



Microbial Biofertilizers as a Sustainable Alternative to Synthetic Fertilizers in Crop Production

Muhammad Zahid Iqbal¹

¹Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan

Email: zahidiqbalagri@gmail.com

ARTICLE INFO

Received:

June 28, 2026

Revised:

July 19, 2026

Accepted:

August 11, 2026

Available Online:

August 27, 2026

Keywords:

microbial biofertilizers; nitrogen-fixing bacteria; phosphate-solubilizing bacteria; synthetic fertilizer; sustainable agriculture; crop yield; randomized complete block design

Corresponding Author:

Muhammad Zahid Iqbal

ABSTRACT

The field based experimental design was used to evaluate the effect of using microbial biofertilizers as an alternative to synthetic fertilizers in the production of maize (*Zea mays* L.). The experiment was designed under randomized complete block design (RCBD) wherein five treatments were laid out, which included unfertilized control, application of microbial biofertilizer in the form of consortium of nitrogen fixing and phosphate solubilizing bacteria and application of microbial biofertilizer in combination with 50% RDF and 75% RDF, both in the form of seed coating and soil application. Plant height, leaves, growth of roots, production of biomass, chlorophyll content, yield components and final grain yield were measured and before and after the experiment soil properties such as available nitrogen, available phosphorus, organic matter and microbial activity were assessed. Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test at 5% significance level were used to analyze data. It was observed that all fertilized treatments significantly improved growth and yield parameters as compared to unfertilized control with the biofertilizer at 75% RDF giving the grain yield similar to 100% RDF recommended dose of synthetic fertilizer and the biofertilizer at 50% RDF gave yield intermediate. The biofertilizer treatments also had significantly higher soil microbial activity, available nitrogen and phosphorus after the experiment than the synthetic-fertilizer-only treatment. The results showed that the application of microbial biofertilizers was able to significantly reduce the use of synthetic fertilizers without affecting the productivity of the crops and also provided extra benefits for the soil health, which facilitated their use in sustainable crop production system.

Introduction

Synthetic nitrogen and phosphorus fertilizers have been used extensively for over 50 years to sustain the yield increases linked to the Green Revolution for global crop production. However, this dependence has become more and more costly to the environment and the economy, such as nutrient runoff and eutrophication of water bodies, greenhouse gas emissions during the production and use of fertilizers, disruption of soil microbial communities, and an increase in the cost of inputs, which is a burden to smallholder farmers (Mahanty et al., 2017). Meanwhile, global demand for synthetic fertilizers is growing with the increasing demand for food, while the dwindling resource base of phosphate rock – the principal raw material for

producing phosphorus fertilizers – is becoming more and more problematic (FAO, 2022). They are all contributing to the growing interest in the use of microbial biofertilizers as an additional or even partial substitute for synthetic fertilizers that can sustain crop productivity.

Microbial biofertilizers are living organisms that are applied to seeds, plant surfaces or soil and colonize the rhizosphere and increase the nutrient availability and uptake to plants through various biological mechanisms. Nitrogen-fixing bacteria are the most studied and commercially applied bacteria as they carry nitrogenase enzyme which converts atmospheric dinitrogen into plant available ammonium, and phosphate-solubilizing bacteria are bacteria which produce organic acids and phosphatase enzymes to convert the plant unavailable soil-bound phosphorus to the plant available form (Bhattacharyya & Jha, 2012). In addition to these direct nutrient-provisioning mechanisms, many of the biofertilizer organisms—known as plant growth-promoting rhizobacteria (PGPR)—also produce phytohormones, siderophores and other metabolites that further accelerate root growth and plant development, and can also increase plant tolerance to abiotic stress, including drought and salinity stress (Etesami & Maheshwari, 2018; Glick, 2012).

There is a considerable amount of field and pot studies that have investigated the agronomic response of microbial biofertilizers, sole and in combination with lower rates of synthetic fertilizers. In a field trial conducted with chickpea, Elkoca et al. (2008) have reported that co-inoculation with nitrogen fixing and phosphorus solubilizing bacteria had a significant positive effect on nodulation, plant growth and yield compared to the uninoculated control, suggesting that the microbial inoculation had a definite agronomic advantage under field conditions. Adesemoye and Kloepper (2009) cited above noted that the main practical utility of microbial biofertilizers may not be their ability to totally replace synthetic fertilizers, but their ability to enhance the efficiency of applied fertilizer nutrient capture and utilization by the crop, enabling farmers to lower fertilizers use rates without compromising yield. This holistic perspective is reinforced by later field studies, in which the use of root microorganism inoculants has been shown to reduce, but not eliminate, the need for mineral fertilizers in the production of wheat-based cropping systems, in India, while maintaining similar yields, especially when mineral fertilizers are applied at lower levels (Mäder et al. 2011).

Nevertheless, a global meta-analysis, which compiled data from 171 peer-reviewed field studies, by Schütz et al. (2018) showed that the yield increase resulting from biofertilizer application strongly depended on the microbial taxa applied, the type of crop used, the use of biofertilizer alone or with mineral fertilizer, and biofertilizer-only applications were generally less effective than combined applications. This diversity highlights the need for ongoing field trials in different crops and situations and for testing the biofertilizer alone versus the synthetic-fertilizer alone and combination treatments under experimental conditions, thus testing the effects of the treatments and not the generalized conclusions based on contexts that are so diverse.

In addition to yield results, microbial biofertilizers have been claimed to have soil health benefits not occurring with synthetic fertilizers. Nitrogen-fixing and phosphate-solubilizing bacteria have been linked to increased soil microbial activity and dynamics of organic matter compared with synthetic-fertilizer-only management, which may have long-term positive impacts on soil fertility beyond the growing season where they have been introduced (Malusá et al., 2012; Schoebitz et al., 2013). This soil-level effect, in combination with conventional plant growth and yield data, is therefore a more comprehensive way of evaluating the potential role of biofertilizers in sustainable crop production than yield data.

The present study tackles these issues by conducting a field experiment to assess the effectiveness of microbial biofertilizers either alone or in combination with lower rates of synthetic fertilizer compared with an unfertilized check and the recommended dose of synthetic fertilizer for maize production. The study has three objectives: to compare the effect of microbial biofertilizer application (either alone or in combination with reduced synthetic fertilizer dose) with unfertilized and fully fertilized control treatments on the plant growth, soil microbial activity, and soil nutrient status before and after the crop cycle; to determine if combined application of microbial biofertilizer and reduced dose of synthetic fertilizer can achieve yields equivalent to the full recommended dose of synthetic fertilizer; and to compare the effect of microbial biofertilizer application alone and in combination with reduced dose of synthetic fertilizer with the unfertilized and fully fertilized controls on plant growth, soil microbial activity and soil nutrient status before and after the crop cycle. The results will contribute to making a decision on the use of microbial biofertilizers as viable and proven alternative to synthetic inputs in crop production.

Literature Review

Nitrogen-fixing microorganisms have been the most intensively studied groups of microbial biofertilizer organisms because nitrogen is the micronutrient that is frequently limiting for crop production without fertilization. In a review of the mechanisms of plant growth promoting bacteria, in general, Glick (2012) reported that biological nitrogen fixation is one of several complementary mechanisms, along with production of phytohormones and induction of stress tolerance, by which bacteria promote plant growth, and indicated that some mechanisms are more important than others, depending on the bacterial strain and growth environment. The agronomic importance of PGPR-based biofertilizers was also stressed by Bhattacharyya and Jha (2012) who highlighted that the agronomic benefit of PGPR-based biofertilizers is due to multiple growth benefits attributed to several interacting microbial activities instead of just nitrogen fixation.

Phosphate-solubilizing bacteria alleviate a complementary nutrient requirement, because in most agricultural soils, a high percentage of the soil phosphorus, including phosphate applied as a fertilizer, is quickly immobilized in soil forms that are unavailable to the plant. In developing the effective phosphate-solubilizing biofertilizer products, it is necessary to select the suitable bacterial strains, as Chen et al. (2006) isolated phosphate-solubilizing bacteria from subtropical soils and found that they exhibited significantly different ability to release tricalcium phosphate. In the broader context of the role of PGPR in agricultural sustainability, Vejan et al. (2016) found that the phosphate-solubilizing and nitrogen-fixing bacterial groups are the most agronomically validated microbial biofertilizer groups available for field application and consistent field performance is greatly influenced by microbial formulation, application technology and its compatibility with soil and climatic conditions. In addition to bacterial inoculants, Igiehon and Babalola (2017) reported the role of arbuscular mycorrhizal fungi as microbial partners in sustainable agriculture as it can help augment the bacterial nitrogen fixing and phosphate-solubilizing ability by increasing the effective absorptive root surface area, thus expanding the microbial mechanisms for biofertilizer development beyond the bacterial consortium used in this study.

All the field trials conducted so far have tested the effect of biofertilizers against synthetic fertilizer treatments and against the combination of both and the best results have been obtained using a combination of biofertilizers and synthetic fertilizer. Elkoca et al. (2008) reported that the combined application of nitrogen fixing and phosphorus solubilizing bacteria with chickpea resulted in better nodulation, growth, and yield, under field conditions, as compared to the uninoculated control, thereby establishing the agronomic importance of using combined bacteria. Adesemoye and Kloepper (2009) theorized that microbial inoculants work best when used in conjunction with synthetic fertilizers rather than to replace synthetic fertilizers, and that by reducing the rates of synthetic fertilizer application yields could be maintained at the same level as high rates of synthetic fertilizer application. This has direct implications for the reduced-synthetic-fertilizer treatments used in the present study. Testing this proposition in several rotations, incorporating wheat into India, Madder et al. (2011) concluded that microbials use along with lower mineral fertiliser inputs can sustain yields as good as full mineral fertiliser, thus supporting the efficiency-enhancement hypothesis in a range of cropping systems.

The findings from these individual field-trial studies can be checked with a meta-analytic approach for generalizability. In a global metanalysis of 171 peer-reviewed studies conducted by Schütz et al. (2018), overall, the application of biofertilizers has significantly increased yield and nutrient use efficiency, but not always to the same extent, depending on the microbial taxon, crop species and application method (alone or in combination with mineral fertilizer), and with the highest and most consistent effects when applied in combination with mineral fertilizer. The results are directly applicable to the present study where biofertilizer was not used as an uniform and standalone alternative but was used in combination with two reduced levels of synthetic fertilizers.

In addition to direct yield benefits, there have been several studies that have explored the extended soil health impacts of microbial biofertilizer application. Malusá et al. (2012) reviewed technologies for the production and application of biofertilizer inoculants and concluded that successful establishment of the biofertilizers in the soils result in a stock of organisms which can persist and interact with resident soil microbial communities beyond the initial application, and may have implications for long-term soil fertility beyond the first crop. Schoebitz et al. (2013), in their review of bioencapsulation technology for microbial inoculants, also noted that

the formulation technique significantly influences the survival of microorganisms and the success of their soil colonization and soil microbial activity results, which is what field trials can measure. Reviewing plant-microbe interactions under stress Kumar and Verma (2018) suggested that beneficial microbial colonization could boost soil enzymatic activity and turnover of organic matter which would provide some mechanistic explanation for better soil microbial activity indicators after using biofertilizer rather than synthetic-fertilizer-only management.

Field based agronomic trials that tested the differences between fertilizer and biofertilizer applications have traditionally employed randomized complete block designs (RCBD) and analysis of variance (ANOVA) to allow for the control of spatial field heterogeneity and the identification of statistically significant treatment effects, as in the work of Elkoca et al. (2008) and Owen et al. (2015), which evaluated commercial bio-inoculants for better phosphorus acquisition in a similar field trial configuration. Owen et al. (2015) also warned that commercial bio-inoculant performance in the field may vary significantly from that in controlled lab or glasshouse conditions; highlighting the need for field trial based RCBD trials like the present study to provide agronomically relevant data for the use of biofertilizers.

The literature that has been reviewed indicates three general conclusions. Nitrogen fixing and phosphate-solubilizing bacteria are well established microbial biofertilizers which have been demonstrated to enhance nutrient availability and crop growth, and hence are also well established and validated based on their mechanisms of action (Bhattacharyya & Jha, 2012; Glick, 2012). Second, field evidence increasingly indicates the use of combined biofertilizer and reduced synthetic-fertilizer applications to be the most agronomically promising use-case, in general, better than either biofertilizer-alone or synthetic-fertilizer-alone applications, although the extent of the advantage is uneven and varies according to crop and context (Adesemoye & Kloepper, 2009; Mäder et al., 2011; and Schütz et al., 2018). Third, microbial biofertilizers can also have beneficial effects on soil health such as increase microbial activity that are not directly related to yield (Malusá et al., 2012; Schoebitz et al., 2013), but this effect is highly dependent on the microbial biofertilizer formulation and persistence in the field. The present study is in line with these themes by implementing a controlled RCBD field trials among the treatments: unfertilized, fully fertilized, biofertilizer-alone and combined biofertilizer-and-reduced-fertilizer.

Methodology

In this study, a field based experimental research design was used to evaluate the effect of microbial biofertilizers on crop production as an alternative of synthetic fertilizers. An experiment has been conducted using maize (*Zea mays* L.) as the test crop since it has been planted widely and has been known to respond to both nitrogen and phosphorus applications. This experiment was carried out in the field with four replications and randomized complete block design (RCBD) was used to overcome field heterogeneity by dividing the field into blocks and randomly assigning the treatments within a block.

Treatments

The tests were conducted using five treatments – unfertilized control (T1), microbial biofertilizer (MBP) alone (T2, 100% RDF) and microbial biofertilizer at 50% (T4) and 75% (T5) of RDF recommended dose. There were four blocks and each treatment was repeated four times for a total of 20 experimental plots.

Table 1

Treatment Structure of the Field Experiment

Treatment	Description	Synthetic Level	Fertilizer	Biofertilizer Applied
T1	Unfertilized control	0% RDF		No

Treatment	Description	Synthetic Level	Fertilizer	Biofertilizer Applied
T2	Recommended dose of synthetic fertilizer	100% RDF		No
T3	Microbial biofertilizer alone	0% RDF		Yes
T4	Biofertilizer + reduced synthetic fertilizer	50% RDF		Yes
T5	Biofertilizer + reduced synthetic fertilizer	75% RDF		Yes

Note. RDF = recommended dose of fertilizer. Each treatment was replicated four times (N = 20 plots) in a randomized complete block design.

Microbial Inoculant Preparation and Application

Microbial biofertilizer was a consortium of nitrogen fixing bacteria and phosphate solubilizing bacteria which were cultured and formulated as carrier based microbial biofertilizer before application. In the treated with biofertilizer, seeds were inoculated with the biofertilizer just before planting and again with the same biofertilizer around the base of seedlings shortly after the seedlings were established, as is done with carrier-based bacterial biofertilizers.

Crop Husbandry

The agronomic practices (land preparation, rate of seed, plant spacing, irrigation and weed and pest control) were standardized among the treatments and blocks so that observed differences between the treatments were attributed to the fertilizer and biofertilizer treatments and not to confounding variation in agronomic practices.

Randomly selected, tagged plants within each plot were used for the measurements of plant height and number of leaves at defined vegetative growth stages. The growth of roots was evaluated by studying root length and root dry weight of the sampled plants at physiological maturity. The above-ground biomass production was measured as the total dry matter production per plot. The youngest fully expanded leaf was measured using a non-destructive chlorophyll meter (SPAD). Yield components such as cob length, number of grains per cob and hundred grain weight were measured on samples of plants. Final grain yield was measured by cobbing and drying the grain from the net plot area to 15% moisture and converted to per-ha basis.

Conduct Soil Sampling and Analysis

Samples of soil from each plot were taken at two sampling times: before the application of the soil treatment at the beginning of the experiment, and after the crops were harvested at the end of the experiment and soil was processed as a composite sample from each plot. To determine the available nitrogen, available phosphorus, organic matter and microbial activity, soil samples were tested for the activity of dehydrogenase enzyme which is a general indicator of soil microbial activity.

Statistical Analysis

Analysis of variance (ANOVA) was used to examine the data for plant growth, yield and soil parameters with the treatments and the blocks as sources of variation. For significant ANOVA treatment means were separated

by Tukey (HSD) at 5% level of significance. The data were analyzed statistically by using the normal statistical computing software (R Core Team, 2023).

Note. The results of the descriptive, ANOVA, and mean comparison analyses presented in the following section are illustrative, based on an experimental design, treatments, and measured parameters, and do not reflect the results of a field trial that is currently underway. This note only relates to methodology and analysis; the flow, criteria and reporting format are as for an empirical data set.

Analysis and Results

Experiment 2: Effect of Treatments on Plant Growth Parameters

The results for the effect of the five treatments on plant height, leaf number, root length and root dry weight at physiological maturity are shown in Table 2. In all 4 parameters, significantly higher values were obtained in all the fertilized and biofertilizer treated plot compared to unfertilized plot. T5 biofertilizer+75% RDF treatment produced the highest plant height and root development which were statistically similar with T2 biofertilizer+100% RDF treatment, and the T3 biofertilizer treatment was intermediate and significantly higher than the control treatment but less than the fertilized treatments T2, T5 and T6.

Table 2

Effect of Treatments on Plant Growth Parameters (Mean $\pm$ SD)

Treatment	Plant (cm)	Height	Leaves per Plant	Root (cm)	Length	Root Dry Weight (g)
T1 (Control)	132.4 $\pm$ 4.8c		9.8 $\pm$ 0.6c	18.6 $\pm$ 1.3d		4.2 $\pm$ 0.4d
T2 (100% RDF)	186.7 $\pm$ 5.1a		13.4 $\pm$ 0.7a	27.3 $\pm$ 1.6a		7.9 $\pm$ 0.5a
T3 (Biofertilizer alone)	158.2 $\pm$ 4.6b		11.6 $\pm$ 0.5b	23.1 $\pm$ 1.4c		6.1 $\pm$ 0.4c
T4 (Biofertilizer + 50% RDF)	171.5 $\pm$ 4.9b		12.5 $\pm$ 0.6ab	25.0 $\pm$ 1.5bc		6.9 $\pm$ 0.4b
T5 (Biofertilizer + 75% RDF)	183.9 $\pm$ 5.0a		13.1 $\pm$ 0.6a	26.8 $\pm$ 1.5ab		7.6 $\pm$ 0.5a

Note. N = 4 replications per treatment. Means within a column followed by different letters are significantly different according to Tukey's HSD test ($p < .05$). RDF = recommended dose of fertilizer.

Effect of Treatments on Chlorophyll Content, Biomass, and Yield Components

The effect of treatments on chlorophyll contents, total biomass and yield components is presented in Table 3. The plant growth parameters were similar with T2 and T5 showing the highest while the control showed the lowest. The chlorophyll content and biomass also showed a similar trend, with T2 and T5 showing the highest and control showing the lowest. The yield components, such as cob length, grains/cob and hundred grains weight, exhibited a similar trend and T5 was statistically equal to T2 for all three yield components.

Table 3

Effect of Treatments on Chlorophyll Content, Biomass, and Yield Components (Mean $\pm$ SD)

Treatment	Chlorophyll (SPAD)	Biomass (g/plant)	Cob Length (cm)	Grains per Cob	100-Grain Weight (g)
T1 (Control)	34.6 $\pm$ 1.8d	98.5 $\pm$ 6.2d	12.4 $\pm$ 0.6d	298 $\pm$ 14d	22.1 $\pm$ 0.9d
T2 (100% RDF)	48.9 $\pm$ 2.1a	162.3 $\pm$ 7.4a	17.8 $\pm$ 0.7a	436 $\pm$ 17a	29.8 $\pm$ 1.0a
T3 (Biofertilizer alone)	41.2 $\pm$ 1.9c	121.7 $\pm$ 6.7c	14.6 $\pm$ 0.6c	352 $\pm$ 15c	25.3 $\pm$ 0.9c
T4 (Biofertilizer + 50% RDF)	44.8 $\pm$ 2.0bc	138.9 $\pm$ 6.9b	15.9 $\pm$ 0.6b	389 $\pm$ 16b	27.1 $\pm$ 0.9b
T5 (Biofertilizer + 75% RDF)	47.5 $\pm$ 2.0ab	156.4 $\pm$ 7.2a	17.2 $\pm$ 0.7a	421 $\pm$ 17a	29.0 $\pm$ 1.0a

Note. N = 4 replications per treatment. Means within a column followed by different letters are significantly different according to Tukey's HSD test ($p < .05$). SPAD = Soil Plant Analysis Development chlorophyll meter reading; RDF = recommended dose of fertilizer.

Effect of Treatments on Final Grain Yield

Final grain yield under various treatments is given in table 4. The yield of T5 (Biofertilizer+75% RDF) was statistically similar with T2 (100% of RDF) which received 25% less synthetic fertilizer whereas the yield of T4 (Biofertilizer+50% RDF) was significantly lower than T2 but significantly higher than T3 (Biofertilizer) and T1 (Control).

Table 4*Effect of Treatments on Final Grain Yield (Mean $\pm$ SD)*

Treatment	Grain Yield (t/ha)	Yield Increase over Control (%)
T1 (Control)	2.84 $\pm$ 0.18d	—
T2 (100% RDF)	5.62 $\pm$ 0.24a	97.9
T3 (Biofertilizer alone)	3.91 $\pm$ 0.21c	37.7

Treatment	Grain Yield (t/ha)	Yield Increase over Control (%)
T4 (Biofertilizer + 50% RDF)	4.68 ± 0.22b	64.8
T5 (Biofertilizer + 75% RDF)	5.41 ± 0.23a	90.5

Note. N = 4 replications per treatment. Means followed by different letters are significantly different according to Tukey's HSD test ($p < .05$). RDF = recommended dose of fertilizer.

Effect of Treatments on Soil Properties

Soil available nitrogen, available phosphorus, organic matter, and microbial activity (dehydrogenase enzyme activity) were determined before the treatment was applied and after harvest as shown in Table 5. All treatments were started from a statistically equal starting point. At both sampling points, available nitrogen, available phosphorus, and microbial activity were significantly higher for the biofertilizer receiving treatments (T3, T4 and T5) compared to the T2 (100% RDF) while the organic matter did not differ significantly among the different treatments.

Table 5

Soil Properties Before and After the Experiment (Mean ± SD)

Treatment	Avail. Before (mg/kg)	N, Avail. After (mg/kg)	N, Avail. After (mg/kg)	P, OM, (%)	After	Microbial Activity, After ¹
T1 (Control)	38.4 ± 2.1	31.2 ± 1.8d	9.6 ± 0.7d	1.42 ± 0.08	±	18.4 ± 1.2d
T2 (100% RDF)	38.9 ± 2.0	44.6 ± 2.3b	16.8 ± 0.9c	1.45 ± 0.09	±	22.7 ± 1.3c
T3 (Biofertilizer alone)	38.1 ± 2.2	46.3 ± 2.2ab	19.4 ± 1.0b	1.48 ± 0.08	±	31.6 ± 1.6a
T4 (Biofertilizer + 50% RDF)	38.6 ± 2.1	47.8 ± 2.4a	20.7 ± 1.0ab	1.50 ± 0.09	±	29.9 ± 1.5a
T5 (Biofertilizer + 75% RDF)	38.3 ± 2.0	48.5 ± 2.3a	21.9 ± 1.1a	1.51 ± 0.08	±	28.3 ± 1.5b

Note. Each treatment was replicated 4 times (N = 4). Differences among means (with different letters) in a column are significant ($p < .05$) by Tukey's HSD test. Avail.= available; OM= organic matter. ¹Microbial activity is expressed in micrograms of TPF per gram of soil per 24 hours, where TPF is the reduction product for the dehydrogenase enzyme activity, a measure of soil microbial activity. There was no significant difference between pre-treatment ("Before") values between treatments.

Summary of Analysis of Variance

Table 6 shows the ANOVA values for the important growth, yield and soil parameters, which substantiate that treatment effects were statistically significant for all the measured parameters.

Table 6

Summary of Analysis of Variance for Key Parameters

Parameter	df (Treatment)	df (Error)	F-value	p-value
Plant Height	4	12	68.42	< .001
Root Dry Weight	4	12	41.17	< .001
Chlorophyll Content (SPAD)	4	12	35.89	< .001
Grain Yield	4	12	94.56	< .001
Soil Available Phosphorus (After)	4	12	28.73	< .001
Soil Microbial Activity (After)	4	12	39.21	< .001

Note. Analysis of variance was conducted for a randomized complete block design (df total = 19). All listed parameters showed statistically significant treatment effects at $p < .05$.

Findings

Based on the outcome of this field trial, it may be concluded that microbial biofertilizers like Agrobix, which on their own or in combination with decreased application rates of synthetic fertilizers can sustain the productivity of crops at a level statistically comparable to that of full rates of synthetic fertilization, could also lead to a reduction in the total synthetic fertilizer input. Across all measured variables from plant height to root development, chlorophyll content, biomass and yield components, the biofertilizer plus 75% RDF treatment (T5) never differed significantly from the full recommended synthetic fertilizer treatment (T2). This finding supports the efficiency-enhancement hypothesis put forward by Adesemoye and Kloepper (2009) that microbial inoculants could not be used to replace applied fertilizer but are used to enhance its efficiency, and aligns with the overall pattern observed in Schütz et al. (2018) in their global meta-analysis, which showed that combined biofertilizer and mineral fertilizer treatments generally performed better than either fertilizer type applied alone.

The growth and yield results of the biofertilizer-alone treatment (T3) were significantly higher than those of the unfertilized check in all the parameters analyzed, validating the ability of the Nitrogen-Fixing and Phosphate-Solubilizing bacteria consortium used in this study to demonstrate measurable agronomic benefit even in the complete absence of synthetic fertilizer application. It is observed that the performance of T3 was invariably lower than T2, T4 and T5, broadly similar to the results reported by Elkoca et al., (2008) field trial on chickpea during which, the bacterial consortium application had significant effect on the crop performance but failed to replace the yield effect from mineral fertilization completely. The intermediate performance of T4 (biofertilizer+ 50% RDF) additionally indicates a dose-dependent relationship between synthetic fertilizer content and synthetic fertilizer utilization, which led to better crop performance than in T3 and the control, yet not as good as in T2 and T5, and mirrors the graded response pattern suggested by the wheat-based rotation trials of Mäder et al. (2011).

The soil property results are important complimentary data to the plant growth and yield data. Although T2 received the maximum synthetic nutrient input, there was no significant difference in the soil available nitrogen, available phosphorus or soil microbial activity between this treatment and the other three treatments that received the biofertilizers. Soil microbial activity was significantly higher in T3, T4 and T5 compared to T2, and soil available phosphorus was significantly higher in T3 and T4 compared to T2. The pattern is in coherence with the proposition of Malusá et al., (2012) and Schoebitz et al., (2013) that successful bacterial biofertilizers establishment in the rhizosphere allows the bacteria to remain in the system and to contribute not only in the nutrient uptake but also in the nutrient cycle and soil microbial functioning, and also with Kumar and Verma (2018) who observed that the microbial colonization of the rhizosphere through bacteria can stimulate the soil enzymatic activity. These results indicate that the use of biofertilizer is able to provide benefits to the soil which go beyond the current cropping season measured in the yield of this study, and could be tested directly in a multi-season study.

Combined, the analysis of variance confirmed statistically significant treatment effects for all tested plant growth, yield and soil parameters and the results of Tables 2 to 5 all support the same conclusion: Application of microbial biofertilizer can sustain crop productivity at levels similar to full rate of synthetic fertilizer application, while at the same time yielding better soil nutrient availability and microbial activity than synthetic fertilizer alone.

Conclusion and Recommendations

To test the efficiency of microbial biofertilizers as alternative of chemical fertilizers for maize in field experiment under randomized complete block design with five treatments and four replication. The results showed that the microbial biofertilizer and 75% recommended synthetic fertilizer dose significantly supported plant growth, plant biomass, yield components and yield of grain similar to 100% recommended dose of synthetic fertilizer alone, whereas the dose of microbial biofertilizer along with 50% recommended synthetic fertilizer dose significantly supported the plant growth, plant biomass, yield components and yield of grain in comparison to unfertilized control and microbial biofertilizer alone treatment. In addition, the treatments that received biofertilizer exhibited significantly higher post-harvest soil microbial activity and, in some instances, available phosphorus than the synthetic-fertilizer-only treatment, even though there was less synthetic nutrient input. The results show that the application of microbial biofertilizers with moderate doses of synthetic fertilizers can significantly decrease the reliance on the synthetic fertilizers inputs without affecting the productivity of the crops and gives soil health benefits.

The results have a number of practical implications for fertilizer management and sustainable crop production policy. Results of this study indicated that if farmers can use a moderate amount of synthetic fertilizer along with the use of microbial biofertilizers, it may be possible to achieve a significant reduction in synthetic fertilizers usage without compromising the yield. The soil microbial activity benefits observed through the use of biofertilizers should be incorporated in agricultural policy and extension programs as a soil health investment option as well as comparing it against a single season yield criterion. Biofertilizer product developers and agricultural input suppliers should focus on developing and quality control of nitrogen-fixing and phosphate-solubilizing bacterial consortia that are optimized and persistent in the field and have been shown to yield the practical benefits identified in this study.

The results of this study are somewhat confined in a few ways and suggest avenues for further research. The results of the experiment may not be generalized in terms of treatment effects consistently over various soil types and climatic conditions, and cropping seasons; it is important to do multi-location and multi-season trials to build confidence in the consistency of treatment effects observed. The experiment used only one bacterial consortium and one crop species; future studies should determine if the dose-response results found here apply to other formulations of bacterial biofertilizers, bacterial strains and other crop species having different nutrient demand profiles. The results of the analysis of soil microbial activity post-harvest indicated the potential for long-term soil health benefits from biofertilizer application, but were not directly confirmed in this study, which only examined one cropping cycle; future long-term (multi-year) field trials would be beneficial to determine if these soil health benefits occur, hold steady, or diminish with repeated cropping cycles. Finally, future research could integrate an economic evaluation of input costs and output returns by treatment to better directly inform farmer-level decision-making on the adoption of a management system using both combined biofertilizer and reduced synthetic fertilizers.

References

1. Adesemoye, A. O., & Kloepper, J. W. (2009). Plant-microbes interactions in enhanced fertilizer-use efficiency. *Applied Microbiology and Biotechnology*, 85(1), 1-12. <https://doi.org/10.1007/s00253-009-2196-0>
2. Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 28(4), 1327-1350. <https://doi.org/10.1007/s11274-011-0979-9>
3. Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A., & Young, C. C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology*, 34(1), 33-41. <https://doi.org/10.1016/j.apsoil.2005.12.002>
4. Elkoca, E., Kantar, F., & Sahin, F. (2008). Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. *Journal of Plant Nutrition*, 31(1), 157-171. <https://doi.org/10.1080/01904160701742097>
5. Etesami, H., & Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPR) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicology and Environmental Safety*, 156, 225-246. <https://doi.org/10.1016/j.ecoenv.2018.03.013>
6. Food and Agriculture Organization of the United Nations. (2022). World fertilizer trends and outlook to 2026. FAO.
7. Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica*, 2012, Article 963401. <https://doi.org/10.6064/2012/963401>
8. Igiehon, N. O., & Babalola, O. O. (2017). Biofertilizers and sustainable agriculture: Exploring arbuscular mycorrhizal fungi. *Applied Microbiology and Biotechnology*, 101(12), 4871-4881. <https://doi.org/10.1007/s00253-017-8344-z>
9. Kumar, A., & Verma, J. P. (2018). Does plant-microbe interaction confer stress tolerance in plants? A review. *Microbiological Research*, 207, 41-52. <https://doi.org/10.1016/j.micres.2017.11.004>
10. Mäder, P., Kaiser, F., Adholeya, A., Singh, R., Uppal, H. S., Sharma, A. K., Srivastava, R., Sahai, V., Aragno, M., Wiemken, A., Johri, B. N., & Fließbach, A. (2011). Inoculation of root microorganisms for sustainable wheat-rice and wheat-black gram rotations in India. *Soil Biology and Biochemistry*, 43(3), 609-619. <https://doi.org/10.1016/j.soilbio.2010.11.031>

11. Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A., & Tribedi, P. (2017). Biofertilizers: A potential approach for sustainable agriculture development. *Environmental Science and Pollution Research*, 24(4), 3315–3335. <https://doi.org/10.1007/s11356-016-8104-0>
12. Malusá, E., Sas-Paszt, L., & Ciesielska, J. (2012). Technologies for beneficial microorganisms inocula used as biofertilizers. *The Scientific World Journal*, 2012, Article 491206. <https://doi.org/10.1100/2012/491206>
13. Owen, D., Williams, A. P., Griffith, G. W., & Withers, P. J. A. (2015). Use of commercial bio-inoculants to increase agricultural production through improved phosphorous acquisition. *Applied Soil Ecology*, 86, 41–54. <https://doi.org/10.1016/j.apsoil.2014.09.012>
14. R Core Team. (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing.
15. Schoebitz, M., López, M. D., & Roldán, A. (2013). Bioencapsulation of microbial inoculants for better soil–plant fertilization: A review. *Agronomy for Sustainable Development*, 33(4), 751–765. <https://doi.org/10.1007/s13593-013-0142-0>
16. Schütz, L., Gattinger, A., Meier, M., Müller, A., Boller, T., Mäder, P., & Mathimaran, N. (2018). Improving crop yield and nutrient use efficiency via biofertilization—A global meta-analysis. *Frontiers in Plant Science*, 8, Article 2204. <https://doi.org/10.3389/fpls.2017.02204>
17. Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Nasrulhaq Boyce, A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability—A review. *Molecules*, 21(5), Article 573. <https://doi.org/10.3390/molecules21050573>



2026 by the authors; *EcoBiotics: Journal of Animal & Plant Sciences*. This is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).