to 12 months.

RESULT TABLE

Table No (A): Effect of different concentrations of Trehalose in Jensen's medium on survival of *Azotobacter chroococcum* colonies at CFU/ml 10^{-9}.

Days	Trehalose 5Mm	Trehalose 10mM	Trehalose 15mM	Trehalose 20mM	Trehalose 25mM	Broth Alone (control)	CD	P- value at 0.05%
0	28.66a±0.035 (26.48)	33.66b±0.09 (48.54)	34.66c±0.13 (52.96)	38.66d±0.08 (70.71)	40.66e±0.02 (79.44)	22.66f±0.03	0.06	5.17E-20
30	28.33a±0.05 (39.35)	31.33b±0.03) (54.11)	34.33c±0.06 (68.86)	35.33d±0.09 (73.78)	40.33e±0.04 (98.38)	20.33f±0.10	0.05	1.56E-21
60	28.33a±0.58 (39.35)	31.33b±0.10 (54.11)	34.33c±0.10 (68.86)	35.33d±0.08 (73.78)	40.33e±0.09 (98.38)	20.33f±0.14	0.19	5.54E-15
90	20.33a±0.06 (45.21)	27.33b±0.04 (95.21)	29.66c±0.11 (111.86)	30.33d±0.07 (116.64)	36.33e±0.05 (159.5)	14.00f±0.60	0.19	1.92E-15
120	20.00a±0.60 (100)	22.00b±0.60 (120.0)	25.66c±0.09 (156.6)	29.00d±0.60 (190.00)	35.00e±0.60 (250.0)	10.00e±0.60	0.4	8.33E-12
150	17.66a±0.06 (120.75)	20.66b±0.14 (158.25)	22.33c±0.08 (179.13)	27.66d±0.13 (245.75)	32.00e±0.16 (300.0)	8.00f±0.60	0.26	6.98E-14

180	**16.33a±0.04 (206.38)**	**19.33b±0.06 (262.66)**	**20.33c±0.052 (281.43)**	**27.33d±0.029 (412.76)**	**31.66e±0.58 (494.0)**	**5.33f±0.08**	**0.18**	**5.34E-16**

Data presented are means of ten readings; values within the same row with different letters are significantly different at 0.05% P-level by Single factor ANOVA test followed by CD & Tukey's test. [Figures in parentheses indicate % increase (+) and % decrease (-) over control; ± : standard error; CD:critical difference P-value: alpha value at 0.05%; Where CFU/ml AT 10^{-9}= Colony forming unit per gram per ml of Trehalose plus Jensen's broth)

Table No (B): Effect of different concentrations of Trehalose in Jensen's medium on survival of *Azotobacter chroococcum* colonies at CFU/ml 10^{-9}.

Days	Trehalose 5Mm	Trehalose 10mM	Trehalose 15mM	Trehalose 20mM	Trehalose 25mM	Broth Alone (control)	CD	P- value at 0.05%
180	**16.33a±0.04 (206.38)**	**19.33b±0.06 (262.66)**	**20.33c±0.052 (281.43)**	**27.33d±0.029 (412.76)**	**31.66e±0.58 (494.0)**	**5.33f±0.08**	**0.18**	**5.34E-16**
210	**14.66a±0.10 (266.5)**	**17.33b±0.07 (333.25)**	**18.66c±0.10 (366.5)**	**25.33d±0.04 (533.25)**	**30.00e±0.07 (650.0)**	**4.00f±0.58**	**0.19**	**2.78E-16**
240	**12.33a±0.08 (311.0)**	**12.33a±0.06 (311.0)**	**15.33b±0.04 (411.0)**	**24.33c±0.04 (711.0)**	**30.00e±0.58 (900.0)**	**3.00f±0.58**	**0.03**	**9.29E-15**
270	**10.33a±0.04 (676.69)**	**9.33b±0.08 (601.5)**	**12.00c±0.58 (802.26)**	**23.33d±0.05 (1664.33)**	**25.66e±0.12 (1829.32)**	**1.33f±0.07**	**0.19**	**3.79E-16**
300	**6.00a±0.58**	**7.33b±0.02**	**10.00c±0.58**	**20.00d±0.58**	**20.66e±0.10**	**0.00f±0.0**	**0.31**	**6.96E-13**
330	**4.66a±0.10**	**6.33b±0.04**	**8.66c±0.05**	**16.00d±0.58**	**17.33e±0.04**	**0.00f±0.0**	**0.18**	**1.30E-14**
360	**3.00a±0.58**	**5.00b±0.58**	**7.00c±0.58**	**12.00d±0.58**	**14.00e±0.58**	**0.00f±0.0**	**0.4**	**2.00E-09**

Data presented are means of ten readings; values within the same row with different letters are significantly different at 0.05% P-level by Single factor ANOVA test followed by CD & Tukey's test. [Figures in parentheses indicate % increase (+) and % decrease (-) over control; ± : standard error; CD:critical difference P-value: alpha value at 0.05%; Where CFU/ml AT 10^{-9}= Colony forming unit per gram per ml of Trehalose plus Jensen's broth).

Graph No 1: Effect of different concentrations of (5mM to 25mM) Trehalose in Jensen's broth on survival of *Azotobacter chroococcum* A1 colonies.

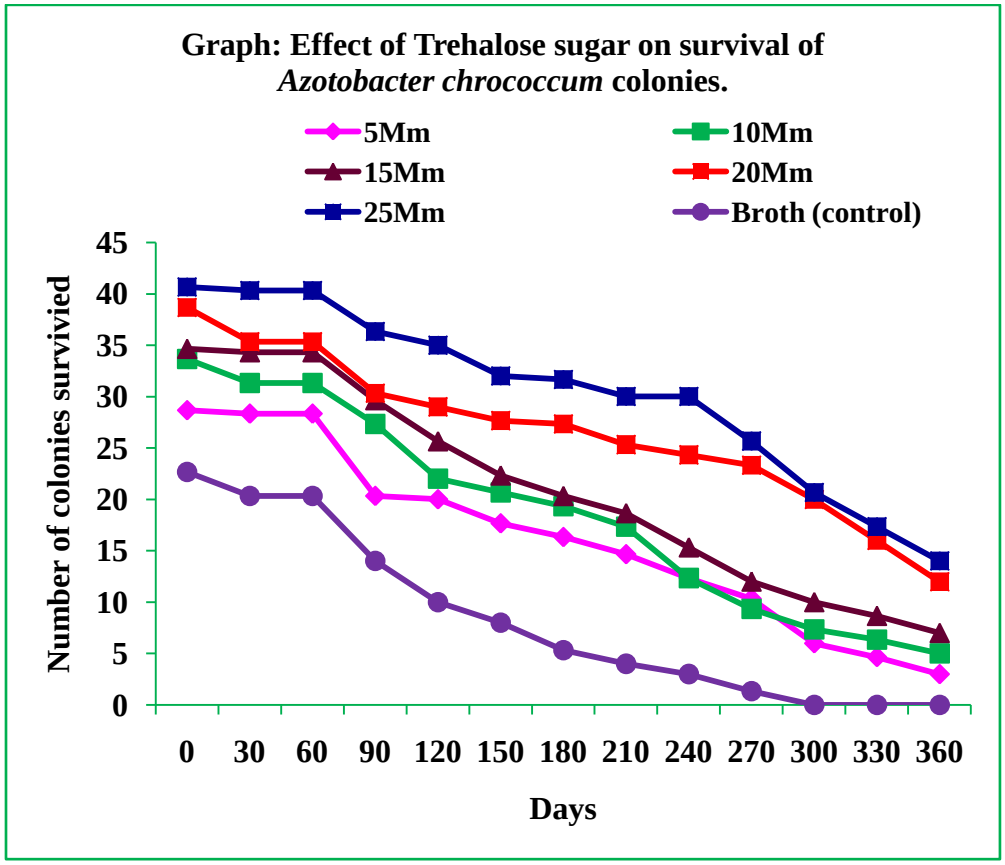
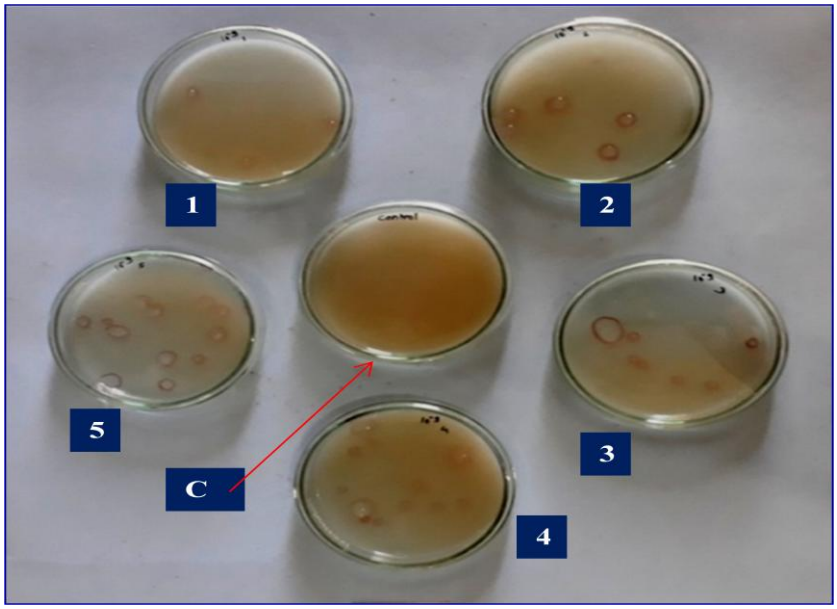


Photo Plate 1: Total viable count of *Azotobacter chroococcum* colonies stored in Jensen broth containing trehalose.



Effect of medium + Trehalose (1 TO 5 mM), where C: control,

III. RESULT AND DISCUSSION

The experimental work was carried out for the survival of Azotobacter in liquid formulations containing Trehalose in different concentration. Many researchers have shown that liquid rhizobial formulations are more beneficial. Hynes *et al.*, 1995, 2001 revealed that the results of liquid rhizobial formulations for the growth and yield of crops are more suitable than that of peat-based products. It was evaluated by using polymers like trehalose along with Jensen's basal medium (Jensen H. 1954). The results are depicted in following tables. Data was tabulated and analyzed statistically, presented in graphical form also. Azotobacter cells in Jenson's broth alone were used as control for liquid formulation. The trehalose was tried in formulation to understand the survival life of Azotobacter and interaction between Azotobacter and formulation materials.

Effect of Trehalose on survival of *Azotobacter chroococcum*:

To increase the shelf life of Azotobacter in liquid formulation the trehalose, was added as supplements to Jensen's broth. This experiment was conducted to test the effect of trehalose on the shelf life of Azotobacter in liquid formulations maintaining proper viable count for longer period of time.

1. Effect of different concentrations Trehalose on survival of *Azotobacter chroococcum A1*: Table No A and B , graph No 1)

Trehalose (mycose/tremalose) is a disaccharide, non-reducing sugar which contains two molecules of glucose. Some plants, bacteria and plants use it for survival under water stress. Different concentrations viz. 10mM to 25mM were prepared and added to the culture of *Azotobacter chroococcum* grown in Jensen's medium. Effect of all concentrations on survival of *Azotobacter* colonies was significant at 0.05% probability level. On zero days highest number of colonies was recorded in medium containing 25mM Trehalose followed by 20mM, 15mM, 10mM and 5mM Trehalose. Survival of the Azotobacter was maintained up to 360 days. It was noted that survival of Azotobacter was concentration correlated. Lowest number of colonies (28.66 on zero day, 3 on 360^{th} day) were observed in formulation containing 5mM Trehalose and highest number of colonies were found (40.66 on zero day,14.00 on 360^{th} day) in formulation containing 25mM Trehalose (10^{-9} CFU/ml). Santhosh (2015) recorded similar results. Regularly Azotobacter colonies in pure broth could be maintained up to 270 days only. In the present work the colonies were remained survive up to 360 days. The trehalose is as enigmatic compound acts as reserve carbohydrate that, may be mobilized during stress (Hounsa et.al.,1988). It is reported to enhance the cell tolerance to desiccation, osmotic and temperature stress. It acts by stabilizing both enzyme and cell membrane (Fillinger et al.,2001) The possible effect of trehalose protective action is that it may be incorporated in to the cell or may induce the synthesis of metabolites that protect against stress(Gomez et al.,2003) which might be the reason for the higher population of Azotobacter cells in trehalose treatment. The presented results were supported by the previous work of Kumaresan and Sivakumar (2019). They stated that Liquid Azotobacter and *Azospirillium* bio-inoculants formulated with trehalose (10mM) promoted long term survival of Azotobacter and *Azospirillium*. In the present work 25mM Trehalose was used as cell protectant which gave better results of Azotobacter survival better than other workers.

IV. Conclusion

It was concluded that, survival of Azotobacter was concentration correlated. Lowest number of colonies (28.66 on zero day, 3 on 360^{th} day) were observed in formulation containing 5mM Trehalose and highest number of colonies were found (40.66 on zero day,14.00 on 360^{th} day) in formulation containing 25mM Trehalose (10^{-9} CFU/ml).

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