Biofertilizers and Sustainable Crop Production: Effects on Soil Health and Crop Yield

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ABSTRACT

In this study, experimental research methods were used to analyze the effect of applying biofertilizer on soil health and crop productivity. To reduce experimental errors, a randomized complete block design (RCBD) was used with four treatments such as control (no application of biofertilizer), 100%, 200%, and 300% application level of biofertilizer. Soil samples were taken before and after the application of the treatment in order to check the changes in organic matter, nitrogen, phosphorus, potassium and microbial activity, and plant growth parameters such as plant height, number of leaves, biomass and end of grain production were measured at regular growth stages. Analysis of variance (ANOVA) was used to analyse the data and post-hoc tests were carried out using Tukey's Honestly Significant Difference (HSD) test at 5% significance level. The results showed that the application of biofertilizers significantly improved soil organic matter, available nitrogen, phosphorus, potassium and microbial activity as compared to the control with the increase in the level of application of the biofertilizer the effect was observed to be increased. Crop growth parameters and final grain yield also showed significant improvement with biofertilizer application compared to the control and the maximum increase in the soil health indicators and grain yield was recorded with the highest dose of biofertilizer application. The study suggests that application of biofertilizer is an effective and sustainable approach to improve the soil fertility and to boost the productivity of the crops and also gives the recommendations for adoption of biofertilizer, integrated nutrient management, further research work etc.

Keywords: biofertilizer, soil health, crop yield.Three Keywords: biofertilizer, soil health, crop yield.

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INTRODUCTION

Global food demand has traditionally been met by dramatic expansion of the application of synthetic chemical fertilizers, which has created long-term problems due to soil degradation, nutrient imbalances, and pollution of the environment. To address these concerns, biofertilizers which are products containing living microorganisms that, when applied to the seed, surface of the host plants, or soil, colonize the rhizosphere and stimulate the growth of the host plants by enhancing the availability of primary nutrients, have been receiving increasing scientific and agronomic interest as an alternative or complement to the conventional chemical fertilization (Vessey, 2003). Since biofertilizers function with a biological process instead of direct nutrient addition, measurable changes in soil biological and physicochemical properties are often part of the agronomic effects of biofertilizers and are key outcomes to consider along with crop yield.

The ways in which the biofertilizers can improve plant growth are many and are well documented in the literature on plant-microbe interaction. One of the most widely investigated groups of the biofertilizer organisms is plant growth promoting rhizobacteria which enhance plant growth by fixing atmospheric nitrogen, soil phosphorus and soil potassium that is not available to the plant, hormone production, and suppression of soil borne pathogens (Vessey, 2003; Bhattacharyya & Jha, 2012). Complementary reviews have highlighted that biofertilizers can positively influence nutrient-use efficiency, particularly by influencing the root architecture and root associated nutrient uptake capacity of plants, providing them with the opportunity to utilize nutrients already available in the soil (Adesemoye & Kloepper, 2009).

The practical agronomic value of these mechanisms is supported by a large amount of experimental field data. Under saline soil condition, application of nitrogen fixing and phosphate solubilizing biofertilizers on barley plants in a controlled field experiment using randomized design significantly enhanced the plant height, spike characteristics, grain yield and uptake of nutrients in addition to the increase in soil available nitrogen and phosphorus and soil microbial population as compared to non-inoculated controls (Alotaibi et al., 2024). Likewise, it has been demonstrated that application of biofertilizers in rice paddy systems with soil salinization significantly enhanced soil available nitrogen, phosphorus and potassium, as well as rice yields and reduced soil electrical conductivity, which seems to mean that application of biofertilizers could also improve soil chemical properties and crop performance at the same time under the environmentally challenging situation of soil salinization (Shan et al., 2023). Overall, a number of reviews that have integrated this broader literature have found biofertilizers to be a reliable method of improving various aspects of soil fertility such as organic matter, nutrient availability and microbial activity and biofertilizers to be an important method for sustaining agricultural intensification in a way that is environmentally sustainable (Mahanty et al., 2017; Itelima et al., 2018).

Even with these pieces of evidence, the dose-response relationship between biofertilizer rate and soil health and yield remains unclear, and the magnitude of response for biofertilizer treatments to various soil health indicators (organic matter, nitrogen, phosphorus, potassium, and microbial activity) in a common experimental design is still unresolved. Most of the previous studies assessed a single rate of biofertilizer application and compared it to a conventional chemical fertilizer or to a zero-fertilizer control experiment (Bargaz et al., 2018; Fasusi et al., 2021), and not by systematically testing multiple rates of biofertilizers in a replicated experimental design in which the effects of different application rates can be distinguished.

To offset the above limitations, the present study was designed as a controlled field experiment using randomized complete block design (RCBD) with four treatments: no biofertilizer (control), 1, 2 and 3 times the recommended rates of biofertilizer application, and four replications with spatial variability in field conditions and minimum experimental error, based on the principles of agricultural experimental design (Fisher, 1935; Gomez & Gomez, 1984). Soil health indicators such as organic matter, nitrogen, phosphorus, potassium and microbial activity were measured before and after treatment application and the treatment means compared using Analysis of Variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. The study aimed to assess the impact of increasing the level of application of biofertilizers on soil health indicators, crop growth parameters and yield of grain, and to establish the optimal level of application of biofertilizers that will have the maximum effect on soil health and crop productivity. The results will offer support to evidence-based biofertilizer dosing in sustainable crop production systems.

LITERATURE REVIEW

Biofertilizers: Mechanism of Action

Biofertilizers are generally defined as formulations consisting of living microorganisms applied to seed, plant surfaces or soil that multiply and colonize the host plant's rhizosphere or interior, and that stimulate plant growth by enhancing the supply or availability of primary nutrients (Vessey, 2003). The plant growth promoting rhizobacteria (PGPR) organisms which are the most widely studied biofertilizers are known to exert their beneficial effects on plants in several ways: through biological nitrogen fixation, mobilizing fixed soil P and K into available forms, by producing phytohormones which stimulate root growth, and through antagonism of soil borne plant pathogens, (Vessey, 2003; Bhattacharyya & Jha, 2012). In addition to these direct nutrient-related mechanisms, biofertilizer organisms have been seen to indirectly improve nutrient-use efficiency in plants by increasing the root surface area which can penetrate deeper into the soil, and retrieve nutrients that would otherwise be beyond plant reach (Adesemoye & Kloepper, 2009).

Nitrogen fixing bacteria like Azotobacter and Rhizobium spp, phosphate-solubilizing bacteria like Bacillus spp and Pseudomonas spp, and potassium-solubilizing bacteria like Bacillus mucilaginosus are some of the microbial taxa commonly used in the formulation of biofertilizers and each of them plays a specific nutrient related function within the rhizosphere microbial community (Bhattacharyya & Jha, 2012). Field trials involving the use of mixtures of N2-fixing and phosphate-solubilizing bacterial biofertilizers have shown that they can yield considerable benefits, not only in terms of plant nutrient uptake, but also measurable changes in soil chemical properties, such as nitrogen and phosphorus availability (Alotaibi et al., 2024).

Soil Health Indicators Affected by Biofertilizers

There is a growing and increasing number of field experimental results showing that the use of biofertilizer has positive effects on several aspects of soil health. Application of biofertilizer using halotolerant microorganisms has been reported to have significantly improved the soil available nitrogen, available phosphorus, and available potassium, and decreased the soil electrical conductivity, which is considered as a good index of salinity stress in saline paddy soils, compared to inorganic fertilization (Shan et al., 2023). Complementary experiments in the barley field under saline soil condition revealed that application of biofertilizer, specifically in combination with moderate dose of chemical fertilizer, caused remarkable enhancement in the soil available nitrogen and available phosphorus content, and soil total microbial counts and dehydrogenase activity, commonly used as a proxy for soil microbial activity (Alotaibi et al., 2024).

Experimental literature has been synthesized to show that biofertilizers significantly increase soil organic matter content and its ability to cycle nutrients, thus increasing long term sustainability of soil fertility management when compared with the use of chemical fertilizers alone (Mahanty et al., 2017; Bargaz et al., 2018). This body of evidence has given rise to biofertilizers being increasingly considered as a management strategy for soil health, and not just a nutrient supplementation approach, and therefore not only for the current growing season (Fasusi et al., 2021).

In addition to their soil properties, biofertilizers have been well documented in various species of crops and environments to enhance crop growth parameters and yield. Bioduplication of chemical NPK fertilizer, in particular, led to a significant increase in the height of the plants, spike length, grain yield, and straw yield over the non-inoculated controls, while reducing the cost of chemical fertilizer use in barley grown under saline field conditions (Alotaibi et al., 2024). The use of biofertilizer, which is a formulation prepared from nitrogen fixing bacteria also has been reported to significantly enhance the plant height, yield components and nutrient levels in harvested grains over the uninoculated control wheat (Kumar et al., 2014).

Experimental studies in the greenhouse carried out early showed that combined, multi-functional biofertilizer formulations containing both nitrogen fixing bacteria and phosphate- and potassium-solubilising microorganisms, plus arbuscular mycorrhizal fungi, could result in better growth of maize compared to uninoculated controls, giving promising evidence for the superiority of combined, multi-functional biofertilizer formulations over single-organism inoculants (Wu et al., 2005). Comparative field studies comparing organic and conventional farming systems have shown that soils under biologically based fertility inputs have a significantly higher microbial biomass and biodiversity than soils under conventional management, and this translates into better long-term soil fertility and crop performance (Mäder et al., 2002).

Specific studies on phosphate-solubilizing bacteria has revealed that when such bacteria is isolated from agricultural soils and used as a biofertilizer, they can significantly enhance the solubilization of poorly accessible soil phosphate, thereby supplying a direct microbiological link between biofertilizer use and soil P liberation and plant growth (Chen et al., 2006).

The qualitative advantages of the application of biofertilizer are known but few attempts have been made to investigate the quantitative changes of the advantages with different levels or rates of application of Biofertilizers in a controlled experiment. Experiments that directly compare different application rates of a biofertilizer under controlled field conditions are valuable, and reviews of biofertiliser research have highlighted that the effectiveness of biofertiliser application can depend on the type, rate and formulation applied, as well as on interactions with existing soil fertility and with any co-applied chemical fertiliser (Bargaz et al., 2018; Itelima et al., 2018). The present study employs a multi-level randomized complete block design, which enables the direct assessment of the relationship between the different application levels of the biofertilizers and the soil health and yield parameters, as opposed to the inference of such relationship from studies in which a single application rate of the biofertilizers is used.

The method: RCBD and ANOVA in Agricultural Experimentation

The randomized complete block design (RCBD) has been widely accepted as a standard experimental design used in field research in agriculture, as it enables to control the systematics variation of the field conditions (soil heterogeneity and micro-topography) by blocking in order to obtain a more precise estimation of the treatment effects (Fisher, 1935; Gomez and Gomez, 1984). In the RCBD, the most widely used inferential test for statistically significant differences among treatment means is Analysis of Variance (ANOVA) and when the overall ANOVA is significant, the multiple comparisons test employed is Tukey's Honestly Significant Difference (HSD) test, which can identify which specific pairs of treatment means differ significantly from each other while appropriately controlling the family-wise error rate (FWER) (Tukey, 1949; Gomez & Gomez, 1984).

Identifying Gaps in the Literature

While there is sound evidence that application of biofertilizer has positive effects on soil health indicators (increase in soil organic matter, nitrogen, phosphorus, potassium, and microbial activity; see Shan et al., 2023; Alotaibi et al., 2024) and crop growth and yield (Kumar et al., 2014; Wu et al., 2005), few studies have quantified the effects of biofertilizer application on all of these soil health indicators together in a replicated field experiment that has been deliberately designed to compare a range of biofertilizer application rates with an untreated control. The present study was designed to overcome this situation by using four treatment RCBD replicated four times to not only test the dose-response effect of applying biofertilizer to this entire array of soil health indicators, but also to crop growth parameters and final grain production, and perform rigorous treatment mean comparisons using ANOVA and Tukey HSD testing.

METHODOLOGY

Research Design

The present study used an experimental research design to study the effect of biofertilizer on soil health and productivity. A randomized complete block design (RCBD) was adopted, which involved four treatments (T0: control (no biofertilizer), T1: low level application of biofertilizer, T2: moderate level application of biofertilizer, and T3: high level application of biofertilizer). As in the usual agricultural field experimental design (RCBD) (Fisher, 1935; Gomez & Gomez, 1984), the division of the experimental area into blocks was done in order to minimize the effect of variations in soil and field topography within the experimental area.

Experimental Site and Treatments.

The experiment was carried out in a field location which had soil and climatic characteristics typical of the cropping system of interest. Four treatments were randomly applied in each of the four blocks to give a total of 16 experimental plots. The biofertilizer applied was the microbial strains (N2-fixing and PHA-solubilizing microbes) which were applied at three increasing rates (T1, T2 and T3) with no application of biofertilizer (T0) used as the experimental control. The same land preparation, seeding and general agronomic management was applied to all plots and the amount of biofertilizer was the only factor experimentally manipulated.

Soil Sampling and Analysis

Soil samples were taken before and after the application of treatment in each experimental plot (baseline and post-treatment) according to standard composite soil sampling practice where multiple soil sub-samples were taken from within each plot and combined to represent the experimental plot. The soil samples were analysed for organic matter content, available nitrogen, available phosphorus, available potassium and microbial activity (by soil dehydrogenase enzyme activity - a standard soil microbial activity indicator), using the standard soil laboratory analytical procedures for each soil parameter.

Crop Growth and Yield Measurements

The crop growth parameters were measured during the growing season at suitable growth stages. At the vegetative growth stage, plant height and number of leaves per plant was determined from a random sample of plants per plot. A sub-sample area was identified in each plot and above-ground biomass was destructively measured at a later stage of growth. Grain yield was determined at physiological maturity after cutting the entire net plot area (excluding the border rows to eliminate edge effects) and standard unit of yield was calculated as yield per hectare.

Data Analysis

Analysis of Variance (ANOVA) was used to analyze data for each soil health indicator and crop growth and yield parameter using Treatment and Block as sources of variation separately for each of the parameters under consideration. An ANOVA F-test was used to determine if treatment had an effect; if so, treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at the 5% significance level ($\alpha = .05$), as is common practice when making multiple mean comparisons after an ANOVA result is statistically significant (Tukey, 1949; Gomez & Gomez, 1984). The normality of the residuals and homogeneity of variance were tested for each DV before ANOVA.

Ethical and Quality Assurance Considerations.

All field and laboratory operations were performed following good agricultural and laboratory practices. Measurements were made using standard soil and plant sampling procedures to reduce measurement error and standard laboratory procedures and calibrated measurement instruments to ensure accuracy and reliability in reporting soil and plant parameter values.

Note. Specific numerical results reported below in the Analysis and Findings sections (soil health indicators, crop growth parameters, grain yield values, ANOVA summary tables, and Tukey's HSD comparisons) are sample values provided to illustrate the reporting format that is appropriate for this experimental design. They are not based on a real field trial; they must be updated with results that are based on an actual field trial before submission of this paper or for any decision-making process.

ANALYSIS

The statistical analysis of the experimental data is presented here. The analysis is then broken down into six steps: (a) describe the baseline soil characteristics by block; (b) compare the post treatment soil health indicators by ANOVA and Tukey's HSD; (c) compare the growth parameters of the crops by ANOVA and Tukey's HSD; (d) compare the final grain yields by ANOVA and Tukey's HSD; (e) summarize overall ANOVA model statistics; and (f) make a brief correlation check between soil health indicators and grain yield.

Baseline Soil Characteristics

Soil samples were taken from all sixteen plots before the treatments were applied to ensure there was no pre-existing systematic difference between the treatments.

Table 1. Baseline (Pre-Treatment) Soil Characteristics by Treatment Group

Soil Parameter	**T0 (Control)**	**T1 (Low)**	**T2 (Moderate)**	**T3 (High)**
Organic Matter (%)	1.82	1.85	1.79	1.83
Available Nitrogen (mg/kg)	48.2	47.6	48.9	48.1
Available Phosphorus (mg/kg)	12.4	12.7	12.1	12.5
Available Potassium (mg/kg)	96.3	97.1	95.8	96.6
Microbial Activity (µg TPF/g soil/24h)	18.6	18.9	18.3	18.7

No significant differences were found among the treatment groups on any of the baseline soil parameters (Table 1, one-way ANOVA, all $p > .05$), confirming that the soil parameters for the treatment groups were approximately equal before the biofertilizers were applied, and that comparisons between the treatment groups after biofertilizers were applied were valid.

Post-Treatment Soil Health Indicators

Means, ANOVA results, and Tukey's HSD grouping for each soil health indicator by treatment are shown in Table 2.

Table 2. Post-Treatment Soil Health Indicators by Treatment, with ANOVA and Tukey's HSD Results

Soil Parameter	T0 (Control)	T1 (Low)	T2 (Moderate)	T3 (High)	F (3, 9)	p
Organic Matter (%)	1.84 c	2.05 bc	2.24 ab	2.41 a	16.82	< .001
Available Nitrogen (mg/kg)	47.9 c	54.3 b	60.1 ab	65.8 a	22.47	< .001
Available Phosphorus (mg/kg)	12.3 c	15.1 b	17.6 ab	19.9 a	19.35	< .001
Available Potassium (mg/kg)	96.0 c	104.2 b	111.7 ab	119.4 a	14.61	< .001
Microbial Activity (µg TPF/g soil/24h)	18.5 c	23.7 b	27.9 ab	31.6 a	25.09	< .001

Note. Means within a row followed by different letters (a, b, c) differ significantly according to Tukey's HSD test at $\alpha = .05$. Means sharing a letter (e.g., 'ab') do not differ significantly from either the group above or below them in ranked order.

As revealed in table 2, treatments were found to be statistically significant for all five soil health indicators (all $p < .001$). For all the indicators, the control (T0) showed the lowest mean value while the other treatments showed an ascending trend and the highest mean value for each of the indicators was noted under T3 (organic matter, available nitrogen, available phosphorus, available potassium and microbial activity). The results of the Tukey's HSD comparisons showed that T3 had a significantly greater soil health score than T0 on all five indicators, and that T2 and T3 did not differ significantly from each other on available nitrogen, available phosphorus or organic matter, indicating a leveling off in the benefits of soil health with the highest level of biofertilizer application for these soil properties.

Crop Growth Parameters

Table 3. Crop Growth Parameters by Treatment, with ANOVA and Tukey's HSD Results

Growth Parameter	T0 (Control)	T1 (Low)	T2 (Moderate)	T3 (High)	F (3, 9)	p
Plant Height (cm)	112.4 c	121.6 b	129.8 ab	136.2 a	18.94	< .001
Number of Leaves (per plant)	9.6 c	10.9 bc	12.1 ab	13.4 a	12.37	.001
Above-Ground Biomass (g/plant)	84.3 c	97.1 b	108.6 ab	119.8 a	21.05	< .001

As presented in the Table 3, all three parameters of the crop growth were significantly influenced by the application of biofertilizer (all $p \leq .001$). The trend of increasing soil health indicators, soil organic carbon and soil aggregation, was also reflected in the trend of plant height, leaf number and above-ground biomass with increasing biofertilizer application level, with the highest application (T3) showing significantly higher values than the lowest (T0) application.

Final Grain Yield

Table 4. Final Grain Yield by Treatment, with ANOVA and Tukey's HSD Results

Treatment	Mean Grain Yield (t/ha)	SD	Tukey's HSD Grouping
T0 (Control)	2.84	0.21	c
T1 (Low biofertilizer)	3.31	0.24	b
T2 (Moderate biofertilizer)	3.72	0.19	ab
T3 (High biofertilizer)	4.05	0.23	a

The ANOVA for final grain yield was statistically significant ($p < .001$), $F(3, 9) = 24.63$, which showed that the level of application of biofertilizer was significant in influencing the grain yield. As presented in the Table 4, Grain yield is improved gradually from control (2.84 t/ha) to higher level of biofertilizer (4.05 t/ha) with the increment of around 42.6% over control. Again, Tukey's HSD comparisons showed that the yield of T3 was significantly higher than that of T0 and T1 and that the yield of T2 and T3 were not significantly different from each other, indicating a leveling off in yield at the higher rates of biofertilizer application.

Relationship Between Soil Health Indicators and Grain Yield

Plot level Pearson correlations were performed between each soil health indicator and final grain yield for all sixteen experimental plots to look at the correlation of increased soil health with increased grain yield.

Table 5. Correlations Between Post-Treatment Soil Health Indicators and Final Grain Yield

Soil Health Indicator	**Correlation with Grain Yield (r)**	**p**
Organic Matter	.71	.002
Available Nitrogen	.76	< .001
Available Phosphorus	.69	.003
Available Potassium	.64	.007
Microbial Activity	.78	< .001

All five soil health indicators were significantly and positively correlated with soil's final grain yield, with microbial activity ($r = .78$) and available nitrogen ($r = .76$) having the strongest correlation, followed by organic matter ($r = .71$), available phosphorus ($r = .69$), and available potassium ($r = .64$) (Table 5). This interpretation is consistent with the pattern observed that the gains in soil biological and chemical fertility due to biofertilizers were directly responsible for the observed gains in crop yield.

FINDINGS

Before the application of biofertilizer, soil was analysed, which indicated that there was no significant difference between the four treatment groups on any soil parameter, thus justifying the use of randomized complete block design and the subsequent treatment comparisons. After application, ANOVA results showed statistically significant differences between the different levels of biofertilizers with the levels getting higher as the biofertilizer levels increased, for all the 5 soil health indicators measured, namely organic matter, available nitrogen, available phosphorus, available potassium and microbial activity. Tukey's HSD comparisons showed that the high level biofertilizer treatment (T3) was significantly different from the untreated control soil (T0) for all soil health indicators, while certain indicators showed T2 was not significantly different than T3, indicating that the marginal benefit of the biofertilizer treatment waned at the higher level tested.

The growth parameters of the crops were closely similar. There was significant increase in all parameters such as plant height, number of leaves and above ground biomass with the increase in the level of biofertilizer, where high level of biofertilizer showed the maximum value compared to the control in all three parameters. The overall trend was the same, with the high-level biofertilizer treatment yielding 4.05 t/ha, which was about 42.6% higher than the 2.84 t/ha for the control, however the moderate- and high-level treatments were not statistically different from each other with Tukey's HSD.

The hypothesized relationship between the improvement of soil health and crop performance was further supported through correlation analysis of all five soil health indicators with final grain yield – all were significantly and positively correlated with final grain yield, and the highest correlations were found with microbial activity and available nitrogen. The pattern indicates that the yield advantage obtained from the biofertilizer treatment was not a secondary effect, but was closely associated with the soil biological and chemical fertility during the time of biofertilizer applications.

Overall, the results suggest that application of biofertilizers led to significant and dose-dependent improvement in the soil health and productivity, with the moderate and high application rates showing the best effects that appeared to level off after a certain point, thereby indicating that there is a limit to the benefits that can be obtained from biofertilizer application beyond that point.

CONCLUSION AND RECOMMENDATIONS

The use of biofertilizer caused statistically significant, dose-dependent increases in soil organic matter, available nitrogen, phosphorus, potassium, and microbial activity, as well as in plant height, leaf number, biomass and final grain yield compared to the untreated control treatment, and the moderate and high level biofertilizer treatments were the most consistently effective with the highest response rates. These results underscore the fact that use of biofertilizers is a viable and biologically sound alternative to soil fertility management and crop production systems that can lead to increased crop productivity while concurrently enhancing soil biological and chemical fertility, and that the yield response to biofertilizer use may be closely related with the increase in biological and chemical fertility of the soil itself. Based on these results, it is recommended that biofertilizers be encouraged at moderate to high application rates as an integrated soil fertility management strategy since the results of the application rates tested in this study showed a diminishing marginal return in the moderate to high application rate, implying that moderate application rates may be more cost effective without compromising benefits. It is also recommended that the use of biofertilizers complement rather than supplant existing soil fertility management practices and that farmers use biofertilizers in conjunction with other soil fertility management practices to decrease their use of chemical fertilizers without compromising long-term soil health and productivity. Finally, the study was conducted at a single field location over one growing season, and future research should be conducted on more than one growing season using different soil types and with different crops, as well as repeat applications of this biofertilizer over time, to determine if the dose-response relationships observed in this study are maintained, intensified, or weakened by continued application of biofertilizers over time.

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