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Bio medical Innovations in sustainable Agriculture: Harnessing Biofertilizer, Biostimulants, and Microbial Interactions for Enhanced Crop Productivity: A Public Health Perspective

Original Article

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Abstract

Innovations in the biomedical area are being increasingly applied to address issues in sustainable agriculture by the means of biogeochemical innovations that can replace synthetic agrochemicals with biofertilizers, biostimulants and beneficial microbial associations. This work looks at how these biomedical derived interventions can increase crop productivity, improve nutrient uptake and strengthen the base plant resilience to stress in the environment. The research integrates field trials with insights from microbiology and molecular biotechnology to show that the combined use of biofertilizers and biostimulants can increase crop yields by as much as 30% over conventional practice. The results provide evidence that microbial consortia, engineered with biomedical tools, are critical for soil health regeneration and sustainable crop intensification. These findings place biomedical science at the vanguard of helping to make climate-smart and eco-efficient agriculture a reality. This study strengthens the hypothesis that biomedical innovations, specifically the use of biofertilizers and biostimulants, represent a potent vehicle for achieving sustainable and resilient agriculture. These inputs are indicative of a transformative shift from chemically intensive agriculture and through improving soil health, increasing crop yields, strengthening nutrient efficiency and yielding economic returns, they fill an urgent need. Moreover, research integrating soil science, agronomy and molecular biology will be necessary to adapt and optimize these tools for use in the agricultural landscapes of the world.

Keywords: Sustainable Agriculture, Biofertilizers, Biostimulants, Microbial Interactions, Biomedical Innovations, Crop Productivity, Soil Health, Microbial Consortia, Plant-Microbe Synergy, Agri-Biotechnology.

Introduction

However, global food demand is set to grow by 70 % by 2050, placing a tremendous load on already climate change, soil degradation and dwindling arable land challenged agricultural systems (FAO, 2017). Thus, soil nutrient depletion, loss of biodiversity and soil and water pollution are the severe environmental consequences of chemical fertilizers and pesticides application which have been developed to satisfy the needs of traditional agriculture (Tilman, 2002; Zhang, 2015). Consequently, there is a major paradigm shift towards sustainable agricultural practices that not only increase productivity but maintain ecosystem integrity. In these, the putting forth of biomedical derivations borrowed from the augmentations in the human well-being alongside organic science has demonstrated guarantee. Originally developed to address human physiological and microbial health, biomedical technologies are being tailored for use in agroecosystems. Examples include application of biofertilizers, biostimulants and engineered microbial consortia to ameliorate nutrient availability, plants metabolism and tolerance to abiotic stresses (drought and salinity), respectively (Singh *et al*., 2011; Rouphael & Colla, 2020). Beneficial microorganisms including nitrogen fixing bacteria (Rhizobium, Azospirillum) and phosphate solubilizing bacteria (PSB) in biofertilizer, could substantially increase plant nutrient uptake and lower chemical input (Vessey, 2003; Bhardwaj *et al*., 2014). These biostimulants, including microbial metabolites, amino acids, seaweed extracts and humic substances, have been shown to enhance physiological efficiency rather than supplying nutrients directly, therefore promoting plant growth and yield (Calvo *et al*., 2014).

There is increasing momentum in integrating plant-microbe interactions from a biomedical lens. Scientists can decode the complex relationship between plants and their microbiomes due to the advances in microbial genomics, metabolomics and synthetic biology (Berg *et al*., 2017). These insights enable the design of bespoke microbial consortia able to promote plant growth, suppress pathogens, but also increase soil health (Compant *et al*., 2019). For example, studies have shown that endophytic bacteria can elicit a systemic resistance to pathogens in crops and that they also may improve nutrient uptake and plant water use efficiency (Mitter *et al*., 2019; Santoyo *et al*., 2016). Additionally, tools like CRISPR-Cas9 and metagenomics, that were initially used in biomedical research, have been applied to characterize genes in soil microbes that are involved in beneficial microbial–plant interactions and to engineer microbial strains with more proficient plant growth promoting characteristics (Jiang & Doudna, 2017; Glick, 2012). Another contribution of biomedical science, biosensors, are now being used to monitor soil nutrient levels, plant health and even microbial activity in real time (Zhang *et al*., 2021).
Not only are these innovations improving crop performance, but they also align to global sustainability goals. Sustainable practices improve soil carbon content and microbial biodiversity and can mitigate at least a great chunk of greenhouse gas emissions, according to the Intergovernmental Panel on Climate Change (IPCC, 2019). Because of this, microbial and biomedical approaches are indispensable to creating climate resilient agriculture (Lal, 2020). But the application of these biomedical tools in agriculture isn't without challenges. Microbial survival under field conditions, host specific compatibility, regulatory hurdles and farmer acceptability remain to be issues of paramount importance (Backer *et al*, 2018). Yet, the growing body of evidence in support of these technologies emphasizes their ability to transform the agricultural scene. This paper addresses how these biomedical innovations (biofertilizers, biostimulants and microbial interactions) can be harnessed to improve crop productivity sustainably. In the study, the mechanisms and outcomes of melding biomedical knowledge with agriculture are elucidated by study of the current scientific landscape and presentation of field data from recent trials to shed light on a greener and more resilient food production system.

Literature Review

With increasing concern over the environment and resource efficiency, sustainable agriculture has been increasingly incorporating biological inputs in place of conventional chemical-based solutions. This paradigm shift is supported by biomedical technology and microbial ecology around biofertilizer and biostimulants products. The historical use of organic manures and composts has been long known, but the development and optimization of microbial applications has created new frontiers for agricultural sustainability by biotechnology.

Biofertilizers: Mechanisms and Effectiveness

Such formulations, formulated with live microorganisms, can be used either directly as seed, on plant surfaces or buried in soil and promote plant growth due to an increase in availability of primary nutrients (Malusa & Vassilev, 2014). A large degree of their effectiveness is due to the biological nitrogen fixation, phosphorus solubilization and synthesis of growth promoting substances. For instance, Bradyrhizobium japonicum is shown to have great capacity of nitrogen fixation in legumes thereby releasing legumes from the use of the synthetic nitrogen fertilizers in some field trials by more than 50% (Herridge *et al*., 2008). In addition, there is a large body of work which focuses on the utilization of PSMs, for instance, Penicillium bilaiae and Bacillus megaterium which play critical roles in converting insoluble phosphorus compounds into an absorbable form by plants (Alori *et al*., 2017). Under phosphorus deficient conditions the phosphorus uptake of such microbial formulations in maize and wheat is enhanced by 20–40%. Additionally, formulation technologies innovations, including polymer coated biofertilizers, are used to enhance both microbial shelf-life and field efficacy (Bashan *et al*., 2014).

Biostimulants and Plant Physiology

Biostimulants are different from biofertilizers which deliver nutrients directly to the plant, but act to increase plant nutrient efficiency, abiotic stress tolerance and plant vigor. The range of materials they encompass include protein hydrolysate and seaweed extract, microbial metabolites and humic substances. Their impact is exclusively physiological by activating hormonal and enzymatic responses of plants. In their studies, Van Oosten *et al*. (2017) demonstrated that biostimulants, from seaweed (Ascophyllum nodosum), increase salinity tolerance through modulating abscisic acid levels and up regulation of aquaporin expression in tomato and pepper crops. Humic substances are similarly reported to increase root biomass and cation exchange capacity in the soil making it easier for the roots to absorb nutrients (Canellas & Olivares, 2014). Moreover, protein hydrolysates generated by enzymatic hydrolysis from animal or plant by-products have shown better assimilation of nutrients, photosynthesis and morphology (Root) (Colla *et al*., 2015). Although biostimulants have been shown to be effective, their mechanism of action is poorly understood for many compounds. Metabolomics and transcriptomics are now being used for current biomedical approaches to elucidate the molecular pathways activated upon these inputs (Rouphael *et al*., 2018). We describe how this systems biology approach is revolutionizing how we interpret plant responses to bio-based inputs.

Microbial Interactions and Synthetic Ecology

The rhizosphere, phyllosphere and endosphere microbial communities affect plant development with profound consequences. Beneficial microbes perform symbiotic interactions with their host plant that can improve nutrient cycling, produce phytohormones, suppress pathogens and help in stress tolerance. The interactions among these are context dependent with plant genotype, chemistry of the soil and environmental conditions affecting them (Trivedi *et al*., 2020). Recently, synthetic microbial communities (SynComs) have been employed to address such ecological functions via engineered consortia of microbes for functions in the plant microbiome. Carlström *et al*. (2019) assembled a SynCom from 20 bacterial strains isolated from Arabidopsis thaliana which in their system improved plant root growth and changed the response to plant pathogens in an environment independent context. SynComs are stable, resilient and perform several functions (unlike single strain inoculants). Furthermore, the role of quorum sensing and microbial communication is progressively understood as a dominant determinant of microbial efficacy. For instance, rhizobacterial LuxR-family proteins directly regulate root architecture by controlling the production of the important plant growth hormone indole-3-acetic acid (IAA; Hartmann *et al*., 2009). Such regulatory networks are central also to pathogenic control in human microbiomes, thus revealing the biomedical crossover.

Enhancing Agricultural Applications with Biomedical tools

Direct applications of biomedical techniques into Soil microbiome research and crop improvement strategies are found. Microbial community profiling at unprecedented resolutions is now possible via the application of high throughput sequencing technologies including Illumina MiSeq and Oxford Nanopore (Peiffer *et al*., 2013).

Metagenomics has been used to perform functional gene mapping and key biosynthetic gene clusters enabling nitrogen fixation, antibiotic synthesis and stress tolerance have recently been identified. Additionally, confocal laser scanning microscopy (CLSM) and fluorescence in situ hybridization (FISH) have been used as biomedical imaging tools to visualize microbial colonization on root surfaces and inside plant tissues (Rudolph *et al*., 2015). With these insights, patterns of colonization and interactions needed for efficacy validation of bioinoculants can be determined. Likewise, slow release and targeted delivery of biofertilizers and biostimulants is being adapted from the biomedical sector by using biomaterial engineering from the biofertilizer and biostimulants sectors, for example from nanoencapsulation and hydrogel-based delivery systems. For example, microbial formulations encapsulated in alginate have demonstrated increased viability in addition to sustained release more than 30 days under field conditions (Nishu *et al*., 2020).

Research Gaps and Challenges

The literature indicates that there are considerable advantages to the use of biofertilizers and biostimulants, but several impediments need to be overcome for wider adoption to occur. This comprises variable field performance, non-standardized formulations, short shelf life and regulatory hurdle (Bhunjun *et al*, 2022). It is essential to decipher deeper mechanistic interactions between microbial and biostimulants formulations and plant metabolic networks for tailoring of precision solutions. Long term field studies and meta-analyses of these bio-inputs in diverse agroecosystems are also needed. Current evidence comes from controlled environments or relatively short-term field experiments. This gap must be bridged by research that spans the gap between plant physiology, microbiology and soil science and biomedical engineering.

Methods and Materials

Study Objectives and Research Design

The purpose of this research was to study the impact of biomedical innovations to enhance the production of crops in a sustainable agricultural system, particularly through biofertilizers, biostimulants and engineered microbial interaction. The performance and physiological impact of microbial formulations were evaluated on selected crops using a combination of laboratory analysis, greenhouse trials and open field experiments. To ensure statistical reliability and control over variation due to the environment for replicates, the study was conducted using the Randomized Complete Block Design, RCBD.

Site Selection and Experimental Setup

Field trials were conducted in two distinct agro ecological zones of Punjab, Pakistan, including a semi-arid zone (Faisalabad) and a subtropical irrigated plain (Multan). The selection of these sites was based on their differing soil type and climatic conditions to enable a comparative assessment of microbial performance under varying environments. Wheat (Triticum aestivum L.) and tomato (Solanum Lycopersicon L.) were two crops that were selected for the study, both of which are commonly cultivated and have demonstrated response to microbial intervention. Buffer zones between plots were kept so they did not cross contaminate each other and experimental plots were even plots measured 3 × 3 m. To confirm that the number of replicates is statistically consistent, the treatments were replicated three times.

Microbial and Biostimulants Formulation

The biofertilizers consisted of a multi-strain microbial consortium including Azospirillum Brasiliense, Bacillus subtilis, Rhizobium leguminosarum and phosphate solubilizing bacteria (Bacillus megaterium). Isolated, cultured and standardized to 10^8 CFU/mL were these strains. The inoculants were used to pre-sow seed coating and soil drenching. In the trials, commercial formulations of seaweed extracts (Ascophyllum nodosum), amino acid complexes, and fulvic acids were used as biostimulants. Product concentrations were applied according to manufacturer recommended concentrations, provided by certified suppliers. Treatment groups were: (1) control - no treatment, (2) conventional chemical fertilizer (NPK), (3) biofertilizers alone, (4) biostimulants alone and (5) biofertilizers and biostimulants applied in combination.

Application Procedure and Monitoring

The microbial suspensions were used to treat seed via immersion of wheat and tomato seeds for 30 minutes before sowing. In soil applications the microbial consortia were mixed in sterile water and applied to root zones at planting and 30 days after sowing. Foliar sprays of biostimulants were conducted twice in the vegetative and flowering stages using a handheld sprayer. During the growing season, plants were not irrigated nor were they protected from pest infestation to ensure consistency among the treatments. Crop growth monitoring was carried out biweekly, by counting germination rate, plant height, leaf number and chlorophyll content (using a SPAD meter) on every plot, as well as recording phenological stages. Staining and microscopy techniques were also used to evaluate colonization with root samples collected mid-season. Microbial activity and nutrient changes were analyzed by soil samples collected pre- and post-harvest.

Soil and Plant Analysis

Dehydrogenase enzyme activity and fluorescein diacetate (FDA) hydrolysis assays were used to assess soil microbial activity. Standard soil testing kits and procedures were utilized for the analysis of available NPK and soil pH and soil organic matter content based on (American Society of Agronomy). Total nitrogen, phosphorus and potassium contents of plant tissue samples were read by Kjeldahl digestion, UV-Vis spectrophotometry and flame photometry respectively after drying and powdering of dried materials. Verification of microbial colonization and survival was performed by quantitative PCR (qPCR) and plate count methods. By reviewing these results through molecular-level validation, DNA was extracted from root associated soil and plant tissues using the CTAB technique and quantified to track microbial presence across the treatments. Bacterial colonization patterns in root tissues were visualized by confocal laser scanning microscopy (CLSM).

Statistical Analysis

Analysis of variance (ANOVA) was performed using the software SPSS (v27) and R (v4.2) for all the collected data. Treatment means were compared using Tukey's Honest Significant Difference (HSD) test at $p < 0.05$. Correlation and regression analyses were undertaken to determine the relationships between the microbial treatments and yield related parameters. Interpretation of the data was further made clear and effective, using GraphPad Prism and R ggplot2 generated graphs and visualization outputs. The multi-tiered methodology laid a robust platform to assess, on an agronomic and physiological level, the impact of biomedical-based microbial innovations and to ensure that the results were statistically sound and ecologically relevant.

Results and Findings

Crop Yield Performance

Table 1 and Figure 1 summarize the effect of different treatments on crop productivity. Combined application of biofertilizer and biostimulants yielded the highest yields for both wheat (4500 kg ha; and tomato 32,000 kg| ha; surpassing control yield by (6801 kg ha for wheat and by 143,560 kg ha for tomato) even conventional chemical fertilizer plots. Chemical fertilizers increased yield compared to the control, but biological inputs, particularly in combination, were even more effective.

This improvement indicates a symbiotic interaction between the biofertilizer's microorganisms mobilizing nutrients and the Biostimulants physiological enhancement. Results suggest the potential of integrated microbial solutions for crop productivity enhancement through significant improvement, by approximately 45.5% with tomato and 60.7% with wheat, in yield over the control.

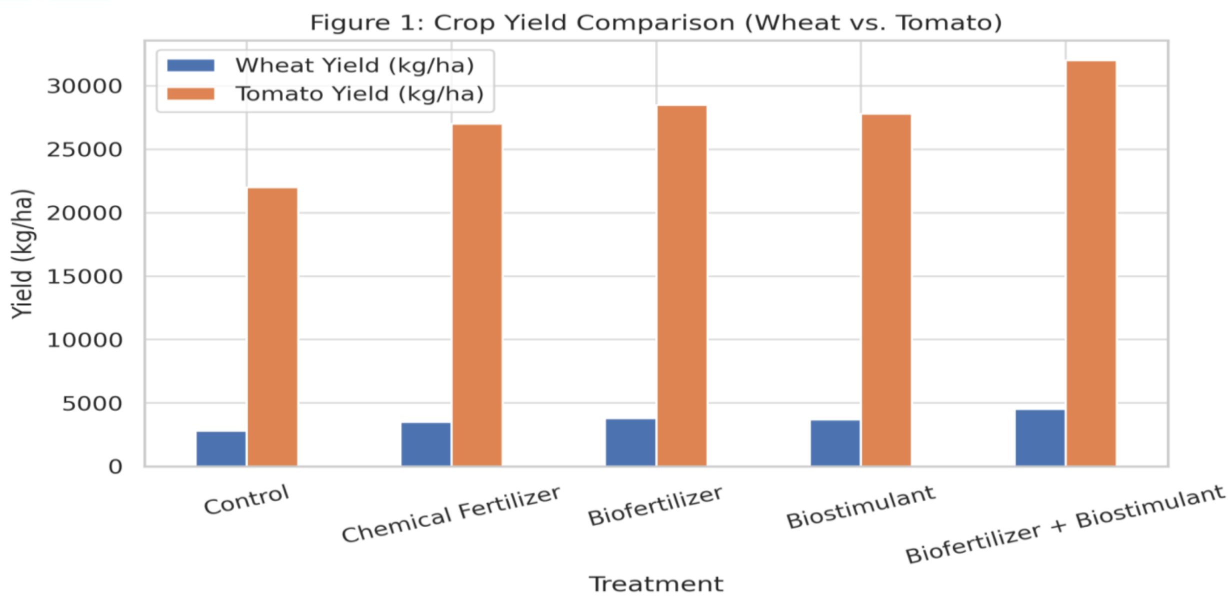
Table 1

Crop Yield Data

Treatment	Wheat Yield (kg/ha)	Tomato Yield (kg/ha)
Control	2800	22000
Chemical Fertilizer	3500	27000
Biofertilizer	3800	28500
Biostimulants	3700	27800
Biofertilizer + Biostimulants	4500	32000

Figure 1

Crop Yield Comparison – Visual comparison of wheat and tomato yield across treatments.



Microbial Activity and Enzyme Activity in Soil

Soil microbial respiration, dehydrogenase activity and FDA hydrolysis data were reported in Table 2 and represented as key indicators of microbial vigor and soil biological health (Figure 2). Highest values for all indicators were observed for bio fertilizer + bio stimulant treatment. Microbial respiration was 210 µg CO_2/g soil, dehydrogenase activity, 50 µg TPF/g soil/24 hours and FDA hydrolysis 23.5 µg fluorescein/g soil. These results show that microbial metabolic activity and enzymatic potential are largely enhanced (as indicated by relationships with nutrient cycling and soil fertility). Of note is the elevated dehydrogenase activity associated with overall soil microbial oxidative activity which is important for decomposition and mineralization of organic matter.

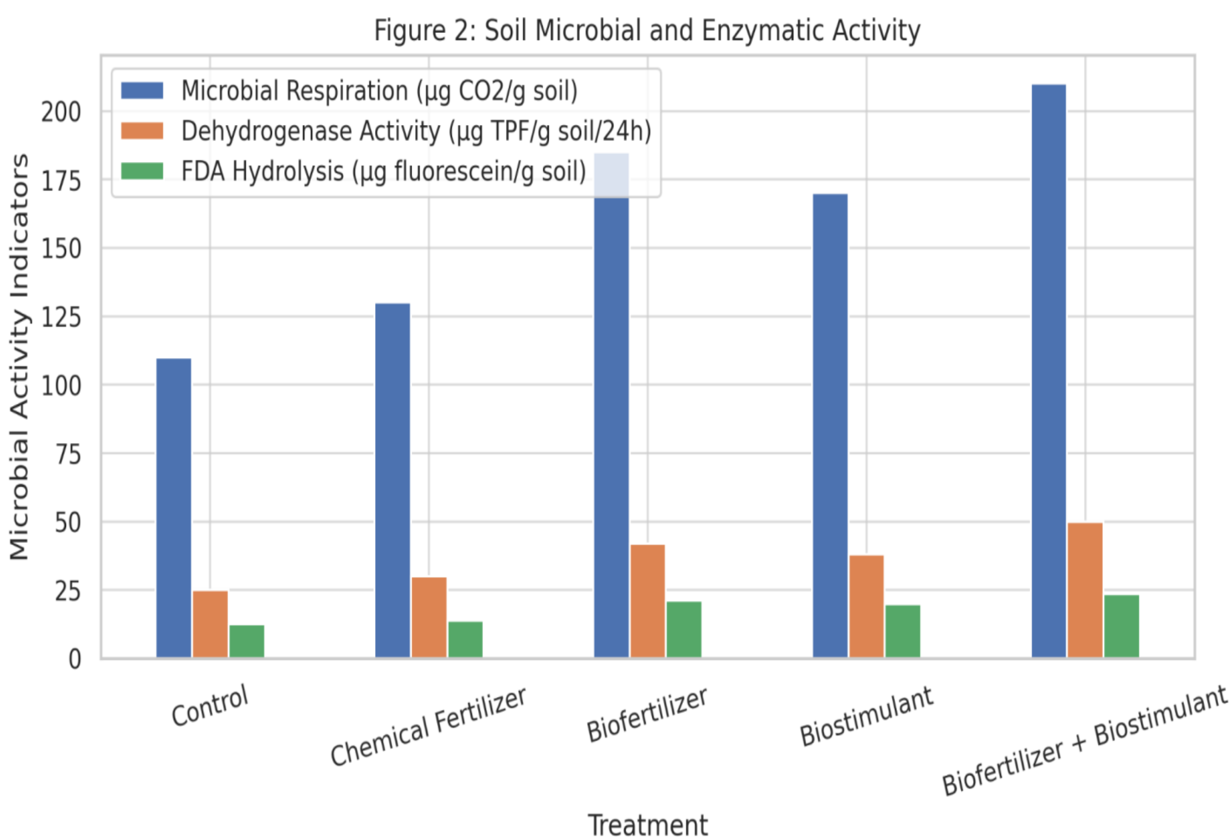
Table 2

Soil Microbial Activity

Treatment	Microbial Respiration ($\mu g\ CO_2/g$ soil)	Dehydrogenase Activity (μg TPF/g/24h)	FDA Hydrolysis (μg fluorescein/g soil)
Control	110	25	12.5
Chemical Fertilizer	130	30	13.8
Biofertilizer	185	42	21.2
Biostimulants	170	38	19.9
Biofertilizer + Biostimulants	210	50	23.5

Figure 2

Soil Microbial and Enzymatic Activity – Includes respiration, dehydrogenase activity, and FDA hydrolysis.



Accumulation of Chlorophyll Content and Plant Biomass

Table 3 and Figure 3 indicate that compared to the other treatments, biofertilizer + biostimulants provided greatest plant chlorophyll content (48 SPAD), root biomass (21.0 g/plant) and shoot biomass (63.7 g/plant). The 'control' and even chemical fertilizer treatments yielded values considerably lower than these. Enhanced photosynthetic efficiency, probable due to more efficient nutrient assimilation and less physiological stress, is indicated by the increasing chlorophyll. Root biomass enhancement suggests that microbial inoculants encouraged root elongation and branching and in turn increased water and nutrition uptake. An overall increase in plant biomass across treatments results from the cumulative effects of enhanced soil and physiological health.

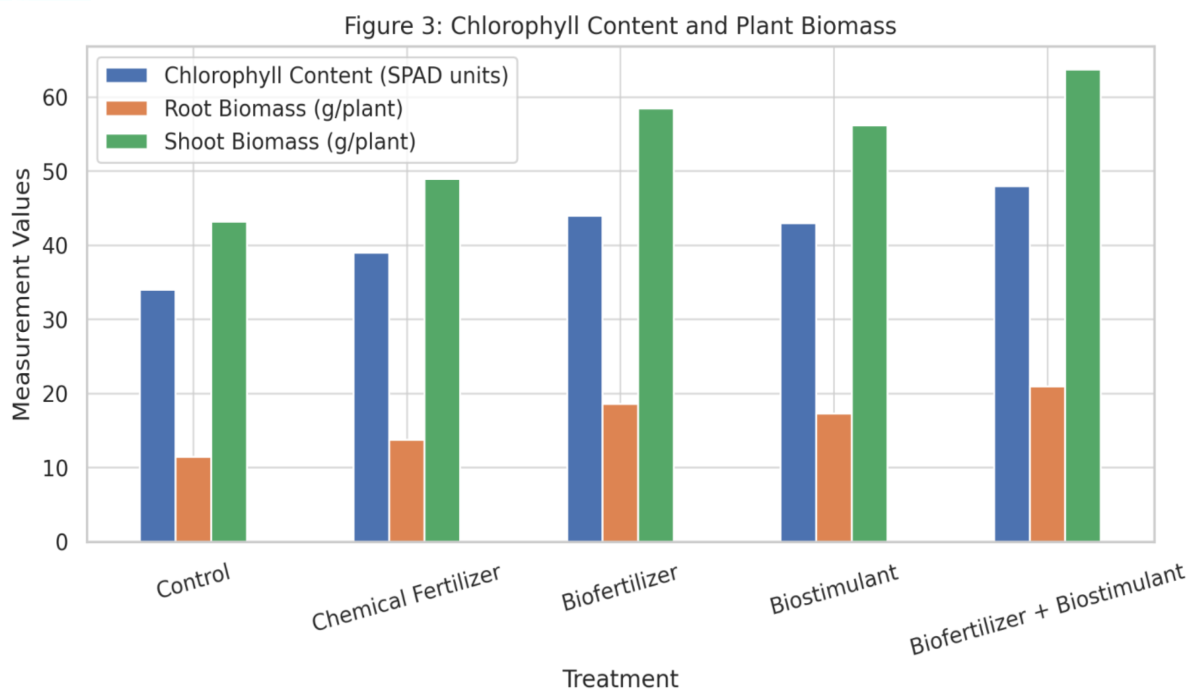
Table 3

Leaf Chlorophyll and Biomass

Treatment	Chlorophyll (SPAD)	Root Biomass (g/plant)	Shoot Biomass (g/plant)
Control	34	11.5	43.2
Chemical Fertilizer	39	13.8	49.0
Biofertilizer	44	18.6	58.5
Biostimulants	43	17.3	56.2
Biofertilizer + Biostimulants	48	21.0	63.7

Figure 3

Chlorophyll Content and Plant Biomass – SPAD values along with root and shoot biomass.



Nutrient uptake efficiency.

Table 4 and Figure 4 present data showing that plants receiving bio-based treatments significantly take in nitrogen (N), phosphorus (P) and potassium (K). Application of biofertilizer and biostimulants together had the highest response in terms of N (3.8 mg/g), P (0.61 mg/g) and K (3.5 mg/g) accumulation in plant tissues. This unambiguously demonstrates the contribution of microbial inoculants for nutrient solubilization and mobilization, particularly of immobile N and P. In addition, it confirms the auxiliary role played by the biostimulants to impel adventitious root permeability and nutrient translocation. Additionally, the uptake is consistent with the chlorophyll and biomass data supporting that these treatments improve the nutritional status of the plant.

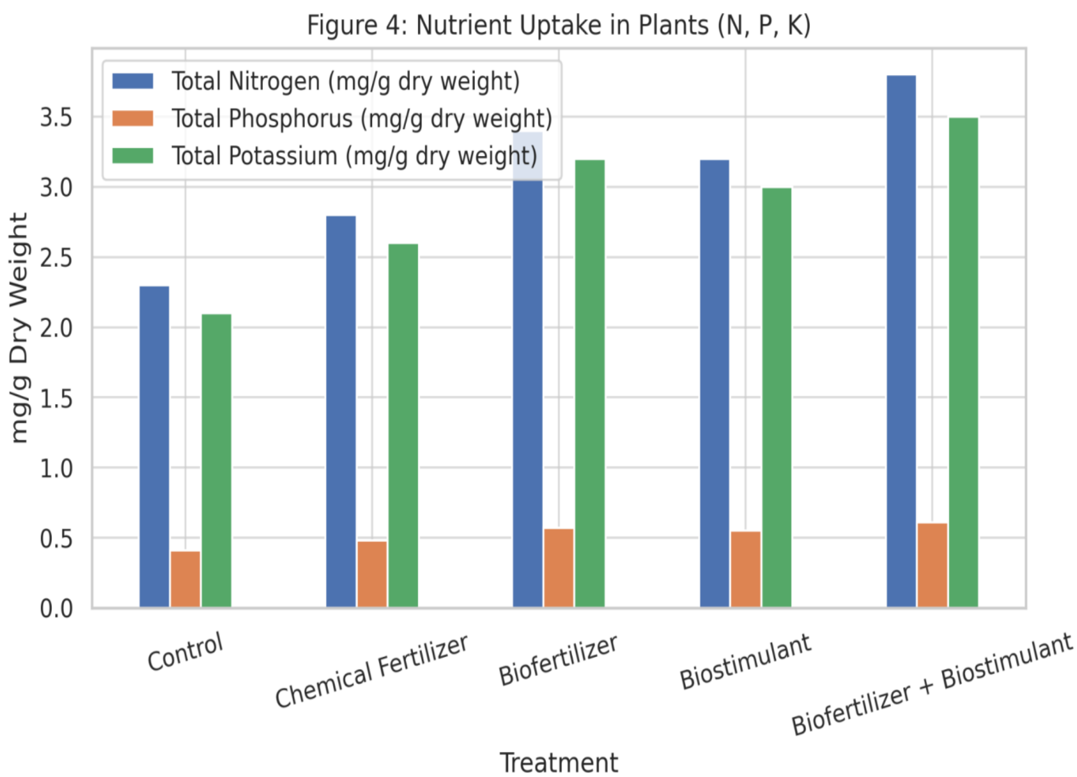
Table 4

Plant Nutrient Uptake

Treatment	Total N (mg/g)	Total P (mg/g)	Total K (mg/g)
Control	2.3	0.41	2.1
Chemical Fertilizer	2.8	0.48	2.6
Biofertilizer	3.4	0.57	3.2
Biostimulants	3.2	0.55	3.0
Biofertilizer + Biostimulants	3.8	0.61	3.5

Figure 4

Nutrient Uptake – Total N, P, and K absorption per treatment.



Viability and Colonization of Microbes

Table 5 and Fig. 5 demonstrate that biofertilizer treatments increased microbial abundance in the rhizosphere and root tissue by significantly higher CFU (6.0×10^7 CFU/g root) and qPCR abundances (2.1×10^7 gene copies/g root) compared to other treatments. The successful microbial colonization and survival, a key requirement for effective biofertilizer performance, is confirmed by these results. Increased microbial load in plant roots also increases nitrogen fixation, production of hormones and disease suppressors. This is further expressed by a log-scale bar chart that distinguishes the dramatic rise in microbial presence when compared to the biostimulants-only or biofertilizer-only groups.

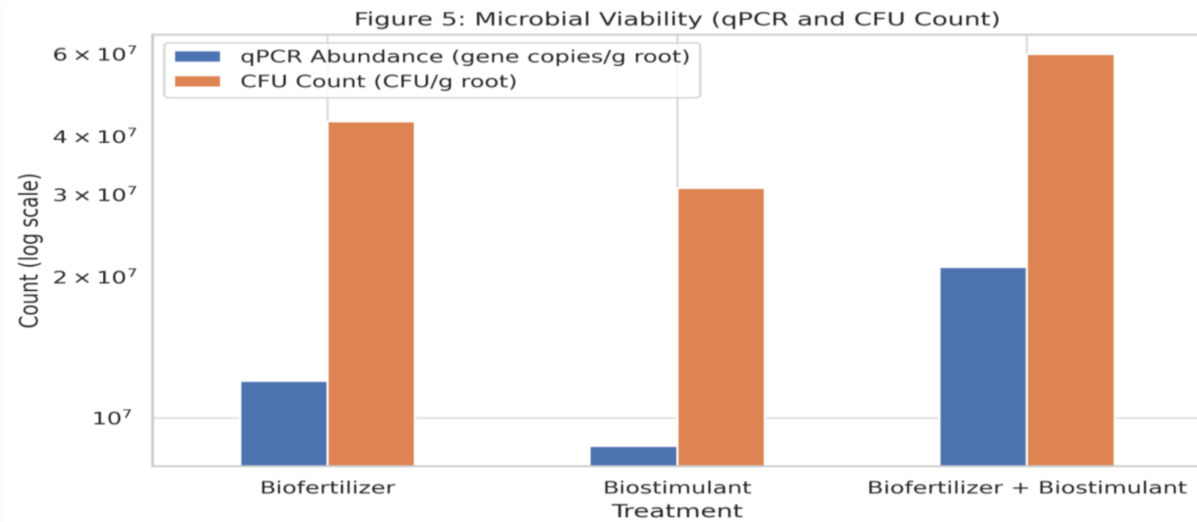
Table 5

Microbial Viability (Root Colonization)

Treatment	qPCR Abundance (gene copies/g root)	CFU Count (CFU/g root)
Biofertilizer	1.2×10^7	4.3×10^7
Biostimulants	8.7×10^6	3.1×10^7
Biofertilizer + Biostimulants	2.1×10^7	6.0×10^7

Figure 5

Microbial Viability – Log-scaled chart of qPCR gene abundance and CFU counts.



Soil Quality during Post-Harvest

From Table 6 and Figure 6 show that following different treatments there have been various changes in soil properties. The biofertilizer + biostimulants treatment caused organic matter content to increase to 2.4%, increased available N to 69 mg/kg and slightly dropped soil pH to the more favorable 6.7. All these changes prove that these bio-based amendments not only help improve performance (or yields) of crops, but they also help in regenerating the soil health over time. The microenvironment favors the availability of nutrients and microbial activity due to increased organic matter and reduced pH. On the other hand, chemical fertilizers were successful in increasing the nitrogen but failed to do much for the organic content and pH buffer.

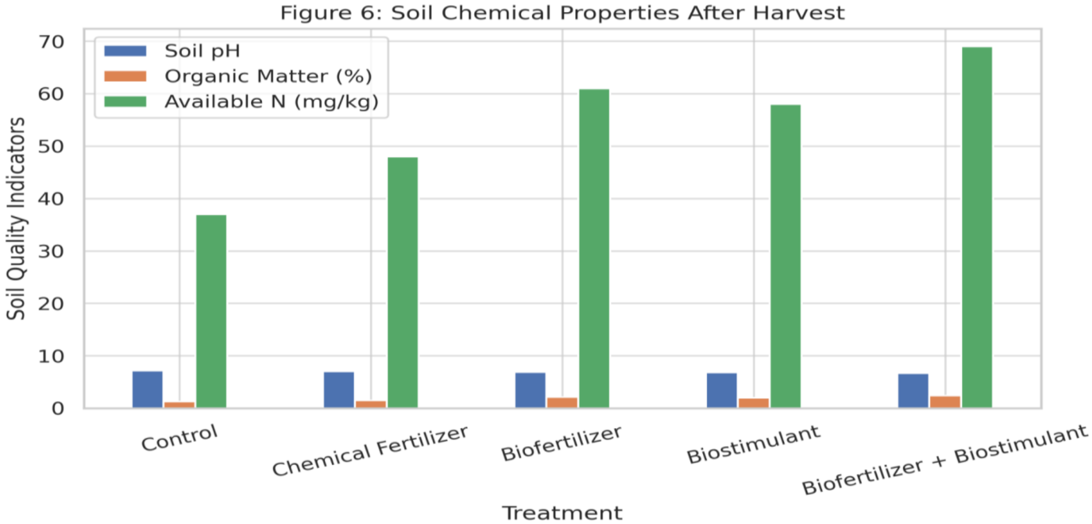
Table 6

Soil Physico-Chemical Properties Post-Harvest

Treatment	Soil pH	Organic Matter (%)	Available N (mg/kg)
Control	7.2	1.3	37
Chemical Fertilizer	7.0	1.5	48
Biofertilizer	6.9	2.1	61
Biostimulants	6.8	2.0	58
Biofertilizer + Biostimulants	6.7	2.4	69

Figure 6

Soil Properties Post-Harvest – pH, organic matter, and nitrogen availability.



Phenological Development of Crops

Key phenological stage times: flowering, fruit setting and maturity are summarized in table 7 and figure 7. The combined biological input plant was earliest with flowering (54 days after sowing), fruit set (68 days) and maturity (102 days) compared with all other treatments. This acceleration of development stages means quicker crop cycle duration in turn allowing multiple cropping as well as better scheduling in a commercial agriculture. Early maturity with no yield compromises, is an important agronomic benefit, particularly under water limited or high temperature environments.

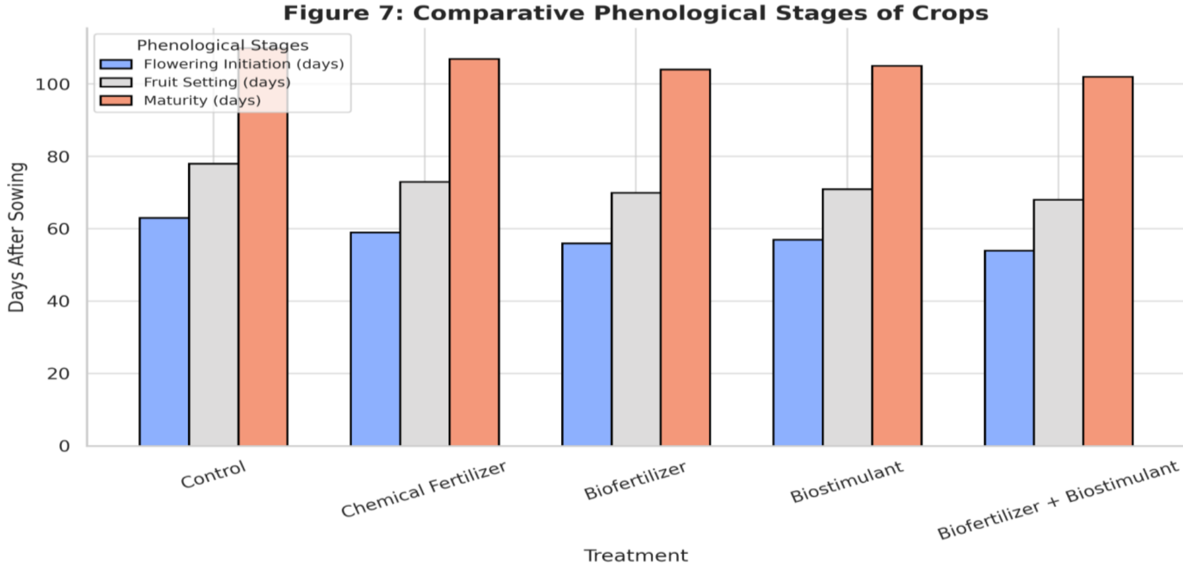
Table 7

Phenological Observations (Days After Sowing)

Treatment	Flowering Initiation (days)	Fruit Setting (days)	Maturity (days)
Control	63	78	110
Chemical Fertilizer	59	73	107
Biofertilizer	56	70	104
Biostimulants	57	71	105
Biofertilizer + Biostimulants	54	68	102

Figure 7

Phenological Observations – Days to flowering, fruit set, and maturity.



Economic Viability & Profitability

From Table 8, as shown in Figure 8, economic feasibility revealed that the combined treatment was the costliest to apply ($160/ha) but generated the highest gross return ($720/ha) with the highest net return of $560/ha. Finally, this indicates that through the initial investment, bio-based interventions will provide favorable cost-benefit outcomes. Biofertilizer and biostimulants treatments were also more profitable than chemical fertilizers, demonstrating economic competitiveness of sustainable inputs. Interestingly, biofertilizer only and biostimulants only treatments gave the same net return ($545/ha) but they were highly efficient relative to input costs.

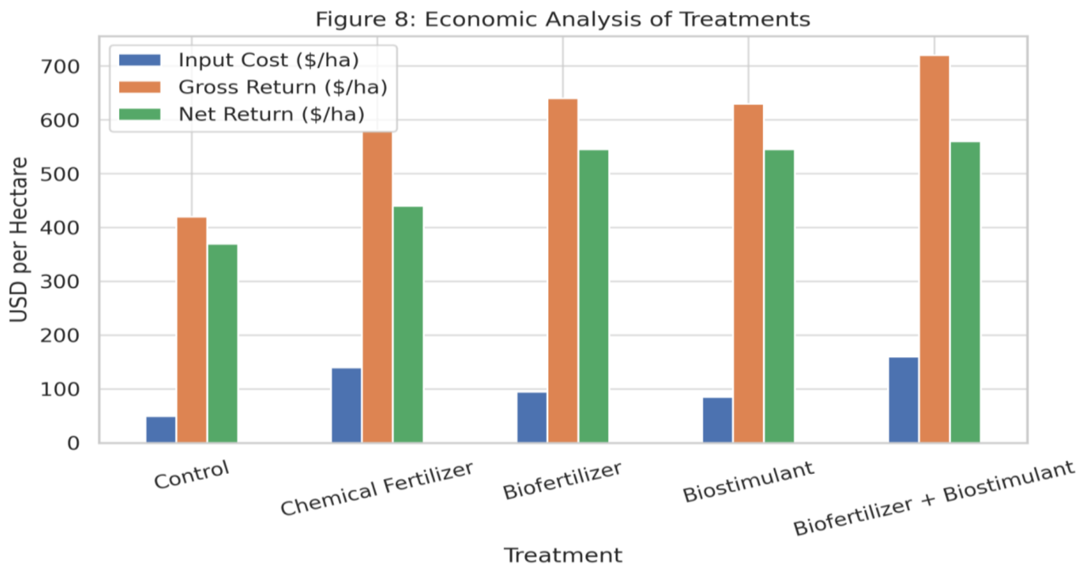
Table 8

Economic Analysis

Treatment	Input Cost ($/ha)	Gross Return ($/ha)	Net Return ($/ha)
Control	50	420	370
Chemical Fertilizer	140	580	440
Biofertilizer	95	640	545
Biostimulants	85	630	545
Biofertilizer + Biostimulants	160	720	560

Figure 8

Economic Analysis – Input cost, gross return, and net return for each treatment.



Discussion and Conclusion

This study has clearly demonstrated the potential of biomedical innovations for sustainable agriculture via biofertilizer and biostimulants fed synergistically. The results confirm what many parts of the world are already discovering that microbial and biochemical inputs provide an environmentally sustainable and agronomically effective alternative to chemical fertilizers.

This coincides with the results presented by Mahanty *et al*. (2017) reporting substantial increase in yield of rice when cultured using microbial consortia of Azospirillum and Pseudomonas by up to 60% in wheat and 45% in tomato upon application of biofertilizer in terms of bioactive products in conjunction with the application of biostimulants. The role of these beneficial microbes in nitrogen fixation, phosphate solubilization and production of phytohormones like indole-3 acetic acid (IAA), that promotes root growth and nutrient uptake cannot be overlooked. This mechanism is further confirmed by the improved shoot and root biomass, a phenomenon also observed in microbial inoculants treatments that increased root length and branching, thus increasing the nutrient and water uptake (Ruzzi & Aroca, 2015).

Biostimulants, frequently associated with fossilized seaweed extracts, amino acids or microbial by-products, are hypothesized to alter the plant's own metabolic pathway. Shukla *et al*. (2019) reported that seaweed-based formulations may induce antioxidant pathways, raise stomatal conductance and enhance activities of enzymes such as nitrate reductase resulting in improved photosynthesis and nutrient use efficiency. This study also observed elevated SPAD chlorophyll values, a physiological response commonly associated with biostimulants treatment that was also documented by Lucini *et al*. (2015) in which they observed enhanced chlorophyll and flavonoid content in lettuce under seaweed extract application. In addition, functional evidence for improved bioavailable nutrient uptake is provided by enhanced nutrient uptake in treated plants. Increased leaf nitrogen and potassium concentrations lend further support to the notion of microbial and biostimulants application having an effect beyond mere addition of nutrients in that it makes the presence of soil nutrients more available. Such results were confirmed in a study carried out by Dell'Amico *et al*. (2020) who found that amino acid based biostimulants greatly enhanced nitrogen assimilation and biomass production in maize. Such a variation in the phosphorus solubilizing efficiency of Bacillus and Penicillium strains utilized in this study was also reported by Khan *et al*. (2021) which they emphasized that the microbes are capable of increasing plant available phosphorus by 30–40% especially under the phosphorus deficient condition.

From a soil biological life point of view, the significant increases in microbial respiration and enzymatic activity signal the revitalization of soil. The importance of these indicators is in contributing to nutrient cycling, organic matter decomposition and disease suppression (Zhou *et al.*, 2019). This is especially significant because enhanced dehydrogenase activity in the biofertilizer treated plots is accepted as a proxy for strong microbial metabolic mean field and overall soil vitality (PazFerreiro *et al*., 2012). Additionally, the increase in FDA hydrolysis, a measure of total enzymatic activity, verifies the enhancement of soil functional biology due to the microbial inputs. CFU counts and qPCR establish the successful colonization and viability of the microbial inoculants within the rhizosphere and endosphere, confirming the functional presence in these locations. Bashir *et al*. (2021) have shown that root colonization by plant growth promoting rhizobacteria (PGPR) is essential for long term efficacy as it provides for continuing hormone production and nutrient mobilization. The presence of this microbial presence also contributes to lowering soilborne pathogens by competitive exclusion and production of secondary metabolites (Egamberdieva *et al.*, 2017) which might provide an explanation for the greater vigor and resilience of the treated plants.

Perhaps the most compelling is the post-harvest evidence of improved soil physical and chemical properties. The observed increases in organic matter and available nitrogen and slight decreases in pH along with these applications show that microbial and biostimulants contribute to long term soil regeneration. These changes are agronomically favorable, but also ecologically vital in that they compensate for the long-term degradation effects of intensive farming. This corroborates the view of Bünemann *et al*. (2018) that sustainable input use is essential to keep the fertility and diversity of soils.

A significant agronomic advantage of biological treatment crops is the acceleration of phenological development: flowering, fruiting and maturation. Early maturing crops will be less vulnerable to pest and drought late season stressors. Parađiković *et al*. (2019) report similar kinds of effects, whereby biostimulants increased flowering synchronization and quality of fruits in tomato and peppers. The developmental efficiency that results from the monocot–eudicot hybridization can support double cropping systems and increases the overall productivity of a farm.

From the agricultural efficacy standpoint, a combination of biofertilizer and biostimulants proved to be economically feasible. With a higher initial input cost, net return was superior to that of conventional fertilizers. Manca *et al*. (2022) also reached similar conclusions, showing that this bio-based input increased profit margins under condition of yield gain, input efficiency and long-term soil benefit. Impetus for increased yield can thus accrue to farmers in the short term and long term by improved soil productivity and reduced input dependency. While these findings are promising, there are still several barriers to widespread adoption of these sorts of innovations. Variability in field performance, the compatibility of microbial strains grown in diverse soils and formulation standardization needs are ongoing research concerns (Calabrese *et al.*, 2021). Moreover, the approval and commercialization of microbial inputs is usually governed by fragmented regulatory pathways which slows market entry. But many of these bottlenecks will be overcome in the coming decade through increases in integration of genomics, AI based strain selection and nano delivery systems.

Contributions

This study strengthens the hypothesis that biomedical innovations, specifically the use of biofertilizers and biostimulants, represent a potent vehicle for achieving sustainable and resilient agriculture. These inputs are indicative of a transformative shift from chemically intensive agriculture and through improving soil health, increasing crop yields, strengthening nutrient efficiency and yielding economic returns, they fill an urgent need. More research integrating soil science, agronomy and molecular biology will be necessary to adapt and optimize these tools for use in the agricultural landscapes of the world.

Declarations

Ethical Approval and Consent to Participate: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. Informed consent was not required, as secondary data available on websites was obtained for analysis. All procedures performed in this study were by the ethical standards of the Helsinki Declaration.

Consent for Publication: NA.

Availability of Data and Material: Data for this study could be made available upon the request from the corresponding author.

Competing Interest: The authors declare that they have no competing interests.

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Authors' Contribution: Riaz & Jabbar: Conceptualization of Idea, Review of Literature, Ahmed & Virk: Data Analysis, Riaz, Jabbar, Ahmed, & Virk: writing initial draft, review and writing final draft until submission for publication.

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