Impact of different carrier material on the viability of *Nostoc* sp. HNBGU 006

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ABSTRACT

Selection of a suitable carrier is the biggest challenge in the successful implementation of bioformulation in agriculture sector. The present study was conducted to evaluate the impact of five different low-cost carrier (neem leaves powder, curry leaves powder, fuller's earth, soil and sand) on the viability of cyanobacteria. The physicochemical properties of all carriers were investigated. Highest Water Holding Capacity (WHC) was recorded in curry leaves powder (183.58%) followed by neem leaves powder (90.94%). The carriers were integrated with the cyanobacteria and the formulation were stored in room temperature for four months. The viability of cyanobacterial cellswas studied by measuring the chlorophyll content of the formulation on monthly basis. The highest increase in chlorophyll content was recorded in neem leaves powder (320%) followed by curry leaves powder (271.53%) and sand (5.12%). Thus, the present investigation highlights the possibility that neem leaves powder can be a suitable carrier for cyanobacterial bioformulation that can be used to enhance the agriculture production.

Keywords: Cyanobacteria, carrier material, bioformulation, biofertilizer

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INTRODUCTION

Plant growth promoting bacteria (PGPB) are considered as soil engineers because they are capable of creating an environment of nutrient rich soil. They are helpful to maintain the soil health and also, enhance the stress tolerance ability of plants (1). It is known from quite a long time that PGPB inoculation in the soil leads to enhancement in crop productivity (2). The integrated nutrient management practices also promote the use of PGPB as source of biofertilizers to reduce our dependency on chemical-based fertilization processes so as to maintain the sustainability in agriculture without compromising productivity (3). These biological substitutes of chemical fertilizer are being continuously encouraged due to its economic feasibility, self-sufficiency and more appreciably, natural origin (4),(1). For example, cyanobacteria have been considered as the renewable biological sources of nitrogen and are immensely used as a biofertilizer in crops much pronounced in rice (5).The use of these organisms to enhance the soil fertility and ameliorate crop productivity is termed as green technology (6).

One major problem with microbial inoculation in the soil is that the bacterial population shrink rapidly if they are inoculated in soil without a proper carrier. The inability of bacteria to sustain in the rhizosphere environment, poor biomass production, physiological state at the time of application, all these factors are responsible for failure of bacterial establishment in the rhizosphere (2). There is always a threshold number of bacterial cells that must be reached by bacterial population so as to impart positive responses in the soil and plant. This number may vary from species to species. Sometimes the application of bacterial inoculum directly into soil do not exhibit expected improvements due to poor handling, short shelf life etc. These are the reason behind the less acceptability of biofertilizer technology by farmers (7).

Thus, to create an appropriate environment intended to provide protection for extended period so as to avoid the depletion of bacterial population, bioformulations of inoculant may be prepared. Formulation is a process in which a bacterial strain is unified with a suitable carrier, any abiotic substance in the form of solid, liquid or gel (2). There are several advantages of integrating PGP microorganisms into a carrier material as it imparts easy handling, provides storage for a longer duration and upgrade the effectiveness of biofertilizer. However, to utilize the potential of bioformulation at its fullest and to support the growth and delivery of inoculants in soil, it is imperative to select suitable carrier. The carrier material should be cheap, easy to handle and sterilize, easily available, should have good moisture absorbance capacity (8). Bacterial inoculants that are integrated with appropriate carrier materials are highly efficient (7). They are also known as biotechnological formulations. The concentration of microbial inoculant in the bioformulation should be large so as to colonize the plant (4).

Many carriers have been used for bacteria inoculants. However, there is less study on suitable carrier for cyanobacteria. Moreover, the suitability of carrier substance differs from species to species

(4). Selection of carrier that do not support the growth and survival of inoculum in the bioformulation may result in the failure of exhibition of desired results in the field. So, focusing on the aforementioned problem, the aim of the present study was to examine the suitability of five different low cost and easily available carriers for bioformulation of heterocystous cyanobacterium, *Nostoc* sp. HNBGU 006. By the selection of proper carrier for cyanobacteria, a desirable environment for the survival and efficient inoculation of cyanobacteria in the soil can be developed. The carriers used in the present study were neem leaves powder, curry leaves powder, soil, fuller's earth (Multani mitti) and sand.These were selected on the basis of their ease of availability. The survivability and stability of cyanobacteria in the respective carrier was observed for a duration of four months and viability study was done on monthly basis by quantifying the chlorophyll content as measure of cyanobacterial proliferation in respective carriers.

MATERIALS AND METHODS

Test organism and cultivation conditions

The cyanobacterium, *Nostoc* sp. HNBGU 006, was selected for the present bioformulation study on the baisis of its in vitro and in vivo plant growth promotion potential in a previous study (unpublished data). The test organism was cultivated in BG11° medium in a culture room at 27 ± 2°C and illuminated with 90 μM photons m^{-2} s^{-1} using white fluorescent lamps under light: dark cycles of 16:8 h (9).

Physicochemical properties of carrier materials

Five different low-cost and easily available material including neem leaves powder, curry leaves powder, soil, fuller's earth and sand were selected to evaluate their potential as a carrier for cyanobacterial bioformulation. The electrical conductivity (EC) and pH of these materials were measured according to the methodology of Page et al.(10). Briefly, the material was suspended in distilled water in the ratio 1:2 and the conductivity of the suspension was measured using conductivity meter (EI 181) Similarly, pH of the suspension was measured using the pH meter (Delux pH meter-EI 101). To determine the water holding capacity (WHC) of different carrier, a known amount of carrier was allowed to saturate with water and left overnight to drain the excess water. The WHC was determined by the formula by Yu et al. given below (11).

Water Holding Capacity (%)- $(mass_{wet} - mass_{dry})/mass_{dry} \times 100\%$

The moisture content of the carrier materials was determined as, 10 g of each carrier material was dried at 70°C for 24 h and was weighed again (12). The moisture content was calculated using the formula-

$M= [(m_1-m_2)/m_2]\times100$ where m_1 = mass before drying, m_2= mass after drying

Preparation of bioformulation

All the carrier material were dried at 70°C for 24 h before use. Curry and neem leaves were ground into fine powder with the help of mixer grinder. The carriers were sterilized properly and mixed with freshly harvested exponential phase cyanobacterial biomass in equal proportion (1:1). The mixing was done in sterile petri plates with the help of sterile glass rod to allow uniformity in the mixture. The mixture was left for curing process in laminar flow hood for 24 hours (13). The resulting slurry was dried, powdered, aliquoted in sterile transparent polythene bags of size (10×7 cm) and sealed properly (5). The polybags were filled up to only three fourth volume to provide proper aeration to the live cells present in bioformulation (14). All the bags were stored at room temperature for four months.

Viability study of bioformulations

The samples were taken periodically for four consecutive months to determine the stability and viability of cyanobacterial cells in different carriers under study by measuring the chlorophyll content of bioformulation. The chlorophyll content is considered as an index of cyanobacterial growth and viability.For this, one gram of sample was scooped out from each bioformulation preparation with help of sterile spatula and was suspended in 4 ml of solvent mixture, acetone: dimethyl sulphoxide (DMSO) (1:1). The solution was shaken thoroughly, incubated in the dark for 48 h at room temperature, filtered with Whatman filter paper and absorbance was measured at 663, 645 and 630 nm by spectrophotometer (EI 2371) (15). The chlorophyll content in the bioformulation was calculated as per the following formula-

$$\text{Chlorophyll (mg/g)} = (11.64\times OD_{663}) - (2.16\times OD_{645}) + (0.10\times OD_{630})$$

Statistical analysis

Statistical analysis wasperformed using the IBM SPSS software trial version 29.0.0.0 (241). One-way analysis of variance (ANOVA) was performed to evaluate the impact of incubation time on cyanobacterial bioformulation. LSD test was used for determination of data significance ($p < 0.05$).

RESULT AND DISCUSSION

Analysis of physicochemical properties

The physicochemical parameters like pH, electrical conductivity (EC), water holding capacity (WHC) and moisture content of any carrier material have a profound impact on the stability, viability and survival of bio-inoculant that is amalgamated with that particular carrier material (16). Table 1. provides the details of the physicochemical properties of the all the carriers that were used in the present study. The results revealed that the carriers were having nearly neutral pH, 6.1-6.7 in nature except for the fuller's earth that was having slightly acidic pH, 5.2. Similar pH was recorded by Maheshwari et al. (19) that investigated soil as a carrier. The pH conditions that are near to neutral supports the growth of huge bioinoculants and responsible for maintaining their viability. Similarly, EC signifies the concentration of soluble salts present in the carrier which is another major factor that

influence the viability (13). The electrical conductivity of the carriers in the present study ranged between 0.008-0.917 dS/m. The highest EC was found in neem leaves powder while the lowest was observed in sand. The moisture content and WHC recorded in the present investigation were also in the acceptable range for carriers. The highest inherent moisture content and WHC were recorded in neem leaves powder (1.59 and 90.94%) and curry leaves powder (1.54 and 183.58%) as compared to other carriers in this study. The desirable WHCin carrier is known to be more than 50% as enzymatic processes are induced by high WHC that results in the organic material degradation. These organic matters serve as a nutrient for bacterial growth (17). The inherent moisture content of the carriers was 0.06-1.59% with the highest being in neem leaves powder. The results indicate that these materials having less than 30% moisture content are suitable for carrier due to their easy transportability and applicability (18). The physicochemical properties of all the selected carrier materials investigated were in desirable range.

Evaluation of viability of bioformulation

The viability study of bioformulations was done by measuring its chlorophyll content at different time interval after preparation. Figure 1. (a-e) shows the chlorophyll content of different carriers measured on monthly basis up to four months. The chlorophyll content measured in neem leaves powder, curry leaves powder and sand on the day of inoculation (0^{th} day) was 61.98 mg/g, 18.76 mg/g and 0.78 mg/g respectively. However, at the end of storage period (120^{th} day), the chlorophyll content increased up to 260.52 mg/g, 69.7 mg/g and 0.82 mg/g respectively. The survival of any organism in the formulation is dependent on the nature of carrier material, the type of organism integrated with the carrier, the physiology of organism and the storage conditions. The carrier material should be sterilized properly before being used as each carrier material has its own microbial flora that can hamper the growth of inoculant resulting in low quality product (18). It was observed that there was significant enhancement in the chlorophyll content on 30^{th} day in all the carriers except for curry leaves powder and sand where reduction in chlorophyll content was observed on 30^{th} day. In case of neem bioformulation, the chlorophyll content increased significantly up to 120^{th} day. The overall enhancement in chlorophyll content from beginning to the end of storage period was recorded to be 320% which was highest among all other carriers. Similarly, Jha and Prasad(5) selected neem as one of carrier for cyanobacterial bioformulation and was found to be effective in enhancing the rice yield in field experiment. The maximum chlorophyll content in soil and fuller's earth was measured on 30^{th} day after which there was reduction in chlorophyll content.The desired population of inoculant even after long storage period can be achieved by enhancing the inoculum concentration at the time preparation of bioformulation (5). Similar findings were reported by (14) where reduction in *Pseudomonas fluorescens* population was observed in soil bioformulation. However, in a field experiment undertaken by (20), highest rice yield was observed in fuller's earth and cyanobacteria bioformulation.There was significant enhancement in chlorophyll content of neem leaves powder,

curry leaves powder and sand bioformulation in which 320%, 271.53% and 5.12% respectively was observed at 120th day.

So, it was observed from the study that the maximum increase in chlorophyll content was supported by neem formulation at the end of storage period. Even low amount of biomass of bioinoculant in the bioformulations exhibited enhanced chlorophyll content at the end of storage period. All the bioformulations in the present study were stored at room temperature that makes the storage economically feasible as refrigeration facilities are not available in most of agricultural systems (13).

CONCLUSION

Concluding the present study, highest increase in chlorophyll content recorded in neem bioformulation suggests that it could be a suitable carrier for the cyanobacterial inoculant *Nostoc* sp. HNBGU 006. The physicochemical properties of this carrier turned out to be an optimum condition that promoted the growth of the inoculant under the period of observation. However, a long-term field study is required to further confirm and validate the findings. These bioformulation can be highly useful in enhancing the agricultural production as it would support the growth of inoculant and can overcome the problem of decline in population due to improper establishment in soil.

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Conflict of interest

None

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Table 1: Details of physicochemical properties of different carriers

S.No	Carrier	pH	Electrical conductivity (dS/m)	Inherent moisture (%)	Water holding capacity (%)
1.	Neem	6.5	0.917	1.594	90.943
2.	Curry	6.0	0.698	1.543	183.576
3.	Soil	6.7	0.015	0.2	8.747
4.	Fuller's earth	5.23	0.803	0.06	86.753
5.	Sand	6.09	0.008	0.12	12.641

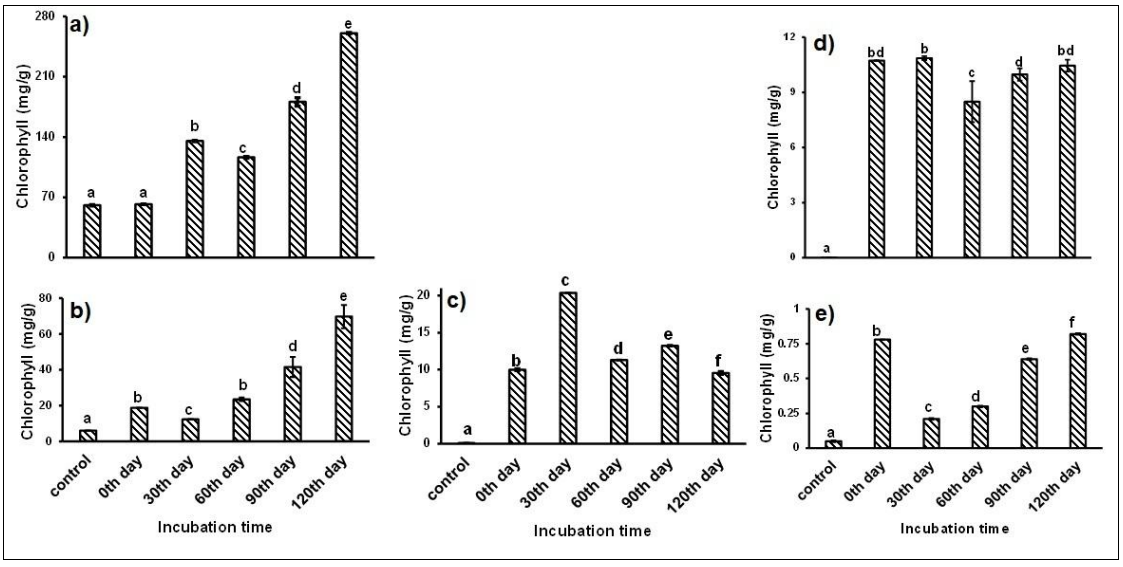

Figure 1: The chlorophyll content of cyanobacterial bioformulation in a) Neem leaf powder b) Curry leaf powder c) Soil d) Fuller's earth e) Sand. The values marked with different letters are significantly different from each other as determined by LSD ($p< 0.05$).