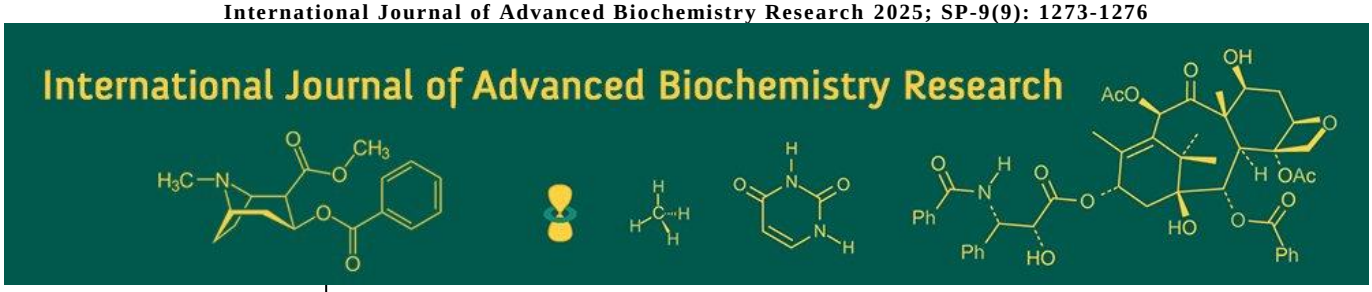

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Liquid formulations of *Bacillus megaterium* and their effects on population shifts and survival studies

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Abstract
Research was conducted to know the significance of liquid formulations with different osmolytes in *Bacillus megaterium.* Growth pattern in *Bacillus megaterium* was plotted by following serial dilution plate count method, that showed the attainment of stationary phase after the 24 hrs of incubation period and further population was decreased after 24 hrs. also its seen that highest population were recorded and continued up to 300 days in the medium containing PVP K-15 + glycerol (log_{10} 8.3 CFU/ml) followed by PEG 6000 + glycerol (log_{10} 6.3 CFU/ml) followed by carrier based inoculant (log_{10} 5.07 CFU/ml). It is seen that the liquid formulations have performed better and also their phosphorus solubilization and in plant growth promotion. Population studies indicate that the inclusion of osmoprotectants during media preparation gave good results.

Keywords: Bacillus megaterium, liquid formulations, osmolytes, population dynamics, survival studies, phosphorus solubilization, plant growth promotion, osmoprotectants

Introduction
Liquid biofertilizer technology offers compelling reasons for its adoption, emphasizing the use of agriculturally important microorganisms in liquid formulations. These formulations contain not only the important microbes and their active ingredients, but also gives protection against harsh environmental conditions thus gives improved shelf life to the cells, and making them ideal for field application. Different approaches exist for developing these liquid formulations, with the common goal of ensuring storage stability, easy delivery, increased persistence, and protection from environmental factors (Lee et al., 2016) [3].
Different types of polymeric additives are being used in liquid inoculant formulation, because they controls the transfer heat into the microbial cells and ability to deform flow under stress condition with high water activities (Mugnier J and Jung G (1985) [5]. The polymeric substances, like, methyl cellulose, gum arabic, polyvinyl pyrrolidone (PVP) and alginate are being normally used as binding agents along with solid based carriers for seed treatments. In the present investigation few of the above mentioned polymeric additives were used get the expected population and also attaining the liquid formulations in a newer perspective as that of holding the efficiency of carrier based formulations.

Materials and Methodology
Isolation of *Bacillus megaterium*
For phosphate solubilizing *Bacillus megaterium*, obtained from the previous study, purified and maintained on modified Sperber's medium.

Optimization of ideal concentration of osmolytes
A study was done to find out the concentration at which the population reaches highest among different osmolytes. In this experiment 4 series of PVP (polyvinyl pyrrollidone), 4 series of polyethylene glycol (PEG) and glycerol at three different concentrations (0.5%, 1.0% and 2.0%) were used along with a control. Flasks having 100 ml broth and at 3 different levels of additives was inoculated with *Bacillus megaterium* culture and incubated. After the incubation period is over (24hrs) serial dilution of upto 10^8 was taken up and colony counts were recorded for each sample.

Optimization of ideal time of addition of osmolytes

To determine the time of addition of osmolyte an experiment was done either at initial or during the stationary phase. From the earlier studies, the concentration which gave maximum population was chosen from each series of osmolyte and was used.

Two methods of experiments were carried out in this aspect. In one method comprised of eight flasks filled with hundred ml broth medium along with the selected concentrations and further inoculated with 24 hour old culture and incubated for about 72 hours. After the incubation period is over, serial dilution plate count technique was followed to record the population count

Similarly, in the second method of experiment, the required osmolytes were sterilized and added into the flasks containing broth cultures only at the stationary phase of the bacterial growth and allowed to incubate up to 72 hrs. Then the population was recorded by following the serial dilution and spread plate method.

Shelf life studies

Preparation of liquid based inoculant

Lignite based *Bacillus* formulations were prepared according to the procedure detailed by Dayamani et al., 2025 [2] and periodically serial dilution was done up to 300 days by spread plate technique and population data was recorded.

Preparation of carrier based inoculant formulations

Lignite based *Bacillus* formulations were prepared according to the procedure detailed by Dayamani et al., 2025 [2].

Results and Discussion

Study of optimum concentration of the osmolytes

PEG-6000 and PVP K-15 at 2% percent gave the highest *Bacillus megaterium* population (CFU 98x10^7/ml).

Plotting the growth curve

Growth pattern was plotted to get the information on reaching the stationary phase of organism by plate count technique. It has shown that population reaches the stationary phase at 24 hrs after inoculation and further population was declined after 24 hrs (Fig. 1). This trend is likely due to the readily availability of nutrients in the nutrient broth and various thickeners, which initially support bacterium growth (Dinesh et al., 2020). These results show that, there is no significant difference among the two different stages of adding the osmoprotectants in the medium. Further it can be concluded that it is better to add up the osmolytes at the time of media preparation, so that can avoid contamination, and process may become simple. These results are supported with Cassidy et al., 1996 [1], reports that, in liquid inoculant formulations, microbial cells come across nutrient stress. Frequency of decrease in viable cells varies with the different strains and also formulation. It is proved that the survival potential of cells was dependent on both the additive types and individual organism responding to the stress.

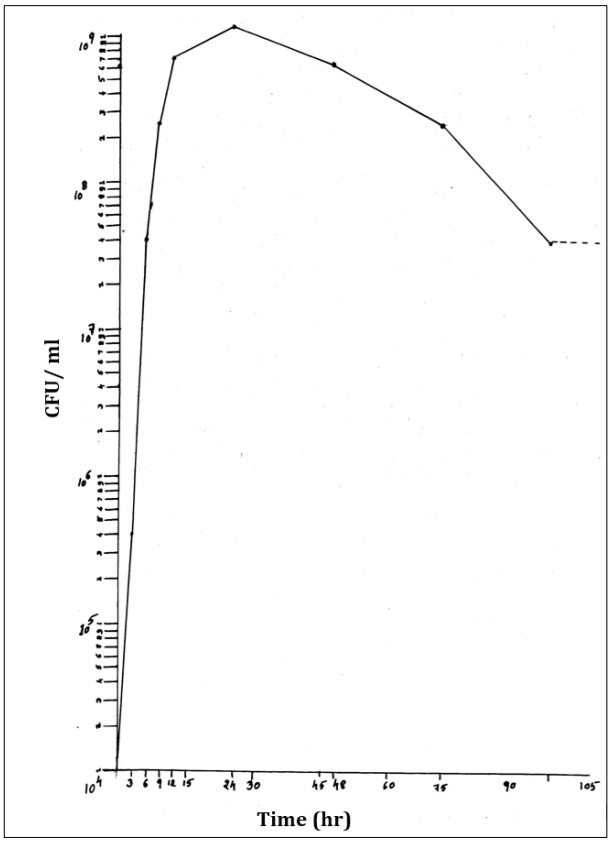

Fig 1: Growth curve of *Bacillus*

Population density of *Bacillus megaterium* as a response by the addition of osmolytes

Table 1 indicates the medium containing different osmolytes to record the population density of *Bacillus megaterium*. From the present investigation, PVP K-15 showed the maximum population in all the three concentrations compared to other osmolytes and control followed by PVP K-90 at 2percent concentrations and PVP K-90 stands next

to this having the population of CFU $63x10^7$/ml at percent concentrations followed by PVP K-25 at 1percent with the population of CFU $54x10^7$ /ml. PEG 400 has showed least population among the osmolytes and PEG 600 has showed less population compared to control at 2percent concentration (Fig 2). These results are supported by Senior et al., 1996. So, quality of microbial inoculants depends primarily on the number of viable cells in the inoculants (Lupwayi, et al., 2000) [4].

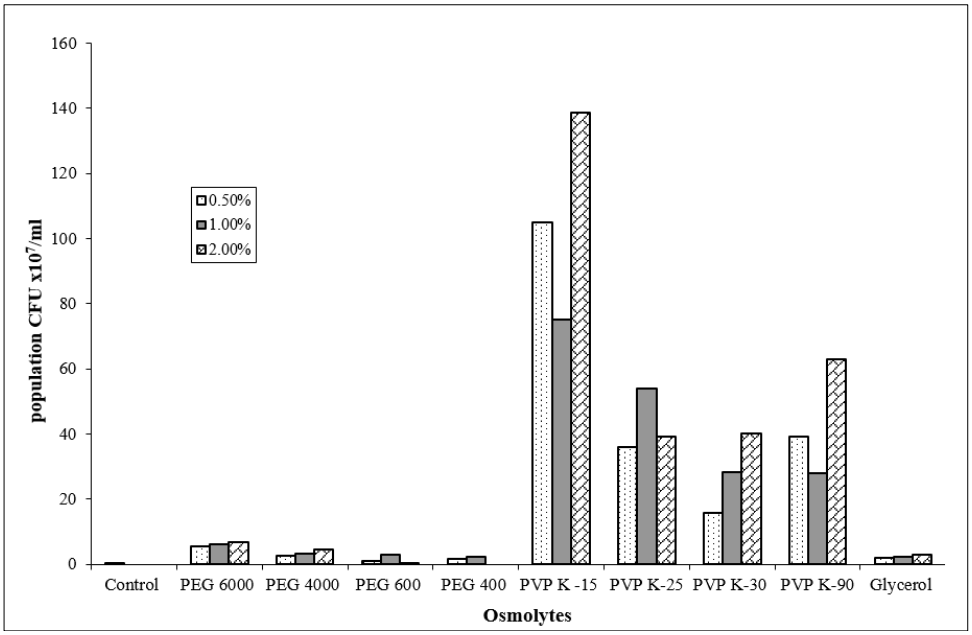

Fig 2: Population density of *Bacillus megaterium* as influenced by the addition of osmolytes in minimal medium

Table 1: Population density of *Bacillus* used for survival studies

Osmolyte concentrations	CFU x 10^8 / ml
Control	56.0
PVP K -15 (2%)	40.0
PEG 6000 (2%)	98.0
Glycerol (2%)	48.0
PVP K -15 (2%) + Glycerol (2%)	208.0
PEG 6000 (2%) + Glycerol (2%)	2.0

Shelf life studies

Population shifts in *Bacillus megaterium* in different inoculant formulations

Changes in population pattern of *Bacillus* in 4 different liquid formulations were studied by comparing with lignite formulation and control up to 300 days of storage (Fig. 3). It is observed that, population density was constant up to initial 30 days in all the formulations and begin to decline slowly. Highest population was observed and was continued up to 10 months in the medium containing PVP K-15 + glycerol (log_{10} 8.3 cfu/ml) followed by PEG 6000 + glycerol (log_{10} 6.3 cfu/ml) followed by lignite based inoculant (log_{10} 5.07 cfu/ml). Studies by Sridhar et al. (2010) [6] supported the role of glycerol in liquid formulations, particularly in maintaining the viability of cells during extended storage periods. Glycerol, along with PVP and glucose, was found to support higher populations of live bacteria and endospores for up to six months of storage.

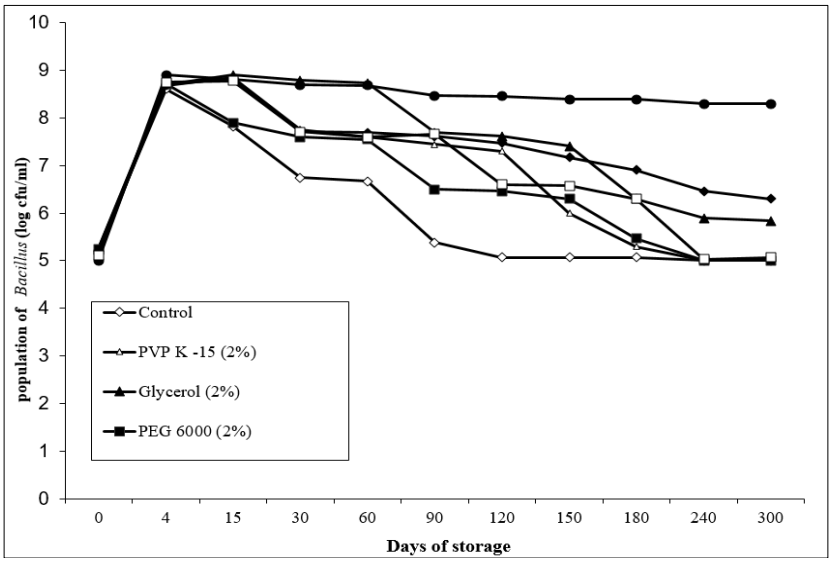

Fig 3: Population dynamics of *Bacillus megaterium* in different inoculant formulations

Conclusion

Maximum population was recorded and was continued up to 10 months of time in PVP K-15 + glycerol (log_{10} 8.3 CFU/ml) followed by PEG 6000 + glycerol (log_{10} 6.3 CFU/ml) followed by carrier based inoculant (log_{10} 5.07 CFU/ml). The results from the present study reveals that the liquid formulations of *Bacillus megaterium* performed better and also they give equal performance level with that of lignite based formulations in phosphate solubilization and plant growth promoting activity.

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