RESEARCH ARTICLE

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

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DOI: 10.18811/ijpen.v9i01.04

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 bio formulations in rural areas through small industrial setups.

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

International Journal of Plant and Environment (2023); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

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 animal 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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How to cite this article: Kumar, P., Singh, R.P. (2023). 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. International Journal of Plant and Environment. 9(1), 24-31.

Conflict of interest: None

Submitted: 14/01/2023 **Accepted:** 28/02/2023 **Published:** 30/03/2023

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, effectivity, and ecological successions 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 (Mącik *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 refineries 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 m^2 (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^{0}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:

Treatments	*Detailed layout of treatments*
NF	No fertilizers
CCF	Recommended dose (RD) of commercial chemical fertilizers (Urea150 kg ha^{-1} and DAP 75 kg ha^{-1})
UBF	Bio-fertilizer i.e., *Azotobacter chroococum* (1.8 kg ha^{-1}) and *Bacillus subtilis* (1.8 kg ha^{-1}) applied in experimental plots.
IBF-I	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.
IBF-II	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.
IBF-III	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.
IBF-IV	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.
IBF-V	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.
IBF-VI	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.

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)-ethylene-diamine 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 $K_2Cr_2O_7$ solution oxidizes oxidizable materials in the soil with the help of heat generated when two volumes of H_2SO_4 are combined with one volume of dichromate sulfuric acid (H_2SO_4) 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 m^2 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 subtlilis* 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 subtlilis* (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 m^2) 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 subtlilis*) 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.

Table 2: Effect of different formulations of organic matrix entrapped biofertilizers and chemical fertilizer on wheat (*Triticum aestivum* L.cv. PBW-343) plant growth on 40, 80 and 120 days after sowing (DAS).

		NF	UBF	IBF-I	IBF-II	IBF-III	IBF-IV	IBF-V	IBF-VI	CCF
SHOOT LENGTH(cm)	40DAS	23 ± .32	29.6 ± 1.33b	30 ± 0.09c	29.2 ± 0.53c	27.6 ± 0.67c	26 ± 0.35c	30.1 ± 0.22c	28.9 ± 0.23c	28.5 ± 0.43c
	80DAS	35 ± .34	35.7 ± 0.94c	43.5 ± 0.47c	42.3 ± 1.65c	45 ± 0.10c	46.5 ± 0.34c	49.5 ± 0.65c	49.3 ± 1.75c	52 ± 0.13c
	120DAS	37 ± 0.20	39.6 ± 2.12 ^{ns}	46.5 ± 0.04c	44.6 ± 0.56c	48 ± 0.54c	47 ± 2.53c	50 ± 0.46c	50.6 ± 1.45c	56.7 ± 0.85c
ROOT LENGTH(cm)	40DAS	3.3 ± 0.75	3.6 ± 0.27a	3.7 ± 0.67 ^{ns}	4.34 ± .36 ^{ns}	4.6 ± 0.33 ^{ns}	4.7 ± 0.41^{ns}	3.3 ± 0.34b	4.4 ± 0.43 ^{ns}	4.85 ± .033 ^{ns}
	80DAS	4.8 ± 0.04	5.2 ± 1.07a	5.3 ± 0.09c	6.1 ± 0.02c	6.1 ± 0.05c	6.3 ± 0.09c	6.3 ± 0.56c	6.5 ±0.09c	6.7 ± 0.05c
	120DAS	6.3 ± 0.07	6.7 ± 0.35c	7.2 ± 0.10c	8.2 ± 0.19c	6.8 ± 0.04c	8.9 ± 0.24c	9 ± 0.32c	9.4 ± .04c	8.4 ± 0.09c
Number of root hair	40DAS	8.5 ± 0.10	12.7 ± 0.86c	12.3 ± 0.34c	12.3 ± 0.09c	10.8 ± 0.14c	13.3 ± 0.09c	13.8 ± 0.35c	11.5 ± 0.13c	11.5 ± 0.08c
	80DAS	14 ± 0.13	16.8 ± 1.17c	18 ± 0.08c	18.5 ± 0.06c	18.3 ± 0.06c	19 ± 0.54c	19.5 ± 0.10c	21.3 ± 0.54c	17.2 ± 0.35c
	120DAS	16 ± 0.04	18.4 ± 1.7c	18 ± 0.02c	18.5 ± 0.53c	18.3 ± 0.75c	19 ± 0.12c	21.5 ± 0.23c	23.3 ± 0.74c	19 ± 0.68c
Number of leaves	40DAS	4.8 ± 0.41	5.3 ± 1.21c	5.2 ± 0.41 ^{ns}	5.8 ± 0.41a	5.2 ± 0.41 ^{ns}	5.2 ± 0.41 ^{ns}	5.5 ± 0.55 ^{ns}	5.8 ± 0.41a	6 ± 0.547b
	80DAS	4.5 ± 0.55	4.8 ± 1.17 ^{ns}	5.5 ± 0.55a	5.2 ± 0.41 ^{ns}	5.2 ± 0.41 ^{ns}	5.7 ± 0.52b	5.7 ± 0.52b	5.5 ± 0.55a	6.8 ± 0.41c
	120DAS	4.5 ± 0.55	5.1 ± 1.83 ^{ns}	5.7 ± 0.52b	5.2 ± 0.41 ^{ns}	5.8 ± 0.41c	5.7 ± 0.52b	5.8 ± 0.41c	5.2 ± 0.41 ^{ns}	6.5 ± 0.55c
Fresh Weight(g)	40DAS	1.2 ± 0.03	1.4 ± 0.04b	1.3 ± 0.04 ^{ns}	1.4 ± 0.15 ^{ns}	1.0 ± .08 ^{ns}	1.5 ± 0.31c	1.8 ± 0.09c	1.5 ± 0.13 ^{ns}	1.5 ± 0.04b
	80DAS	2.4 ± 0.23	2.3 ± 1.50a	2.7 ± 0.12 ^{ns}	3.1 ± 0.26b	3.9 ± 0.11c	5.6 ± 0.10c	8.7 ± 0.46c	11.2 ± 0.04c	8.1 ± 0.66c
	120DAS	2.2 ± 0.05	2.3 ± 2.02c	2.3 ± 0.05b	2.7 ± 0.10c	3.5 ± 0.09c	5.5 ± 0.43c	8.2 ± 0.21c	10.8 ± 0.32c	7 ± 0.08c
Dry Weight(g)	40DAS	0.4 ± 0.02	0.5 ± 0.12b	0.6 ± 0.02a	0.6 ± 0.04b	0.4 ± 0.12 ^{ns}	0.6 ± 0.12c	0.7 ± 0.02c	0.5 ± 0.13 ^{ns}	0.6 ± 0.04b
	80DAS	1.2 ± 0.12	1.1 ± 0.25 ^{ns}	1.2 ± 0.12 ^{ns}	1.7 ± .04 ^{ns}	1.7± 0.04a	2.0 ± 0.08	2.2 ± 0.03c	2.3 ± 0.03c	2.7 ± 0.01c
	120DAS	1.5 ± 0.10	1.5 ± 0.15ns	1.6± 0.19a	2.5 ± .10c	2.6 ± 0.09c	2.7 ± 0.10c	2.7 ± 0.08c	2.9 ± 0.24c	3.8 ± 0.09c

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 subtlilis* 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; 60kg 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

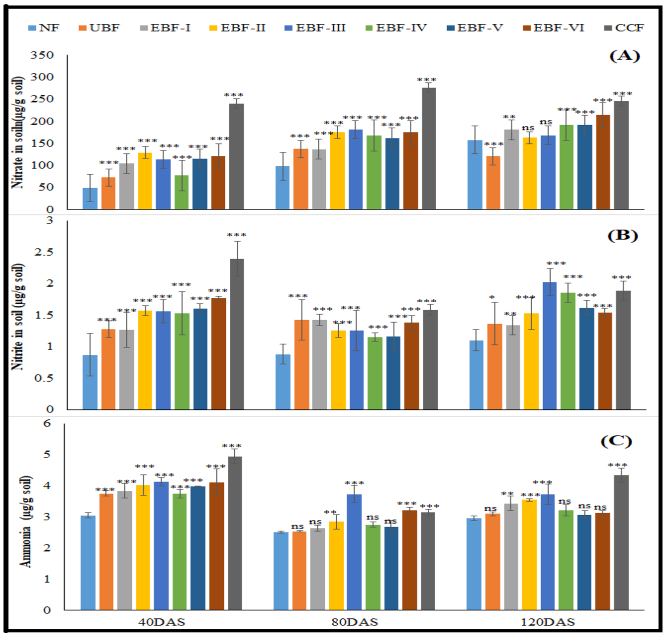

Fig. 1: Levels of nitrate (A), nitrite (B), and ammonium (C) in the soil of experiment *T. aestivum* applied with different fertilizer forms at 40, 80, and 120 DAS.

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 biofertilizers. 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. One-way ANOVA analyzed data 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 subtlilis* 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, vermi compost 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, vermi-compost 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)

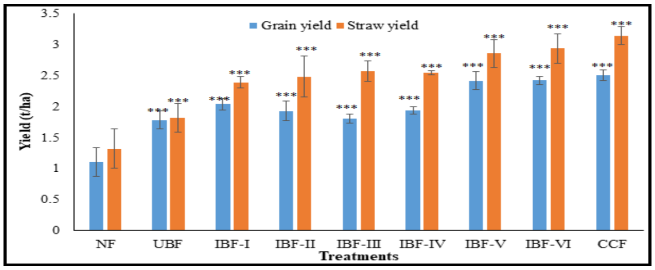

Fig. 2: Effect of different fertilizers forms on and grain and straw yield.

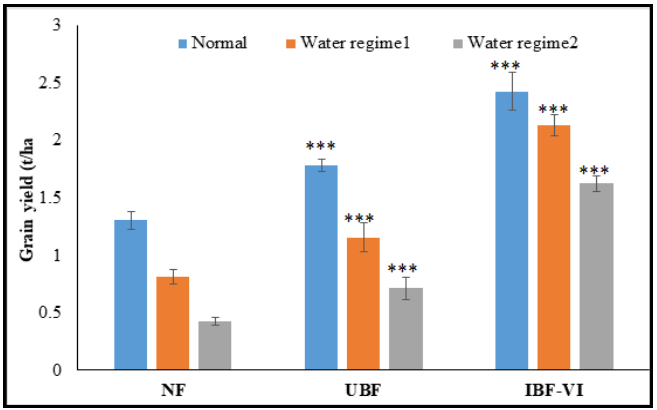

Fig. 3: Effect of different treatments under different water regime grain yield of wheat.

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 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 subtlilis* 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); recommended dose of Conventional chemical fertilizers (Urea;150 kg ha^{-1} DAP; 60kg ha^{-1}, CCF)

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 subtlilis* 1.8 kg ha^{-1}, UBF); 3) Organic matrix immobilized biofertilizer (clay soil, Pressmud and Molasses for organic matrix, IBF-VI)

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

		NF	UBF	IBF-I	IBF-II	IBF-III	IBF-IV	IBF-V	IBF-VI	CCF
Potassium (µg/g)	SS	110.2 ± 2.71	109.4 ± 5.20^{ns}	128.9 ± 3.90b	119.6 ± 3.4$^{0\ n}$s	104.5 ± 4.87^{ns}	99.2 ± 7.1 ^{ns}	98.3 ± 6.4 ^{a}	131.8 ± 5.80^{c}	102.8 ± 5.20 ^{ns}
	H	89.2 ± 3.70	93.4 ± 6.40 ^{ns}	87.9 ± 5.20 ^{ns}	98.6 ± 4.90^{b}	96.2 ± 3.87^{a}	92.2 ± 2.3 ^{ns}	95.3 ± 3.1^{ns}	103.3 ± 4.40 ^{c}	103.2 ± 4.30^{c}
Phosphate (µg/g)	SS	15.3 ±.38	24.6 ± 0.44^{c}	24.6 ± 1.02^{c}	23.5 ± 0.34^{c}	24.6 ± 1.75^{c}	27.6 ± 3.1c	18.1 ± 2.43 ^{ns}	21.4 ± 3.23^{b}	46.7 ± 4.32^{c}
	H	20.3 ± 1.14	28.6 ± 2.90^{b}	31.6 ± 1.56^{b}	32.5 ± 4.60^{b}	30.3 ± 1.11^{b}	26.6 ± 1.9b	29.5 ± 4.34^{b}	31.4 ± 7.32^{b}	33.7 ± 1.24^{b}
Organic carbon(%)	SS	1.5 ± 0.02	4.9 ± 0.09^{c}	6.0 ± 0.16^{c}	2.6 ± 0.04^{c}	2.9 ± 0.10^{c}	4.5 ± 0.05c	4.6 ± 0.37^{c}	4.5 ± 0.53^{c}	3.0 ± 0.24^{c}
	H	2.1 ± 0.04	3.2 ± 0.03^{c}	3.8 ± 0.08^{c}	3.4 ± 0.27^{c}	3.4 ± 0.14^{c}	2.9 ± 0.29c	3.5 ± 0.32^{c}	3.3 ± 0.04^{c}	3.2 ± 0.09^{c}
pH	SS	8.2 ± 0.190	8.2 ± 0.18 ^{ns}	8.2 ± 0.39 ^{ns}	8.1 ± 0.26 ^{ns}	8.1 ± 0.32^{ns}	8.2 ± 0.42 ^{ns}	8.2 ± 0.07 ^{ns}	8.1 ± 0.09 ^{ns}	8.1 ± 0.32 ^{ns}
	H	8.1 ± 0.08	8.2 ± 0.26 ^{ns}	8.1 ± 0.09 ^{ns}	8.0 ± 0.05 ^{ns}	8.1 ± 0.27^{ns}	8.03 ± 0.13 ^{ns}	8.1 ± 0.39 ^{ns}	8.1 ± 0.12 ^{ns}	8.2 ± 0.04^{a}
conductivity (µsimen)	SS	22.1 ± 0.03	31.3 ± 1.23^{c}	17.9 ± 0.74^{c}	39.9 ± 2.33^{c}	32.2 ± 2.23^{c}	12.7 ± 0.84c	20.4 ± 1.95^{ns}	15.3 ± 0.54^{c}	19.0 ± 1.95^{b}
	H	27.6 ± 0.13	31.8 ± 3.43^{c}	21.6 ± 0.87^{c}	28.7 ± 1.43^{ns}	19.5 ± 1.34^{c}	21.8 ± 0.94c	22.5 ± 0.87^{c}	23.4 ± 0.33^{c}	23.0 ± 0.34^{c}

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, 2.5 and 1.49-time fold decrement in wheat yield productivity, respectively, compared to regular and water regime 2 (Fig. 3).

DISCUSSION

It is clear 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 (Klimczyk *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 chroococcum* 1.8 kg ha^{-1} and *Bacillus subtlilis* 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); 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 effectivity 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). Significantly enhanced nitrite levels were observed in organic matrix immobilized fertilizers (IBF-V and IBF-VI) treated plant leaves demonstrated greater nitrate assimilation during the vegetative and reproductive stages, resulting in increased plant growth and seed output. Different water regimes showed that immobilized biofertilizers were to increase the water stress tolerance of wheat plants, which may be due to production of certain organic substances and regulating gene expression. *Bacillus subtalis* improve water potential by increasing sugars, amino acids, and organic acids 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.

Acknowledgements

Pawan Kumar would like to acknowledge the Department of Biotechnology (DBT), MHRD, New Delhi, India, for financial support in the form of JRF and highly obliged to Laboratory of DES for experimental facilities. Dr. Rana Pratap Singh is appreciative of the financial assistance provided by the MRP project, which it received from UGC New Delhi.

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.

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