Assessing the Efficacy of Different Formulations of Organic Granules Immobilizing Azotobacter chroococcum and Bacillus subtilis on Productivity and Yield of Wheat (Triticum aestivum L.) at Different Water Regimes

Pawan Kumar¹ and Rana Pratap Singh¹*

ABSTRACT

Microbial biofertilizers can be effective alternatives to fulfill plants’ nutritional requirements, as chemical fertilizers are unsustainable and pose a threat to the environment and human beings. However, farmers hardly adopt these bioinoculants due to the uncertainty of their effectiveness in field conditions. This study used porous dry organic materials from agro-waste and process byproducts from agro-industries that can act as suitable carriers and protect the microbes during storage, marketing, and field application. Various formulations were made for Azotobacter chroococcum and Bacillus subtilis by a combination of Pressmud, cow dung manure as the organic matrix, jiggery, molasses, and serous gum as the binder, and clay as the stabilizer. Azotobacter chroococcum and Bacillus subtilis increased straw and grain yield in wheat (Triticum aestivum L.) over the unimmobilized biofertilizers and other matrixes and binders in irrigated as well as water-stressed cropping conditions. The immobilized biofertilizers significantly increased soil fertility and nutrient availability compared to the unimmobilized PGPR. The formulation IBF-VI showed 35.9 and a 61.21% increase in grain and straw yields, respectively, over unimmobilized PGPRs. Interestingly, the biofertilizers immobilized in the organic matrix have supported similar grain and straw yield recorded for the synthetic chemical fertilizer’s urea and DAP. The performance of selected immobilized biofertilizers was further examined under water stress. The studies show that immobilizing microbial biofertilizers in waste press mud and molasses for granule production improves the performance of microbial biofertilizers. These compounds are abundant and inexpensive, and small-scale entrepreneurs can supply them on modest scale. The findings provide a new potential for producing and marketing effective bioformulations in rural areas through small industrial setups.

Keywords: Organic matrix, PGPR, Immobilized biofertilizer, Water regime

INTRODUCTION

The use of agrochemicals, including synthetic fertilizers, increased manifold after adopting the green revolution in agriculture. The excessive loading of chemical fertilizers has caused a reduction in the soil biodiversity, increased the cultivation cost, and induced toxicity causing health hazards to animals and human consumers (Tal 2018). Though the availability of plant nutrients during cropping is essential for a good crop yield, synthetic fertilizers are no longer considered ecologically sustainable and in tune with the emission cuts and achieving sustainable development goals. Organic fertilizers, bio-fertilizers with plant growth-promoting rhizobacteria (PGPRs), slow-release fertilizers, etc., have been developed as possible alternatives to lessen chemical fertilizers (Chakraborty and Akhtar, 2021; Rai et al., 2017). Soil-born beneficial microbes have been considered a viable alternative to chemical fertilizers which can maintain increased soil fertility and good crop yield sustainably (Hafez et al., 2021).

In recent years, biofertilizers are becoming a vital part of the integrated nutrient delivery system to increase crop output ecologically (Batista and Singh, 2021). Multiple strains of Azotobacter, Azospirillum, Acetobacter, Bacillus, Bradyrhizobium, Pseudomonas, and Rhizobium, etc., have been employed as bio-fertilizers for cereals, pulses, vegetables, oilseeds, cotton, sugarcane, and some other crops (Kumar and Singh, 2021). However, these bioinoculants have not been adopted by the farming community on a large scale possibly due to the problems related to their efficacy in different agro-climatic conditions. Microbial fertilizers require specific environmental conditions in order to survive and develop. These conditions include temperature, moisture, pH, and nutrient availability. If these conditions are not met, the microorganisms’ development and activity may be hampered (Kumar and Singh, 2021). Together with microbial fertilizers, other bacteria in the soil

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may compete. If the soil already contains a large population of microorganisms, additional microbial fertilizers may be difficult to establish themselves and so be useless. Most important if the fertilizer is not applied appropriately or at the appropriate time, it may fail to establish itself in the soil and provide the desired benefits. The viability, effectiveness, and ecological successes of these PGPRs after their application in the crop fields have yet to be extensively studied (Chojnacka et al., 2020; Kumar and Singh, 2021). The application of carriers may provide specific microenvironments and protection to these microbes during storage and field application and may increase the effectiveness of biofertilizers by elongating the shelf life (Maček et al., 2020). A good carrier material should be non-toxic, efficient moisture absorber with good buffering capacity, easy to process, and inexpensive with easy availability (Kumar et al., 2012, 2015; Kumar et al., 2014; Sohaib et al., 2019). Rice husk, farmyard manure (FYM), Pressmud, charcoal, peat, and lignite have been reported to be suitable carrier materials (David et al., 2018).

Pressmud is a solid waste by-product produced at a rate of 3% for every ton of sugarcane crushed in a sugar mill during the clarification of cane juice. It can be used as organic fertilizer as it possesses a good amount of organic carbon, phosphorus, NPK, and other micronutrients (Sahu, 2018). The sugarcane industry dumps it out due to the storage problem and causes nuisance and environmental pollution. Though Pressmud is non-toxic organic waste, available in large amounts, has good moisture absorption capacity, is sterilizable, full of nutrients, and is cost-effective. These properties make it a suitable carrier for the beneficial soil microbes used as bioinoculants (Rawat et al., 2020). The molasses (pH approx. 6) is another viscous, dark, and sugar-rich byproduct of sugar refinery consisting of sucrose (32%), glucose (10.5%), fructose (8%), nitrogen (0.98%), vitamins and trace elements (Eliodório et al., 2019 and Garcha et al., 2019). The waste molasses is acidic, dark brown, and has high chemical and biochemical oxygen demands (BOD) (COD) (De Godoi et al., 2019) and hence is considered a notorious byproduct if dropped into the environment as waste. We have used cowdung, acacia gum, vermicompost and some other local agrowaste to prepare PGPR immobilized nutritional granules which have increased the productivity of wheat, rice, mustard and tomato in the experimental plots (Dahiya et al., 2004; Kumar et al., 2014; Ashok et al., 2015; Minj and Singh, 2015; Rai et al., 2017).

This study was undertaken to design granules with Pressmud and immobilizing the commercially available biofertilizers A. chroococcum and B. subtilis to provide a nutritious coat to the microbes, which may act as a buffer zone, and reduce negative impacts of the environmental stress such as pH, water, temperature, and salinity etc. in the field conditions. The key objective of this study is to find a non-toxic organic carrier of local origin available in bulk to small-scale entrepreneurs to enhance the effectiveness of bioinoculants under environmental stresses. It is intended to empower small-scale entrepreneurs and small-scale landholders to develop cooperative efforts to prepare efficient bioinoculants for local uses.

**Material And Methods**

**Experimental Design**

The experiments were carried out at Babasaheb Bhimrao Ambedkar University’s environmental field station in Lucknow, India. Lucknow is situated at latitudes 26.30 and 27.10 North and longitudes 80.30 and 81.13 East, 123 meters above sea level. It experiences a cool, dry winter from December to February and a warm subtropical climate overall. A local trader in Lucknow provided the certified wheat seeds (Triticum et al. cv. PBW-343). The trials were carried out in two consecutive years across two winter seasons, 2017-2018 and 2018-19. The experimental design included multiple treatments; each duplicated three times in a randomized block pattern (RBD). The field blocks were maintained in a 6 m² (4 x 1.5) rectangle.

**Immobilization of Biofertilizers in Organic Matrices**

All gathered materials (cow dung manure, Pressmud, and clay) were dried individually in a 60-70°C oven for three days before being powdered in a grinder and mixer. Biofertilizers were immobilized using these materials as a supporting matrix. Biotech Park, Lucknow, provided the biofertilizers such A. chroococcum and B. subtilis immobilized in charcoal as a carrier. These supporting matrices were mixed in a 1:1 ratio, i.e., 112.5 kg ha⁻¹ clay soil and 112.5 kg ha⁻¹ (cow dung manure or Pressmud) and as a binder, Acacia gum (commercial saresh), jiggery, and molasses were used. The thick aqueous binder paste was made, and the mixture was blended and constantly agitated. A total of 303.6 kg ha⁻¹ of IBF granules containing biofertilizer were applied as a basal application at the time of sowing. All treatments’ detailed layouts are shown in Table 1.

**Table 1:** The detailed layout of commercial chemical fertilizer, organic matrix immobilized chemical fertilizer and organic immobilized commercial biofertilizers treatments are as follows:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Detailed layout of treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF</td>
<td>No fertilizers</td>
</tr>
<tr>
<td>CCF</td>
<td>Recommended dose (RD) of commercial chemical fertilizers (Urea 150 kg ha⁻¹ and DAP 75 kg ha⁻¹)</td>
</tr>
<tr>
<td>UBF</td>
<td>Bio-fertilizer i.e., Azotobacter chroococcum (1.8 kg ha⁻¹) and Bacillus subtilis (1.8 kg ha⁻¹) applied in experimental plots.</td>
</tr>
<tr>
<td>IBF-I</td>
<td>Organic matrix immobilized bio-fertilizer (303.6 kg ha⁻¹) containing clay soil (112.5 kg ha⁻¹) + Cow dung manure (112.5 kg ha⁻¹); 1:1 with 25% binder saresh gum (75 kg ha⁻¹) + UBF applied in experimental plots.</td>
</tr>
<tr>
<td>IBF-II</td>
<td>Organic matrix immobilized bio-fertilizer (303.6 kg ha⁻¹) containing clay soil (112.5 kg ha⁻¹) + Pressmud (112.5 kg ha⁻¹); 1:1 with 25% binder saresh gum (75 kg ha⁻¹) + UBF applied in experimental plots.</td>
</tr>
<tr>
<td>IBF-III</td>
<td>Organic matrix immobilized bio-fertilizer (303.6 kg ha⁻¹) containing clay soil (112.5 kg ha⁻¹) + Cow dung manure (112.5 kg ha⁻¹); 1:1 with 25% binder jiggery (75 kg ha⁻¹) + UBF applied in experimental plots.</td>
</tr>
<tr>
<td>IBF-IV</td>
<td>Organic matrix immobilized bio-fertilizer (303.6 kg ha⁻¹) containing clay soil (112.5 kg ha⁻¹) + Pressmud (112.5 kg ha⁻¹); 1:1 with 25% binder jiggery (75 kg ha⁻¹) + UBF applied in experimental plots.</td>
</tr>
<tr>
<td>IBF-V</td>
<td>Organic matrix immobilized bio-fertilizer (303.6 kg ha⁻¹) containing clay soil (112.5 kg ha⁻¹) + Cow dung manure (112.5 kg ha⁻¹); 1:1 with 25% binder Molasses (75 kg ha⁻¹) + UBF applied in experimental plots.</td>
</tr>
<tr>
<td>IBF-VI</td>
<td>Organic matrix immobilized bio-fertilizer (303.6 kg ha⁻¹) containing clay soil (112.5 kg ha⁻¹) + Pressmud (112.5 kg ha⁻¹); 1:1 with 25% binder Molasses (75 kg ha⁻¹) + UBF applied in experimental plots.</td>
</tr>
</tbody>
</table>
All combinations in Table 1 were irrigated at Crown root + Tillering + Booting + Earing + Milking stages of wheat for Normal irrigation level. The treatment NF, UBF, and IBF-VI were also kept on water regimes 1 (Crown root + Booting + Earing + Milking) and 2 (Crown root + Booting + Milking).

Maintenance of Crop Growth Conditions
Wheat (Triticum aestivum L.cv. PBW-343) seeds were sowed at a depth of 4-5 cm, and thinned plants were carried out after 25 days of sowing. A distance of 10 cm was maintained between the plants. Hoeing-cum-weeding was done after one month of sowing. The crop was harvested when the color of the silique changed from green to light yellow. After proper labeling, the harvested crop was tied in bundles and kept for sun drying.

Measurement of Root and Shoot Length, Number of Leaves, Number of Roots, Fresh and Dry Plant Weights
A meter scale measured root and shoot length in plants at 40, 80, and 120 days after sowing (DAS). The number of leaves and roots was physically counted at regular intervals. Plant components were carefully removed from growing plants, cleaned in deionized water, and blotted on filter paper to dry. A single-pan electronic balance was used to determine the fresh weight of roots and shoots. The tissues were oven dried at 70°C until they reached a consistent dry weight.

Measurement of The Yield Parameters
Grain and straw yield (t ha−1): Each plot’s grain and straw yield were recorded in tones after cleaning the threshold produced and expressed in t ha−1.

Estimation of Nitrate, Nitrite, Ammonium, Phosphate, Potassium, and Organic Carbon Content In Soil and Plant Tissues
The nitrate content of soil and leaves was determined using a 5 percent salicylic acid solution in concentrated sulfuric acid and 2N sodium hydroxide, as described by Cataldu et al. (1975). Stevens and Oaks (1973) used a homogenate of the material with sulphanilamide and N-(1-Naphthyl)-ethylenediamine dihydrochloride to determine the nitrite concentration in soil and leaves. Weatherburn (1967) described a method for estimating ammonium content in soil and leaves that used Nessler’s reagent. The Dickman and Bray (1940) method was also used to determine the phosphorus content of soil and leaves. A UV-visible spectrophotometer measured the absorbance of nitrate, nitrite, ammonium, and phosphate solutions at 410, 540, 420, and 680 nm, respectively. A flame-photometer was used to estimate the potassium content of soil (Osborn and Johns 1951). Some other parameters, i.e., pH and conductivity, were measured with the help of pH and conductivity meters, respectively. The Walkley-Black (1934) chromic acid wet oxidation method was used to calculate the organic carbon content of the soil. A 1N K₂Cr₂O₇ solution oxidizes oxidizable materials in the soil with the help of heat generated when two volumes of H₂SO₄ are combined with one volume of dichromate sulfuric acid (H₂SO₄) to heat the dilution. Standard ferrous ammonium sulfate titrates the excess chromate left after C oxidation.

Statistical Analysis
Each experimental plot (n = 6) was reproduced three times with two determinations in each condition. One-way ANOVA was used to evaluate the results (GraphPad Prism6 package and MS Excel). A one-way ANOVA is employed at p 0.05 to examine the statistical significance of different treatments. Significant differences between the treatments are indicated by values followed by various symbols.

Results
Effect of Organic Matrix Immobilized Microbial Biofertilizers and Chemical Fertilizers on Wheat Growth, Productivity, and Yield (Triticum Aestivum L-Cv 343) in An Experimental Plot.
In 6 m² plots (1.5 x 4 m) in the rabi seasons of 2017-18 and 2018-19 (6 Dec to 7 April) and twelve different types of nutrient-providing formulations were prepared either by microbial bio-fertilizers (Azotobacter chroococcum and Bacillus subtilis) at the rate of 1.8 kg ha⁻¹ each in a 1:1 ratio) or chemical fertilizers (urea 150 kg ha⁻¹ and Diammonium phosphate 75 kg ha⁻¹) immobilized in the organic matrix using organic manures (cow dung compost /Pressmud) and organic binders (jiggery/molasses/saresh gum) (please see material and methods for details). All the treatments were applied in a random block design with three replicates. The plots with commercial charcoal-based bio-fertilizers and soluble chemical fertilizers were maintained as controls. In the controlled plots, a free form of bio-fertilizer Azotobacter chroococcum (1.8 kg ha⁻¹) and Bacillus subtilis (1.8 kg ha⁻¹) or urea (150 kg ha⁻¹) and DAP (kg ha⁻¹) chemicals were applied once as a basal dose. To screen out the most efficient formulation of organic matrix-based granular fertilizer Each plot (6 m²) received 182.16 g of immobilized bio-fertilizer (EBF) as a base dose on the soil’s surface.

Growth Parameters
Our results showed that immobilized microbial bio-fertilizers (Azotobacter chroococcum and Bacillus subtilis) in a consortium (1:1) performed better than conventional chemical fertilizers. It appears that bio-fertilizers produce significantly less root biomass in wheat under similar agro-climatic conditions than chemical fertilizers, urea, and DAP. The increased shoot length growth was observed in chemical fertilizers and bio-fertilizers over unimmobilized fertilizers and no fertilizers. In immobilized bio-fertilizer formulations, IBF-V and IBF-VI performed better than other closely performing formulations. The IBF-V and IBF-VI showed a 26.32% and 27.89% increase in shoot length over un-immobilized bio-fertilizer and 35.14% and 36.8% over no fertilizer. Immobilization of bio-fertilizer enhanced root length can be seen in all immobilized treatments over unimmobilized and control (NF) at all stages of plants. The immobilized chemical fertilizer caused a significant increase in root biomass over the recommended dose of CCFs, bio-fertilizer, and immobilized biofertilizer. At all three stages of the plant, the same dose of biofertilizers consistently caused an increase in root development. (40, 80, and 120 DAS). The immobilized formulation shows a significant increase in most of the growth parameters shown in Table 2.
All the values are means of three replicates with two determinations (n = 6) ±SD. Data was analyzed by one-way ANOVA at p < 0.05. Value followed by ns, a, b, and c shows p > 0.05, p < 0.05, p < 0.01, and p < 0.001, respectively, where p < 0.05 are significant differences between the treatments. 1) No added fertilizer = NF(CONTROL); 2) Free form of biofertilizers (Azotobacter chroococcum 1.8 kg ha⁻¹ and Bacillus subtilis 1.8 kg ha⁻¹, UBF); 3) Organic matrix immobilized biofertilizer (clay soil, vermicompost and saresh gum for organic matrix, IBF-I); 4) Organic matrix immobilized biofertilizer (clay soil, Pressmud and saresh gum for organic matrix, IBF-II); 5) Organic matrix immobilized biofertilizer (clay soil, vermicompost and jiggery for organic matrix, IBF-III); 6) Organic matrix immobilized biofertilizer (clay soil, Pressmud and jiggery for organic matrix, IBF-IV); 7) Organic matrix immobilized biofertilizer (clay soil, vermicompost and Molasses for organic matrix, IBF-V); 8) Organic matrix immobilized biofertilizer (clay soil, Pressmud and Molasses for organic matrix, IBF-VI); a recommended dose of Conventional chemical fertilizers (UREA:150 kg ha⁻¹, DAP; 60 kg ha⁻¹, CCF)  

Changes in physicochemical properties of soil in different treatments  
At sowing time, the experimental plots’ soil pH was slightly basic (8.23 to 8.3), but a decrease in pH was recorded to some extent after applying immobilized biofertilizer experimental plots. Compared to no fertilizer, conventional chemical fertilizers, and biofertilizers used in their free form, the immobilized biofertilizers significantly decreased soil pH (Table 3). On the other hand, during crop cultivation, the soil’s water retention capacity increased, which was higher in plots treated with immobilized fertilizers (IBF-V and IBF-VI). Compared to no fertilizers or standard chemical fertilizers, organic matrix immobilized fertilizers considerably increased the soil’s organic carbon (percent). In addition, in the presence of immobilized fertilizers, total N, available P, and soluble K were considerably higher at harvest than in the other treatments. 

Nitrate, nitrite, and ammonium levels in soil and leaves in treatments  
It was demonstrated that nitrate content increased in rhizosphere soil and fresh leaves of wheat plants for all the treatments of immobilized organic matrix-based bio-fertilizers and chemical fertilizers compared to no fertilizer and unimmobilized. Applying organic matrix immobilized bio-fertilizers IBF-VI over un-immobilized bio-fertilizer increased the average soil nitrate content by 67.58%, 27.52%, and 77.54%, respectively, on 40, 80, and 120 DAS. The immobilized chemical fertilizer-treated experimental plots reflected more availability of nitrate content than the free soluble chemical fertilizer-treated experimental plots. The nitrate content in the leaves of wheat plants treated with IBF-V and IBF-VI was significantly higher than unimmobilized bio-fertilizer and more closely performing than immobilized bio-fertilizer after 120 days. The nitrite level in the immobilized chemical fertilizer treated field was higher than the free form of chemical fertilizer at each interval (Fig. 1). The nitrite content in wheat leaves
Increasing Efficacy of Microbial Biofertilizer by Immobilizing in an Organic Matrix

Applied with organic matrix immobilized chemical fertilizers or immobilized bio-fertilizer was slightly higher than free-form fertilizers on 40, 80, and 120 DAS. The soil ammonium content was increased by 38.34% and 12.67% on 40 and 120 DAS, respectively, with the application of immobilized bio-fertilizer IBF-VI over unimmobilized bio-fertilizers. The organic matrix immobilized formulation of bio-fertilizer IBF-V and IBF-VI gave better results than closely performing other formulations of immobilized bio-fertilizer and chemical fertilizer. The ammonium content of wheat leaves was 49.5%, 74.1%, and 59.53% higher in IBF-VI treated plants than in un-immobilized bio-fertilizer treated plants.

Grain and straw yield
The grain and straw yields (t ha⁻¹) showed a significant increase in the application of IBF-V and IBF-VI over the NF and UBF (Fig. 2). Grain yield and straw yield increased by 119.07 and 122.26%, 35.9 and 61.21%, and 0.34 and 2.6%, respectively, when IBF-VI was used instead of NF, UBF, and IBF-V. The yield obtained in IBF-V and IBF-VI was approximately equal, slightly lower (3.02%) than in C Fig. No. 2.

All the values are means of three replicates with two determinations (n = 6) ±SD. Data analyzed by one-way ANOVA at p < 0.05. Value followed by ns, a, b, and c shows p > 0.05, p < 0.05, < 0.01, and < 0.001, respectively, where p < 0.05 are significant differences between the treatments.

Grain and straw yield

![Fig. 2: Effect of different fertilizers forms on and grain and straw yield.](image)

All the values are means of three replicates with two determinations (n = 6) ±SD. Data analyzed by one-way ANOVA at p < 0.05. Value followed by ns, a, b, and c shows p > 0.05, p < 0.05, < 0.01, and < 0.001, respectively, where p < 0.05 are significant differences between the treatments. 1) No added fertilizer = NF (CONTROL); 2) Free form of biofertilizers (Azotobacter chroococcum 1.8 kg ha⁻¹ and Bacillus subtilis 1.8 kg ha⁻¹, UBF); 3) Organic matrix immobilized biofertilizer (clay soil, vermicompost and saresh gum for organic matrix, IBF-II); 4) Organic matrix immobilized biofertilizer (clay soil, Pressmud and jiggery for organic matrix, IBF-IV); 5) Organic matrix immobilized biofertilizer (clay soil, vermicompost and Molasses for organic matrix, IBF-VI); recommended dose of Conventional chemical fertilizers (Urea; 150 kg ha⁻¹ DAP; 60 kg ha⁻¹, CCF)

All the values are means of three replicates with two determinations (n = 6) ±SD. Data analyzed by one-way ANOVA at p < 0.05. Value followed by ns, a, b, and c shows p > 0.05, p < 0.05, < 0.01, and < 0.001, respectively, where p < 0.05 are significant differences between the treatments.

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All the values are means of three replicates with two determinations (n = 6) ±SD. Data analyzed by one-way ANOVA at p < 0.05. Value followed by ns, a, b, and c shows p > 0.05, p < 0.05, < 0.01, and < 0.001, respectively, where p < 0.05 are significant differences between the treatments.
Effect on the productivity of wheat in different water regimes

The growth and productivity of wheat varied accordingly, as irrigation intensity was reduced for all the treatments. The control (NF) and unimmobilized biofertilizer (UBF) showed maximum downfall in growth and productivity of wheat on decreasing water regime. Although the immobilized biofertilizer (IBF-VI) also showed a reduction in growth and productivity but had a lower fold rate. The NF, UBF, and IBF-VI showed 3.25 and 1.49-time fold decrement in wheat yield productivity, respectively, compared to regular and water regime 2 (Fig. 3).

**Discussion**

It is well-known that about 30–50% of applied N fertilizers are taken up by crops and that a large portion (50-70%) is lost from the agricultural field due to surface runoff, nitrate, nitrite, and ammonium leaching, as well as ammonia volatilization and NOx gas emissions (Klimczuk et al., 2021). All the values are means of three replicates with two determinations(n=6) ±SD. Data were analyzed by one-way ANOVA at p < 0.05. Value followed by ns, a, b, and c shows p>0.05, p<0.05, p<0.01, and p<0.001, respectively, where p<0.05 are significant differences between the treatments. 1) No added fertilizer=NF(CONTROL); 2) Free form of biofertilizers (Azotobacter chroococum 1.8 kg ha⁻¹ and Bacillus subtilis 1.8 kg ha⁻¹, UBF); 3) Organic matrix immobilized biofertilizer (clay soil, vermicompost and saresh gum for organic matrix, IBF-I); 4) Organic matrix immobilized biofertilizer (clay soil, Pressmud and saresh gum for organic matrix, IBF-II); 5) Organic matrix immobilized biofertilizer (clay soil, vermicompost and jiggery for organic matrix, IBF-III); 6) Organic matrix immobilized biofertilizer (clay soil, Pressmud and jiggery for organic matrix, IBF-IV); 7) Organic matrix immobilized biofertilizer (clay soil, Pressmud and Molasses for organic matrix, IBF-V); 8) Organic matrix immobilized biofertilizer (clay soil, Pressmud and Molasses for organic matrix, IBF-VI); recommended dose of Conventional chemical fertilizers (Urea;150 kg ha⁻¹ DAP; 60kg ha⁻¹, CCF).

Gaseous nitrogen losses harm ecosystems, decrease water quality, and contribute to global warming (Xu et al., 2020). Biofertilizers have recently been recognized as a sustainable alternative to chemical fertilizers for increasing soil fertility and crop yield (Kour et al., 2020). Biofertilizers have evolved as an essential component of integrated nutrient management programs in recent years, and they hold great promise for increasing agricultural productivity while causing minimal environmental damage. As biofertilizers for cereals, pulses, vegetables, oilseeds, cotton, sugarcane, wheat, and other crops, strains of Azotobacter, Azospirillum, Acetobacter, Bacillus, Bradyrhizobium, Pseudomonas and Rhizobium have been created. (Kumar and Singh 2021). In a previous study, we investigated the pattern of release of nutrients and doses of biofertilizer to optimize the effectiveness of biofertilizer (Kumar et al., 2013) and optimized the doses of biofertilizer (Rai et al., 2017). This suggests that the nutrients in this formulation are released more slowly than in free soluble forms or only organic matrix without microbes or nutrients and that a triple dose of the recommended dose of Azotobacter chroococum and Bacillus subtilis performed better. Higher levels of nitrate, nitrite, ammonium, and phosphate were found in rhizospheric soil and wheat leaves after applying several organic matrix-bound biofertilizers at 40, 80, and 120 DAS. As assessed at crop harvest, these fertilizers increased soil physicochemical qualities and nutrient availability (Table 1). Increased nitrate levels in plant leaves show that these fertilizers boosted root nitrogen uptake. (Kumar et al., 2013; Rai et al., 2017).

### Table 3: Effect of different formulations on wheat (Triticum aestivum L.cv.PBW-343) planted soil

<table>
<thead>
<tr>
<th></th>
<th>NF</th>
<th>UBF</th>
<th>IBF-I</th>
<th>IBF-II</th>
<th>IBF-III</th>
<th>IBF-IV</th>
<th>IBF-V</th>
<th>IBF-VI</th>
<th>CCF</th>
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<tbody>
<tr>
<td>Potassium (µg/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SS</td>
<td>110.2±2.71</td>
<td>109.4±5.20</td>
<td>128.9±3.90b</td>
<td>119.6±3.44</td>
<td>104.5±4.87</td>
<td>99.2±7.1</td>
<td>98.3±6.44</td>
<td>131.8±5.80</td>
<td>102.8±5.20</td>
</tr>
<tr>
<td>H</td>
<td>89.2±3.70</td>
<td>93.4±6.40</td>
<td>87.9±5.20</td>
<td>98.6±4.90b</td>
<td>96.2±3.87</td>
<td>92.2±2.3</td>
<td>95.3±3.14</td>
<td>103.3±4.40</td>
<td>103.2±4.30</td>
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<tr>
<td>Phosphate (µg/g)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>15.3±38</td>
<td>24.6±4.4c</td>
<td>24.6±1.02c</td>
<td>23.5±0.34c</td>
<td>24.6±1.75c</td>
<td>27.6±3.1c</td>
<td>18.1±2.43</td>
<td>21.4±3.23</td>
<td>24.6±4.32c</td>
</tr>
<tr>
<td>H</td>
<td>20.3±1.14</td>
<td>28.6±2.90b</td>
<td>31.6±1.56b</td>
<td>32.5±4.60b</td>
<td>30.3±1.11b</td>
<td>26.6±1.9b</td>
<td>29.5±4.34b</td>
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<td>33.7±1.24b</td>
</tr>
<tr>
<td>Organic carbon(%)</td>
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<tr>
<td>SS</td>
<td>1.5±0.02</td>
<td>4.9±0.09c</td>
<td>6.0±0.16c</td>
<td>2.6±0.04c</td>
<td>2.9±0.10c</td>
<td>4.5±0.05c</td>
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<td>3.0±0.24c</td>
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<td>H</td>
<td>2.1±0.04</td>
<td>3.2±0.03c</td>
<td>3.8±0.08c</td>
<td>3.4±0.27c</td>
<td>3.4±0.14c</td>
<td>2.9±0.29c</td>
<td>3.5±0.32c</td>
<td>3.3±0.04c</td>
<td>3.2±0.09c</td>
</tr>
<tr>
<td>pH</td>
<td>8.2±0.190</td>
<td>8.2±0.18c</td>
<td>8.2±0.39c</td>
<td>8.1±0.26c</td>
<td>8.1±0.32c</td>
<td>8.2±0.42c</td>
<td>8.2±0.07c</td>
<td>8.1±0.09c</td>
<td>8.1±0.32c</td>
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<td>Conductivity (µsimen)</td>
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<tr>
<td>SS</td>
<td>22.1±0.03</td>
<td>31.3±1.23c</td>
<td>17.9±0.74c</td>
<td>39.9±2.33c</td>
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<td>12.7±0.84c</td>
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<td>15.3±0.54c</td>
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<tr>
<td>H</td>
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<td>21.6±0.87c</td>
<td>28.7±1.43c</td>
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<td>23.4±0.33c</td>
<td>23.0±0.34c</td>
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in plant tissue by improving photosynthetic efficiency and regulating the ethylene signaling pathway by regulating the expression of a regulatory component (CTR1) (Liu et al., 2013; Barnawal et al., 2017). The azotobacter produces ACC deaminase, which decreases plant ethylene levels and helps in tolerating any type of stress in plants, e.g., water, salinity, pH, temperature, etc. (Aasfar et al., 2021). The carrier Pressmud and cow dung manure act as good moisture holders and provide nutrition to the microbes, which supports the growth of microbes at even low availability of water. Our results showed that Pressmud could be used as an excellent organic material for the immobilization of microbes as it also consists of sugar, crude wax, fiber, fats, crude protein, and ash comprising oxides of Si, Ca, P, Mg, and K, which are suitable for microbes as well as plant growth.

**Conclusions**

The results suggested that organic matrix immobilized biofertilizers performed other treatments in terms of wheat growth and yield and that they can be further optimized for Wheat-growing northern Indian states which have alluvial soil with semi-tropical agro-climatic conditions. The organic matrix formulation combination of Pressmud: molasses and cow dung manure: molasses performed better than other formulations. Pressmud and molasses are waste products generated in the sugarcane industry during sugar production. It is dumped near sugar industries as a landfill, causing environmental problems. If we can use it to produce this type of granule on a large-scale commercial basis, we can reduce this waste and generate an economy from it. One of the primary benefits of employing press mud and jiggery as granular carriers is that they are both organic and biodegradable, meaning that soil microbes can quickly digest them. This allows for gradual nutrient release, enhancing soil quality and boosting plant development over time. The water-holding ability of Pressmud can also be optimized further so that it can be used in low-water irrigation areas with effective biofertilizer activity. Furthermore, the use of microbial biofertilizers might minimize the requirement for synthetic fertilizers, which can be costly and have significant environmental effects. Farmers may increase the health of their land and crops while lowering their environmental impact by utilizing natural and sustainable alternatives. For a reliable and sustainable supply chain to support your small-industrial granule production raw material availability should be on a large scale and continuous with easy access, filled with Pressmud and molasses.

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**Authors Contributions**

**Pawan Kumar:** Investigation, Conceptualization, Methodology, Formal analysis, Validation, Data curation, Writing - original draft. **Rana Pratap Singh:** Project administration, Supervision, Writing - review and editing, Resources.

**References**


Increasing Efficacy of Microbial Biofertilizer by Immobilizing in an Organic Matrix


