Agronomic feasibility of biostimulant products in dryland wheat crops in Minas Gerais, Brazil

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Abstract

Fungi and bacteria are among the most widely used biological agents in Brazilian agricultural practices. However, their application in wheat under rainfed tropical conditions remains limited. This study was developed to evaluate the feasibility of applying biological products as inoculants in rainfed wheat cultivation in the southern region of Minas Gerais state. Field trials were carried out over two crop seasons (2021 and 2022) at two and three locations, respectively. Four biological products and a control were tested in this study. The experimental design was a randomized complete block design with four replicates. Seven agronomic traits, such as thousand grain weight and grain yield per hectare, were evaluated. Statistical analyses included combined mixed model analysis, followed by the multilocation GGE biplot and AMMI analysis and a principal components (PCA) multi-trait analysis, using R software. No significant differences were observed among treatments, likely influenced by suboptimal environmental conditions during the study periods. As an initial assessment under field conditions, it was observed that the biological products did not significantly enhance rainfed wheat productivity.

Keywords: *Triticum aestivum* L. *Azospirillum. Bacillus.* Biological. Fertilizers.

Introduction

In Brazil, wheat (*Triticum aestivum* L.) consumption exceeds 13 million tons annually, but only 7.5 million tons were produced in 2025, resulting in a reliance on imports (CONAB, 2026). Although the South Region accounts for 85 % of national wheat production, Minas Gerais has emerged as a key producer in the Southeast, with approximately 430 thousand tons harvested in 2025. Expanding wheat cultivation under tropical conditions may help to reduce dependence on imports and contribute to more sustainable production systems through crop rotation and succession. The rising costs of inputs and societal demands for sustainable practices have intensified interest in biological products, including biostimulants and microbial inoculants, which may enhance plant development, reduce input use, and mitigate environmental impacts (Klepa *et al.*, 2024; Prando *et al.*, 2024).

Recent meta-analyses and field studies of microbial inoculants and bio-effectors indicate consistent, although variable, benefits for crop performance and nutrient-use efficiency (Schütz *et al.*, 2018; Nkebiwe *et al.*, 2024). A global meta-analysis of biofertilizers reported an average yield increase of ~16 % across crops and climates, highlighting the capacity of microbial products to improve nutrient acquisition and productivity when properly matched to soil and management conditions (Schütz *et al.*, 2018). More targeted syntheses have since refined these estimates for specific bio-effectors and crops: a 2024 meta-analysis of bio-effectors involving

maize, wheat and tomato reported an overall growth/yield increase of 9.3 % but emphasized strong context-dependence (crop, growth stage, and environmental conditions), reinforcing that effect sizes vary between controlled and field conditions (Nkebiwe *et al.*, 2024).

Experimental research supports mechanistic pathways for these outcomes: inoculation with plant growth-promoting rhizobacteria (PGPR) may increase plant-available N, P and K, stimulate root growth and modulate stress responses, enabling yield gains even under reduced fertilizer regimes (Wang *et al.*, 2020). Importantly for tropical production systems, results from field trials with *Azospirillum brasilense* indicate that seed inoculation can improve biomass, grain yield and nitrogen-use efficiency under tropical conditions, pointing to practical routes for integrating biological products in resource-limited and warm environments (Galindo *et al*., 2022), while *Bacillus* and *Pseudomonas* species contribute to nutrient solubilization and biocontrol, collectively enhancing plant vigor and stress tolerance (Araújo *et al.*, 2021; 2025).

Despite these promising results, reproducibility across environments, especially in heterogeneous tropical agroecosystems, remains a major challenge and calls for cautious interpretation of individual positive reports. Meta-analyses and standardized multi-site experiments consistently note high variability in responses, driven by interactions of soil properties, native microbiomes, product formulation, application timing, and abiotic stressors (Schütz *et al.*, 2018; Nkebiwe *et al.*, 2024). This inherent variability underscores the critical need for robust, localized field validation under specific environmental and management conditions where these products are intended for use to better understand their performance in complex tropical contexts.

Therefore, while peer-reviewed evidence supports the potential applicability of biological products to enhance wheat productivity and nutrient efficiency in tropical climates, the literature also indicates that benefits are not universal; they are conditional on clear product–environment–management matching and on rigorous, replicated field validation. This synthesis motivates the present study to (i) evaluate biological product performance for wheat under tropical field conditions, and (ii) assess how agronomic management and prevailing climatic conditions influence product performance variability within these diverse tropical field environments.

Material and methods

The experiments were carried out on three grain producing farms located in the Southern Minas Gerais and Campo das Vertentes regions of the state of Minas Gerais, Brazil. One of the farms, Fazenda 3W (L1) is a private property located in the municipality of Itutinga, Minas Gerais, at 21°24'39"S and 44°39'53"W, with an altitude of 1,072 m. The other two sites are located in the municipality of Lavras, at approximately 900 m above sea level. The second (L2) is a private property owned by Armazém Souza & Figueiredo (Fazenda ASF), located at 21°12'21"S and 44°58'08"W and the last one (L3) belongs to the Federal University of Lavras (Fazenda Muquém) and it is situated at 21°12'5"S and 44°58'47"W. In 2021, experiments were installed at the first two locations (L1 and L2), while all three locations (L1, L2 and L3) were included in 2022, totaling five evaluation environments. For all locations the wheat cultivar used was BRS 264.

The experiments were carried out using a randomized complete block design (RCBD). For each treatment, four replications were used, and each experimental plot consisted of fifteen rows (spaced of 0.20 m) measuring 5 m in length, totaling 15 m^2 and just five rows and three meters in length in the middle were considered for the evaluations and harvest. The control treatment

(NA = water application) was included as a check with the same number of replications of the regular treatments. Sowing was carried out in April 2021 and for the 2022 growing season, sowing was performed in March. Throughout the experimental period, all crop management practices were carried out in accordance with technical recommendations for wheat cultivation (Cunha, Caieirão, 2023).

The treatments consisted of five different biologicals: AZ – *Azospirillum brasilense* AbV6 (Azotrop® – Biotrop), BH – *Bacillus subtilis* + *Bacillus megaterium* (Biomaphos® – Biotrop), ST – Mineral fertilizer containing Ca, S, Mo, K_2O, Zn (Stimutrop® – Biotrop), BP – *Pseudomonas fluorescens* CCTB03 + *A. brasilense* AbV6 (Biofree® – Biotrop), Control – No product applied (water only).

The products were received from the owners few days before the applications for each year to guarantee their viability. The foliar applications were done at the tillering stage (stage 21 – Zadoks *et al.*, 1974). It was performed using a CO_2-pressurized backpack sprayer. The sprayer featured a 3-meter-long boom with six nozzles spaced 50 cm apart, equipped with an anti-drip system and quick couplers. The system included a 2 kg CO_2 cylinder, a backpack support system, a pressure regulator, a pressure gauge (0–100 psi), and a suction tube fitted with a safety valve 60 psi and quick coupling. Applications were performed at an operating pressure of 30 psi, calibrated to deliver a spray volume of 200 L ha^{-1}.

The regional climate, according to the Koppen climate classification is Cwa, subtropical humid, with hot and humid summers and cold and dry winters (Dantas *et al*., 2007), mean temperatures ranging from 18° to 22°C, and a mean annual rainfall of 1650 mm. The dry season stretches from May to September, and the rainy season normally lasts until late March or early April.

The meteorological data of air temperature (maximum, minimum and average) and rainfall were collected from a meteorological station linked to the Meteorology National Institute (INMET - Brazil), located at Universidade Federal de Lavras approximately 1 km from the experiments L2 and L3.

The water balance was calculated on a ten-day scale to obtain values of water deficit and surplus during the wheat production cycle in the 2021 and 2022 seasons. The calculation considered the model proposed by Thornthwaite and Mather (1955), which accounts for the interaction between soil, plant, and atmosphere using data on precipitation, soil water holding capacity (WHC), and crop evapotranspiration (ETc). The WHC was defined as 100 mm based on the soil's physical-water characteristics in the experimental area. The ETc calculation utilized the equation: ETc = Kc x ETo, where Kc represents the crop coefficient, and ETo is the reference evapotranspiration. The wheat's ETo was estimated using the Thornthwaite model (Thornthwaite, 1948) (Figure 1).

The following agronomic traits were evaluated: plant height (PH, in cm), number of spikes per meter (NSM), number of spikelets per spike (NSS), number of grains per spike (NGS), thousand-grain weight (TGW, in g), number of tillers per plant (NTP) and grain yield estimation (YLD, in kg ha^{-1}). The evaluations were carried out based on the procedures described in Scheeren *et al.* (2015).

The data were first analyzed individually for each environment. Subsequently, a joint analysis was performed using a linear mixed model, as follows:

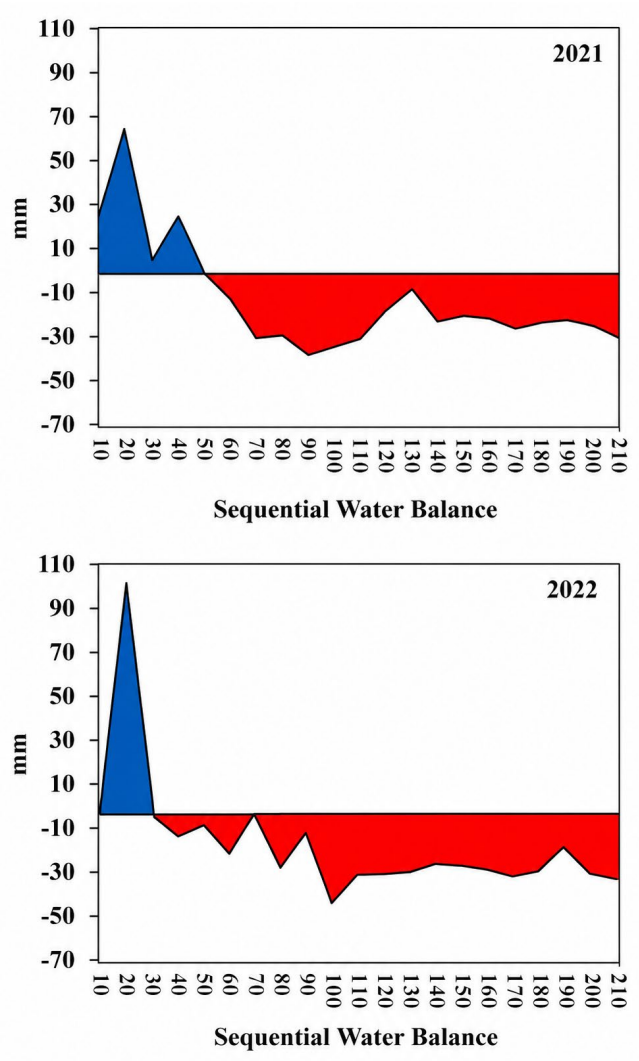
$$y = X\beta + Zu + e$$

Where: y is the vector of phenotypic observations; β is the vector of fixed effects, including treatments; u is the vector of random effects, including environments, blocks within environments, and the treatment × environment interaction; X and Z are the incidence matrices for fixed and random effects, respectively; and e is the vector of residual errors. The random effects were assumed to be normally distributed with mean zero and variance components estimated by restricted maximum likelihood (REML). The significance of fixed treatment effects was tested using an F-test, whereas random effects were tested using likelihood ratio tests.

The performance of treatments (biological products) was evaluated across multiple environments (locations and years) using GGE Biplot analysis, which simultaneously accounts for the mean treatment effect and the treatment × environment interaction (G + GE).

For YLD, a manual Additive Main Effects and Multiplicative Interaction (AMMI) decomposition was performed, from which the treatment × environment interaction (GEI) matrix was extracted. Scores for the first two principal components (PC1 and PC2) were then calculated and used to compute the AMMI Stability Value (ASV). This metric quantifies the stability of each treatment based on the proportion of variation explained by PC1 and PC2. In addition, the Yield

Figure 1. Sequential water balance of wheat on a decadal scale in different sowing seasons for the 2021 and 2022 crops.



Source: authors (2023).

Stability Index (YSI) was obtained by integrating yield performance with stability (ASV), allowing for the identification of the most desirable treatments. To facilitate direct comparisons among treatments, a standardized measure of yield superiority, the Z-score, was also calculated.

For all the evaluated traits, a Principal Component Analysis (PCA) was performed using the adjusted means (from the LMM), aiming to explore the association among variables and the separation of treatments in the multivariate space.

All statistical analyses and graphical representations were performed using R® software (R CORE TEAM, 2025), using the package metan (Olivoto; Lucio, 2020). The principal component analysis was performed for the environment jointly with the package *factoextra*.

Results and discussion

The comprehensive evaluation of four distinct biological products and a control, conducted over two crop seasons (2021 and 2022) across multiple locations in the southern region of Minas Gerais, revealed no statistically significant differences among the treatments for all of the evaluated agronomic traits (Table 1).

This outcome, while initially surprising given the potential of biological inoculants, is not uncommon in field studies, often highlighting the inherent variability and context-dependency of their performance in complex agroecosystems (Marques *et al.*, 2023). Crucially, this finding must be critically contextualized within the prevailing environmental conditions encountered during experimental periods, mainly drought conditions. The 2021 and 2022 crop seasons were characterized by unfavorable environmental conditions for both optimal wheat development and the expected efficacy of the applied biological products. Specifically, cumulative rainfall levels were low, and average temperatures frequently deviated from the optimal range for microbial activity, as vividly depicted in the sequential water balance (Figure 2), thereby likely limiting the full expression of the biological products' potential (Nardino *et al.*, 2023; Teixeira *et al.*,

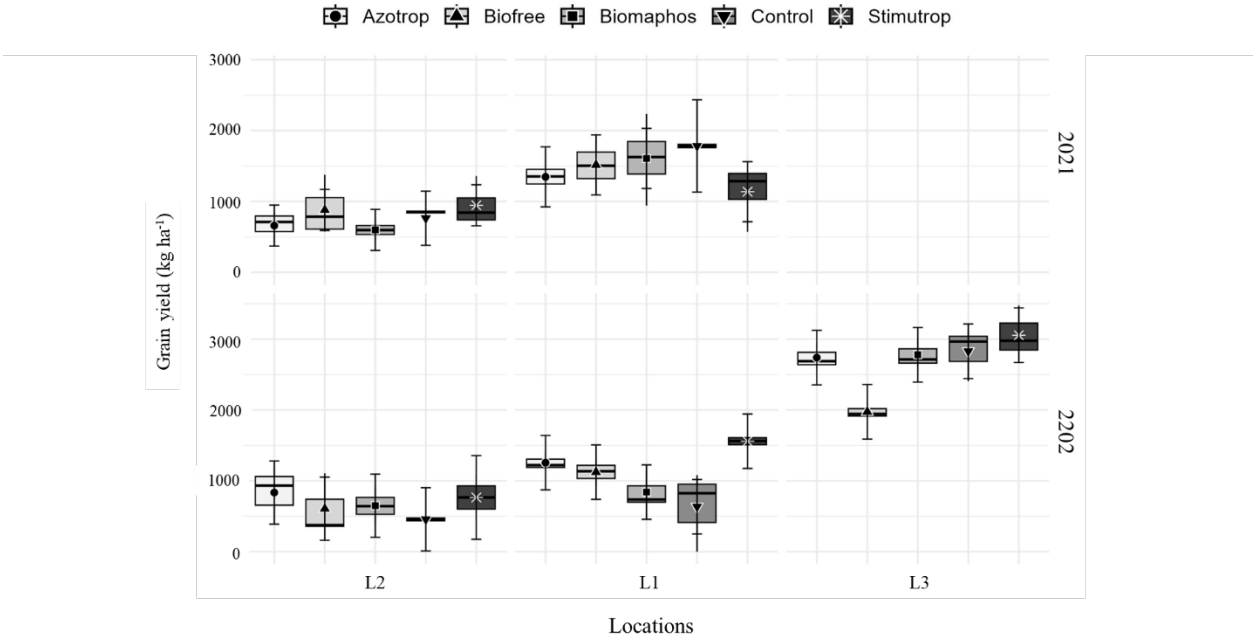
Table 1. p-values from the likelihood ratio test (LRT) of random effects and the F-test of fixed effects, along with statistical parameters from the joint analysis of five biological treatments in wheat, evaluated for plant height (PH, in cm), number of spikes per meter (NSM), number of spikelets per spike (NSS), number of grains per spike (NGS), thousand-grain weight (TGW, in g), number of tillers per plant (NTP) and grain yield (YLD, in kg ha^{-1}) across five locations in 2021 and 2022 crop years.

Source of Variation	PH	NSM	NSS	NGS	TGW	NTP	YLD
Treatments (T)	0.285^{ns}	0.227^{ns}	0.176^{ns}	0.490^{ns}	0.473^{ns}	0.143^{ns}	0.483^{ns}
Year (Y)	1.000^{ns}	1.000^{ns}	0.225^{ns}	1.000^{ns}	0.414^{ns}	1.000^{ns}	1.000^{ns}
Environment (E)/ Y	0.000**	0.000**	0.603^{ns}	0.000**	0.000**	0.000**	0.000**
Rep/E/Y	0.999^{ns}	0.700^{ns}	1.000^{ns}	0.925^{ns}	0.999^{ns}	0.999^{ns}	1.000^{ns}
Mean	56.42	48.62	13.32	34.52	44.94	3.73	1326.97
CV(%)	5.77	17.38	25.34	16.32	7.52	56.46	26.96

^{ns},**: non-significant and significant, respectively, at 1 % as determined by likelihood ratio test (LRT) and F-test; CV: residual coefficient of variation.

Source: authors (2023).

Figure 2. Boxplots of grain yield (kg ha^{-1}) for wheat measured in four biologicals products and the check under five locations in 2021 and 2022 crop years.



Source: authors (2025).

2023). These include the potential inefficiency or lack of viability of the biological products used, given that no prior viability assessment was conducted; the possible interference or dominance of native soil microbial populations, in the absence of recovery or establishment tests for the applied biological agents; and limitations related to the imprecise characterization of yield components (e.g., NSM and NTP), which may have affected both the sensitivity of the analysis and the interpretation of the results.

Many plants growth-promoting rhizobacteria (PGPRs), such as *Azospirillum brasilense* and *Bacillus* spp. used in this study, rely heavily on adequate soil moisture for their survival, motility, effective colonization of the rhizosphere, and subsequent metabolic activity (Kasim *et al.*, 2021). Critical microbial functions, including biological nitrogen fixation (BNF), nutrient solubilization (e.g., phosphate), and the production of phytohormones, are known to be significantly attenuated or even inhibited under water stress conditions, as demonstrated for *Azospirillum brasilense* (Marques *et al.*, 2023). While PGPR inoculation has shown significant yield enhancement in other tropical crop systems (Gagné-Bourque *et al.*, 2016), these successes are typically observed under environmental conditions conducive to robust microbial proliferation and sustained plant growth.

Cumulative rainfall levels were low, respectively and average temperatures remained different from the optimal range for microbial activity, limiting the expression of their potential (Figure 1). The variation among locations, particularly in 2022, was high, reflecting not only the climatic conditions but also the influence of soil characteristics and agronomic practices, including the adoption of integrated systems, crop succession, and differences in sowing time (Aparecido *et al.*, 2024).

Regarding YLD, the mean values observed in this study across the 2021 and 2022 seasons were considered low even for the region, averaging approximately 1,300 kg ha^{-1} (Table 1, Figure 2). This figure falls below typical expectations,

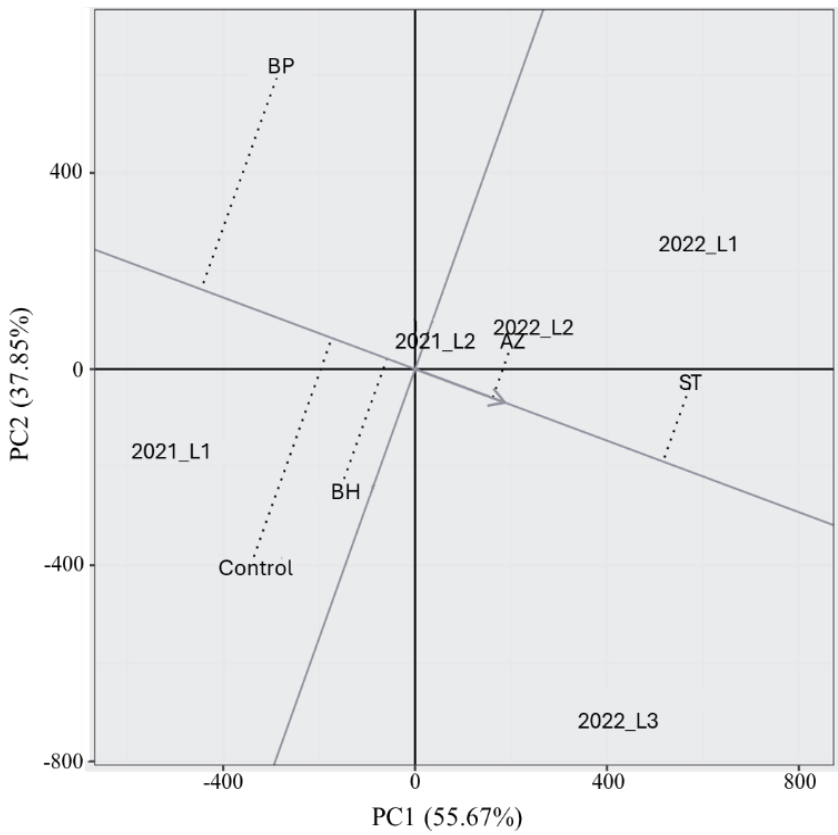
often reported around 3,000 kg ha^{-1} for national averages and approximately 2,000 kg ha^{-1} for the regional mean (CONAB, 2025).

For instance, inoculation with *Azospirillum brasilense* has been reported to mitigate the effects of water stress in maize (Marques *et al.*, 2023) and to enhance drought tolerance in wheat seedlings (Kasim *et al.*, 2021). Similarly, *Bacillus subtilis* has been associated with drought stress alleviation in various crops (Gagné-Bourque *et al.*, 2016), while *Bacillus megaterium*, also included in our treatments, has been associated with improved maize tolerance to combined drought and high-temperature stress (Romero-Munar *et al*., 2023).

Due to high interaction between Locations/ Year for a better understanding of the results a multilocation analyses GGE were used (Figure 3). The results of the multivariate analysis via the main components (PCA) for the control and treatments revealed that two PCAs explained 93.5 % of the total variation. In this case, AZ has the more stable performance, and ST has the highest adaptability for the study achieved.

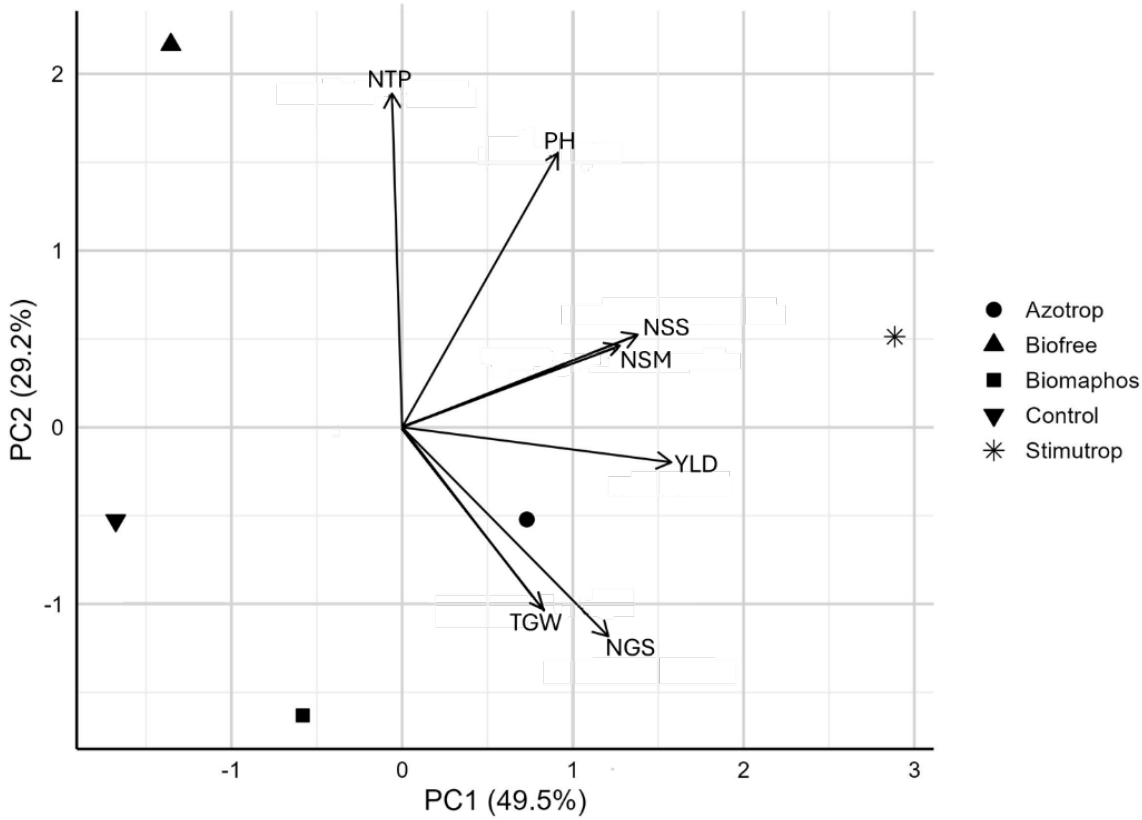
In the principal component analysis (PCA), regarding the association of the evaluated traits to the products, it reflects the development of the plants under the exposed conditions (Figure 4). According to these results, the NSS and NSM were high associated between them as also TGW and NGS were high associated. Such aspects can be observed due to the proximity of the axes, demonstrating interaction of these traits.

Figure 3. GGE biplot (Mean vs. Stability) of grain yield (kg ha^{-1}) for four biologicals products, Azotrop® (AZ), Biomaphos® (BH), Stimutrop® (ST) Stimutrop®, Biofree® (BP) and control evaluated in wheat across five locations (L_i) in the 2021 and 2022 crop years.



Source: authors (2025).

Figure 4. Principal component analysis based on wheat traits as plant height (PH, in cm), number of spikes per meter (NSM), number of spikelets per spike (NSS), number of grains per spike (NGS), thousand-grain weight (TGW, in g), number of tillers per plant (NTP) and grain yield (YLD, in kg ha^{-1}) evaluated under five locations in 2021 and 2022 crop years.



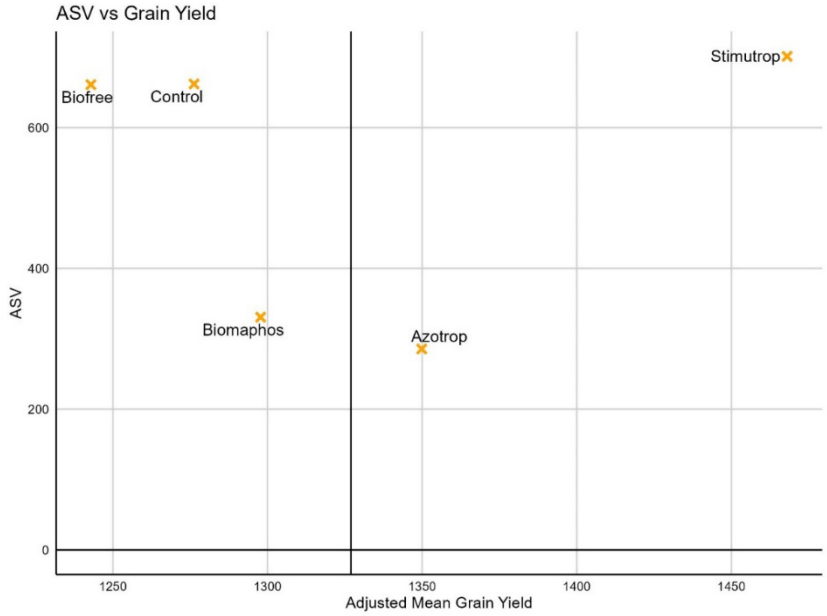
Source: authors (2025).

Considering the products in the PCA, it's evident that NSS, NSM and YLD were influenced by ST as the performance of AZ influences most TGW and NGS (Figure 4). This is one of the main issues that arise in studies involving biological products and the evaluated traits, as they affect productivity components. Santos (2018) and Cadore *et al.* (2016) reported that inoculation enhanced root system establishment and seedling vigor, resulting in superior grain productivity. Similarly, Okon and Labandera-Gonzales (1994) demonstrated that approximately 60 % of experiments using *Azospirillum* spp. promoted yield increments, largely attributed to improved root development and enhanced nutrient uptake. In the case of fungal inoculants, significant benefits have also been observed at early growth stages.

Considering ASV (AMMI Stability Value) and Z-score, there were found differences of biological treatments, specifically the ST treatment showed the highest YLD (1468 kg ha^{-1}) and Z-score, indicating promising potential (Figures 5 and 6). However, this same treatment had lowest stability index (ASV = 701.60), indicating high interaction with the environment and lower predictability of performance.

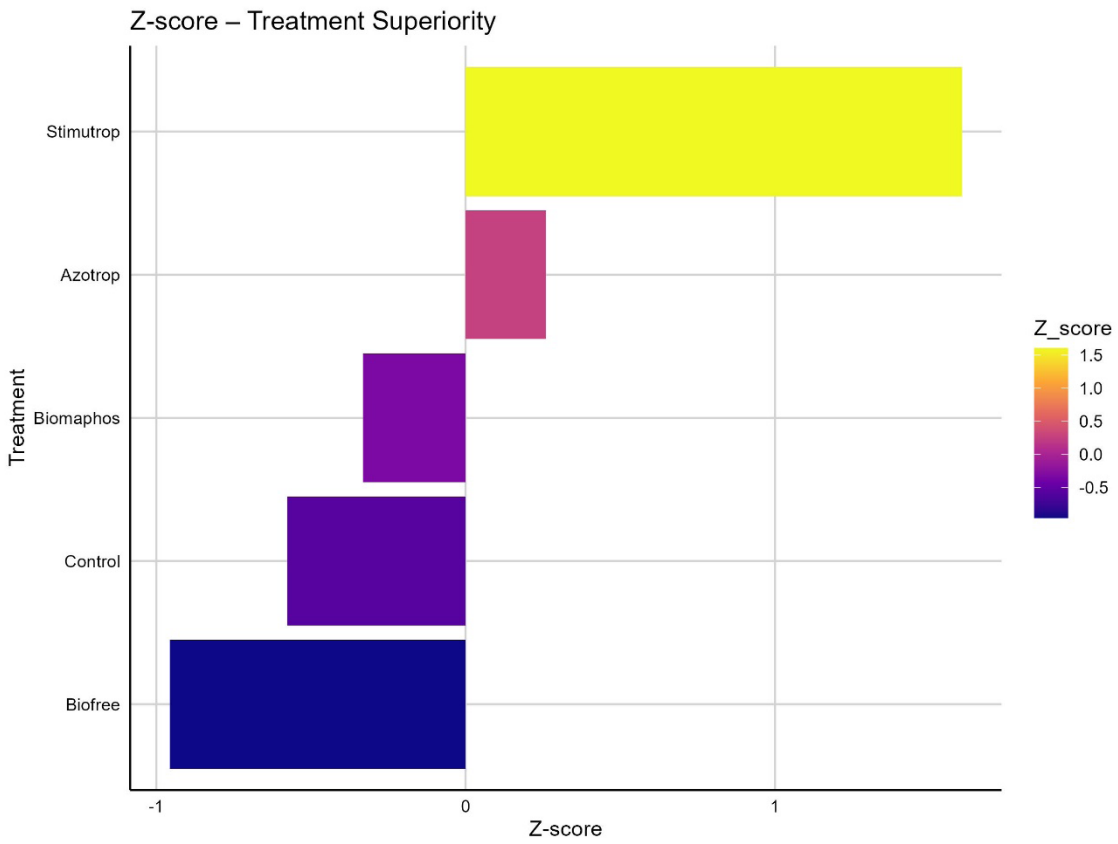
The specific strain selection within these biological products is also a critical determinant of efficacy. Different strains, even within the same species, may exhibit significant genetic and physiological variability, leading to diverse capacities for rhizosphere colonization, production of plant growth-promoting metabolites, and tolerance to environmental

Figure 5. AMMI Stability Value (ASV) of five biological treatments in wheat, evaluated for grain yield (YLD, kg ha^{-1}), across five locations in 2021 and 2022 crop years.



Source: authors (2025).

Figure 6. Z-score of five biological treatments in wheat, evaluated for grain yield (YLD, kg ha^{-1}), across five locations in 2021 and 2022 crop years.



Source: authors (2025).

stresses (Nadeem *et al.*, 2014). Some strains are known to produce a broader spectrum or higher concentrations of stress-protective compounds (e.g., exopolysaccharides, osmolytes, antioxidants) or phytohormones under adverse conditions, making them more effective in stress mitigation (Chandra *et al.*, 2018). Therefore, the lack of consistent responses observed in this study suggests that the microbial strains present in the evaluated products were not able to fully mitigate the effects of the drought and high-temperature conditions encountered during the field trials. Environmental constraints may have limited microbial establishment, activity, or plant–microbe interactions, thereby reducing the potential benefits of inoculation under these conditions.

These findings contribute to an evidence-based framework for the adoption of biological products in wheat production. Furthermore, they highlight the need for future studies to refine experimental approaches by integrating nitrogen management strategies and evaluating crop- and environment-specific bio-products, with the aim of improving response consistency and enhancing effectiveness under tropical growing conditions.

Conclusions

Under the edaphoclimatic conditions evaluated during the 2021 and 2022 growing seasons, the biological products did not consistently improve wheat performance. The variability in product responses among environments suggests that their effectiveness was strongly influenced by prevailing climatic conditions.

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