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Synergy between biostimulants and nitrogen fertilization: effects on the physiology and yield of soybean (*Glycine max* L.)

Sinergia entre bioestimulantes y fertilización nitrogenada: efectos en la fisiología y el rendimiento del cultivo de soya (*Glycine max* L.)

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ABSTRACT

Soybean (*Glycine max* [L.] Merr.) is a key crop for global food security, but its productivity is challenged by climate variability and the heavy reliance on synthetic fertilizers. Biostimulants offer a sustainable alternative to improve nutrient efficiency and crop performance. This study aimed to evaluate the individual and combined effects of three biostimulant formulations: one based on amino acids (Codafol®), one on chitosan (QuitoMax®), and one on micronutrients (CBFert®) on soybean var. 'INCASoy-36', compared with conventional nitrogen fertilization under field conditions. The study was conducted at the Basic Scientific and Technological Unit of Los Palacios, Pinar del Río, Cuba, belonging to the National Institute of Agricultural Sciences (latitude 22.571898° N, longitude 83.238144° W). A randomized block design with eight treatments, including full nitrogen fertilization and different biostimulant combinations, was established. General physiological and agronomic indicators were assessed to determine treatment effects on growth and yield. Combined biostimulant applications improved vegetative development, chlorophyll status, and yield components, achieving results comparable to full nitrogen fertilization. Conversely, individually applied biostimulants produced limited responses and did not equal the performance of the integrated approach. Significant differences were observed among treatments, particularly in pod formation and grain filling. Overall, the coordinated use of amino-acid-based (Codafol®), chitosan-based (QuitoMax®), and micronutrient-based (CBFert®) biostimulants demonstrated a clear synergy that enhanced soybean productivity while allowing a 70% reduction in synthetic nitrogen fertilization without significant yield loss. These findings highlight the synergistic potential of biostimulant integration as an effective strategy for more sustainable production systems.

Palabras clave:
Aminoácidos
Fijación biológica de nitrógeno
Quitosano
Rendimiento de grano
Eficiencia de nutriente

RESUMEN

La soya (*Glycine max* [L.] Merr.) es un cultivo fundamental para la seguridad alimentaria mundial, pero su productividad se ve amenazada por la variabilidad climática y la gran dependencia de los fertilizantes sintéticos. Los bioestimulantes ofrecen una alternativa sostenible para mejorar la eficiencia de los nutrientes y el rendimiento de los cultivos. El objetivo de este estudio fue evaluar los efectos individuales y combinados de tres formulaciones de bioestimulantes: una a base de aminoácidos (Codafol®), otra de quitosano (QuitoMax®) y otra de micronutrientes (CBFert®) en la variedad de soja INCASoy-36, en comparación con la fertilización nitrogenada convencional en condiciones de campo. El estudio se llevó a cabo en la Unidad Científico-Técnica Básica de Los Palacios, Pinar del Río, Cuba, perteneciente al Instituto Nacional de Ciencias Agrícolas (latitud 22,571898° N, longitud 83,238144° O). Se estableció un diseño de bloques aleatorios con ocho tratamientos, incluyendo la fertilización completa con nitrógeno y diferentes combinaciones de bioestimulantes. Se evaluaron indicadores fisiológicos y agronómicos generales para determinar los efectos de los tratamientos sobre el crecimiento y el rendimiento. Las aplicaciones combinadas de bioestimulantes mejoraron el desarrollo vegetativo, el estado de la clorofila y los componentes del rendimiento, logrando resultados comparables a los de la fertilización completa con nitrógeno. Por el contrario, los bioestimulantes aplicados individualmente produjeron respuestas limitadas y no igualaron el rendimiento del enfoque integrado. Se observaron diferencias significativas entre los tratamientos, especialmente en la formación de vainas y el llenado del grano. En general, el uso coordinado de bioestimulantes a base de aminoácidos (Codafol®), a base de quitosano (QuitoMax®) y a base de micronutrientes (CBFert®) demostró una clara sinergia que mejoró la productividad de la soja, al tiempo que permitió una reducción del 70% en la fertilización con nitrógeno sintético sin que se produjera una disminución significativa del rendimiento. Estos resultados ponen de relieve el potencial sinérgico de la integración de bioestimulantes como estrategia eficaz para lograr sistemas de producción más sostenibles.

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Legumes play a pivotal role in global food systems due to their high nutritional density, protein contribution, and capacity to enhance soil fertility through biological nitrogen fixation. They currently account for 12-15% of the global cultivated area and contribute more than one-quarter of primary crop production, reinforcing their significance for food security and sustainable agricultural resilience (FAO 2023). Their symbiosis with rhizobia reduces dependence on nitrogen fertilizers, an advantage for production systems increasingly constrained by climate change, soil degradation, and resource limitations (IPCC 2023).

Soybean (*Glycine max* [L.] Merr.) is one of the most economically important legumes worldwide. Global production has increased eightfold over the past five decades, driven by sustained yield improvements (Pérez-Pizá et al. 2020) and expansion of cultivated areas (Song et al. 2021). In Cuba, it is valued for its diverse industrial and food uses (Castillo-Ortiz et al. 2024). Despite its importance, production remains vulnerable to extreme climatic events, recurrent droughts, and rising input costs, particularly fertilizers and agrochemicals (FAO 2023; IPCC 2023). These pressures highlight the need for more efficient and resilient production strategies.

Biostimulants have emerged as promising tools to enhance crop performance under both optimal and stress conditions. These products commonly formulated from amino acids, chitosan, or marine algae extracts can improve nutrient uptake, modulate plant hormonal responses, and increase tolerance to abiotic stress (Rouphael and Colla 2020). Experimental evidence shows that biostimulants stimulate photosynthesis, strengthen natural defense mechanisms, and promote root and shoot development, contributing to greater yield stability (Calvo et al. 2014). Foliar application offers additional advantages due to rapid tissue absorption and direct interaction with metabolic pathways.

In this context, biostimulants derived from chitosan (QuitoMax®), aminoacids (CBFert®), and algae extracts (Codafol®) offer distinct but complementary mechanisms of action that warrant evaluation under real production conditions. Therefore, the aim of this study was to evaluate the individual and combined effects of foliar biostimulants (chitosan-based, amino-acid-based, and algae-extract-based) and nitrogen fertilization on the growth and yield of soybean (var. 'INCASoy-36') under field conditions in Cuba.

MATERIALS AND METHODS

Study site and soil characterization

The study was conducted from January to April 2022 at the Basic Scientific and Technological Unit of Los Palacios, Pinar del Río, Cuba, belonging to the National Institute of Agricultural Sciences (Latitude 22.571898° N, Longitude 83.238144° W**)**. The soil was classified as Ferruginous Petroferric Nodular Gleysol (Hernández et al. 2015). A grid sampling (0-20 cm) was performed before sowing. Soil physicochemical properties were analyzed at the Provincial Soil Laboratory of Pinar del Río following national standard procedures: pH (NC ISO 10390:1999), organic matter by wet oxidation (NC 51:1999), exchangeable cations extracted with ammonium acetate (NC 65:2000), and available phosphorus determined using the Oniani method (NC 52:1999).

Daily meteorological information (temperature, relative humidity, and rainfall) was obtained from the official Weather Station No. 317, "Paso Real de San Diego," located 7.2 km from the study area. During the study period, typical climatic conditions for the region were observed, with a mean temperature of 24.5±1.8 °C, average relative humidity of 78±5%, and cumulative precipitation of 342 mm (Figure 1). These values remained within the historical range for the area (INSMET 2022).

Experimental design and treatments

A completely randomized block design with three replicates was established. The experimental units consisted of 10 m² plots (2.5 m wide × 4 m long), each with three furrows spaced 0.70 m apart and 4 m long. Eight fertilization-biostimulant treatments were evaluated, integrating mineral N fertilization and foliar biostimulation. The soybean cultivar 'INCASoy-36' was selected due to its regional adaptation.

In all treatments, a basal nitrogen application of 40 kg N ha^{-1} (78 kg of urea) was applied before planting, equivalent to 30% of the total application (260 kg of urea). The biostimulant foliar products (Codafol®, QuitoMax®, and CBFert®) were applied during the vegetative stage at the recommended intervals to establish the treatments used in the study (Table 1).

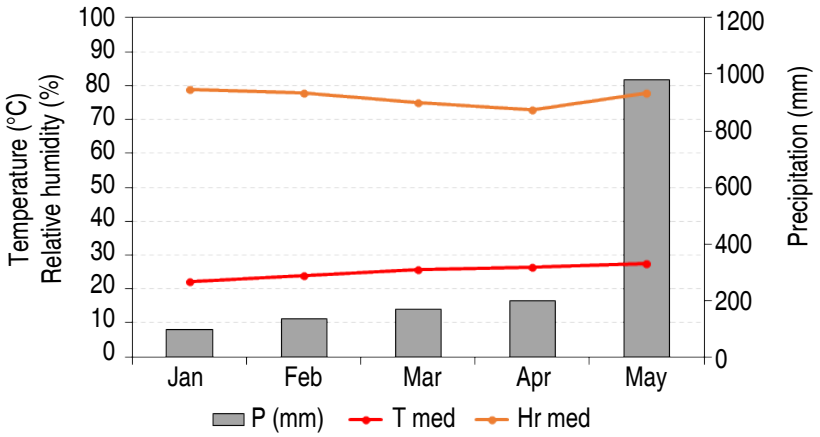


Figure 1. Behavior of meteorological variables: mean temperature (T mean), average relative humidity (RH mean), and monthly cumulative precipitation (P) during the period from January 2022 to May 2022 (Latitude 22.571898° N, Longitude 83.238144° W).

Table 1. Fertilizer and biostimulant treatments.

Treatment	Description
T1 (Control)	Full mineral N fertilization with urea (260,8 kg ha^{-1} = 120 kg N ha^{-1})
T2	Reduced N+Codafol® (2 L ha^{-1})
T3	Reduced N+QuitoMax® (100 mL ha^{-1})
T4	Reduced N+CBFert® (2.8 L ha^{-1})
T5	Reduced N+CBFert®+QuitoMax®
T6	Reduced N+Codafol®+CBFert®
T7	Reduced N+Codafol®+QuitoMax®
T8	Reduced N+Codafol®+CBFert®+QuitoMax®.

Crop management

Sowing was carried out using no-till planting, with a planting density of 285,000 plants per hectare, a row spacing of 0.70 m, and an in-row spacing of 0.05 m. Weed control consisted of two manual weeding sessions 15 and 30 days after planting, following national guidelines for soybean management (MINAG 2023). Foliar applications were carried out using manual backpack sprayers equipped with flat fan nozzles to ensure uniform coverage. Applications were made at an approximate working pressure of 2.0–2.5 bar, with a spray volume of 200 L ha^{-1}. Spraying was carried out in the early morning hours (8:00–10:00 a.m.), under favorable environmental conditions, with low wind speeds and moderate temperatures, in order to minimize drift and maximize foliar absorption efficiency.

Measurements of physiological and yield variables

Evaluation of physiological and productive variables

In each experimental unit, 10 plants were randomly selected, resulting in a total of 30 individuals per treatment. SPAD values (related to chlorophyll content in the leaf) was measured in the upper third of the foliage during the flowering period (at 45 and 65 days after germination). Five readings were taken per plant and subsequently averaged to obtain representative value.

Plant height (cm) was measured at 75 days after germination (DAG) on representative plants from the central rows of each plot, using a graduated ruler from the base of the stem at soil level to the vegetative apex.

At harvest maturity (114 DAG), plants were carefully uprooted to preserve the integrity of the root system. Roots were gently washed with running water to remove adhering soil, and total root system length (cm) was measured from the stem base to the longest root using a

millimeter ruler. The number of pods and grains per plant was determined by manual counting on the same plants. For grain yield (t ha^{-1}) estimation, a useful area of 5.6 m^2, corresponding to the central rows of each plot, was manually harvested to avoid border effects. The harvested plant material was manually threshed, and the grain was dried to a standard moisture content of 14%. Grain weight was then recorded and extrapolated to a hectare basis to calculate yield.

Statistical analysis

Data were first evaluated for normality (Shapiro–Wilk) and homoscedasticity (Levene). A two-way ANOVA (P<0.05) was performed for all agronomic and the physiological variables. Treatment means were compared using Tukey's test (α=0.05). Analyses were conducted using standard statistical software SPSS v. 22 for Windows.

RESULTS AND DISCUSSION

The results of the soil analysis of the ferruginous petroferric nodular Gleysol (Table 2) showed a pH in KCl of 6.29, classifying it as slightly acidic, and an organic matter content of 3.87%. The levels of exchangeable bases (Ca^{2+}: 9.60±0.261; Mg^{2+}: 0.69±0.039; K^{+}: 0.15± 0.007 cmol kg^{-1}) and available phosphorus (11.19± 0.021 mg kg^{-1}) were classified as low.

Table 2. Properties Characterizing the Fertility of the Arable Horizon (0-0.20 m) of the Ferruginous Petropheric Nodular Gleysol Soil (n=5) where the experiment was conducted.

Property	Property	Property	Property	Desv. S.
pH (KCl)		6.29	6.29	0.063
MO	%	3.87	3.90	0.071
Ca^{2+}		9.60	9.60	0.261
Mg^{2+}	cmol kg^{-1}	0.69	0.70	0.039
Na^{+}		0.12	0.12	0.014
K^{+}		0.15	0.15	0.007
P Available	mg kg^{-1}	11.19	11.19	0.021

Soil analysis revealed a slightly acidic pH, a condition considered optimal for enhancing nutrient availability and promoting microbial activity in the soil (Cheng et al. 2023). The organic matter content (3.87%) exceeded the ranges commonly reported for soils with similar characteristics (2.1-3.2%). This elevated concentration is likely attributable to the implementation of conservation agriculture practices, such as crop rotation with cover crops and the return of organic residues to the soil, which are known to promote the accumulation of exchangeable bases (Teng et al. 2024). However, the levels of exchangeable bases (Ca^{2+}, Mg^{2+}, and K^{+}) and the available phosphorus content were classified as low, highlighting the need for supplemental fertilization to achieve optimal agronomic yields.

Climatic conditions influenced soybean development, as average temperatures slightly below 23.5 °C during the vegetative stages contributed to a prolonged growth period. This thermal regime is reported to favor vegetative growth in the 'INCASoy-36' variety, although it may extend the overall crop cycle (Rojan-Herrera et al. 2020).

Physiological and agronomic variables

SPAD values revealed significant differences (P<0.05) among the evaluated treatments at both 45 and 65 days after germination (DAG). At 45 DAG, treatment T1, corresponding to conventional nitrogen fertilization, exhibited the highest SPAD values (33.2), with no significant differences compared to treatment T8 (32.1), which included the combined application of the biostimulants Codafol®, QuitoMax® and CBFert® (Figure 2).

In contrast, treatments T2 and T3, which consisted of the individual application of Codafol® and QuitoMax®, respectively, exhibited the lowest SPAD values (27.8 and 28.42), with no significant difference between them (Figure 2). These results may be associated with lower efficiency in the uptake of nitrogen and other essential nutrients required for optimal performance of the photosynthetic apparatus at this early developmental stage. In treatment T4 (CBFert® alone), a 4.15% increase in SPAD values was observed, reaching 29.60. However, the combined treatments T5, T6, and T7 did not show significant differences among themselves

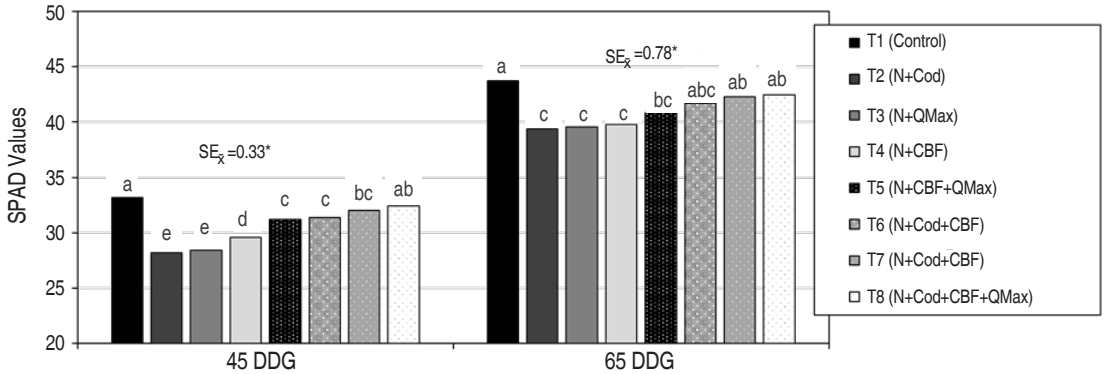


Figure 2. SPAD Values of soybean plants subjected to individual and combined application of Codafol®, QuitoMax® and CBFert®. Bars with unequal letters differ significantly according to Tukey's multiple range test for *P*<0.05, n=30.

(*P*<0.05), although their values were lower compared to T1 and T8.

At 65 days after sowing (DAS), a general increase in SPAD values was recorded (Figure 2), which corresponds to the continuous growth of the plants and the increased demand for photosynthetic activity necessary to meet energy needs and the synthesis of photoassimilates in metabolism, based on the soil and climate conditions in which they are grown (Amoanimaa-Dede et al. 2022). Treatments T1 (43.4) and T8 (43.0) stood out, with no significant differences observed between them (*P*<0.05). This result indicates that the triple combination of biostimulants in treatment T8 was able to match the efficacy of conventional nitrogen fertilization (T1) during the advanced stages of crop development. This result may be attributable to the accumulation and stimulation of metabolites through the frequent and synergistic application of the bioproducts.

Treatments with individual applications (T2–T4) showed significantly lower values (T2=38.9; T3=39.4; T4=39.79), with no significant differences among them, but statistically different from T1 and T8 (T1=43.72; T8=42.43). Meanwhile, the double combinations (T5-T7) showed intermediate results, with T7 (42.1) and T6 (41.6) not differing from T1 and T8. These results suggest that the individual application of the evaluated biostimulants was insufficient to match T1, while conventional fertilization ensured the highest chlorophyll levels. Nevertheless, the biostimulant combination (T8) represents a promising alternative to reduce dependence on nitrogen fertilizers without compromising the crop's nutritional status.

The application of 30% of the basal nitrogen fertilization, combined with different biostimulants (Codafol®, QuitoMax® and CBFert®), either individually or in combination, induced changes in the growth and development of soybean plants. In particular, variations were observed in SPAD values. The high SPAD values recorded in treatment T1 are consistent with previous studies, which attribute this effect to the immediate availability of nitrogen supplied by urea, a key nutrient in chlorophyll synthesis (Zhang et al. 2022).

This physiological response suggests that integrated formulations (T8) activate complementary mechanisms. For example, chitosan (QuitoMax®) could enhance the root assimilation of residual nitrogen, while amino acids (CBFert®) and seaweed extracts (Codafol®) would optimize its conversion into functional chlorophyll. Thus, these combinations not only mitigate the risk of nitrate leaching, a major environmental concern in intensive agriculture resulting from nitrogen fertilization, but also build resilience at the biochemical foundation of the crop (Li et al. 2024).

Analysis of plant height, root length, number of pods per plant, and number of grains per plant revealed a differential response to the applied treatments (Table 3). Regarding plant height, treatments T8 and T1 (control) recorded the highest averages, with no statistically significant differences between them (*P*<0.05).

Treatments with individual applications of biostimulants (T2-T4) showed significant reductions in plant height, with T4 (CBFert®) exhibiting the lowest height at 66.10 cm,

Table 3. Response of Soybean Plants to Individual and Combined Applications of Codafol®, QuitoMax®, and CBFert®.

	Treatments	Plant Height (cm)	Root Length (cm)	Pods per Plant	Grains per Plant
T1	Control	76.23±0.24a	21.12±0.05^{a}	62.26±0.16^{a}	134.98±0.26^{a}
T2	N+Cod	68.50±0.29^{d}	16.57±0.06^{e}	53.80±0.14^{d}	113.15±0.32^{d}
T3	N+QMax	69.26±0.17^{cd}	17.15±0.04^{d}	54.66±0.18^{d}	111.18±0.35^{d}
T4	N+CBF	66.10±0.14^{e}	17.43±0.04^{d}	53.93±0.16^{d}	111.61±0.43^{d}
T5	N+CBF+QMax	70.93±0.22^{bc}	19.16±0.06^{c}	55.0±0.16^{d}	124.15±0.21^{c}
T6	N+Cod+CBF	69.96±0.20^{cd}	19.95±0.05^{b}	56.73±0.15^{c}	128.19±0.33^{b}
T7	N+Cod+QMax	72.40±0.22^{b}	20.31±0.07^{b}	58.46±0.16^{b}	130.48±0.31^{b}
T8	N+Cod+CBF+QMax	77.80±0.45^{a}	21.06±0.05^{a}	59.33±0.16^{b}	135.51±0.26^{a}
$SE_{\bar{x}}$		0.72*	0.16*	0.46*	0.89*

± Confidence Intervals; $SE_{\bar{x}}$ = Standard Error of the Mean.

representing a 13.29% decrease compared to T1. The double combinations (T5-T7) displayed intermediate values without significant differences among them (69.27-72.40 cm), consistent with the chlorophyll content observed in the experiment.

It is essential to note that soybeans can associate with Rhizobium species that are capable of providing nitrogen under conditions of nutrient deficiency (Mahmud et al. 2020). However, it has been demonstrated that under conditions of good soil fertility, symbiosis with growth-promoting bacteria also occurs in this crop, as well as stimulating symbiosis through the application of biostimulants (Mahmud et al. 2020; Cheng et al. 2023). Therefore, the response observed in T8 could stimulate biological nitrogen fixation. Accordingly, the increase in SPAD values at 65 DAG aligns with reports linking this process to foliar chlorophyll retention during grain filling (Hajibarat and Saidi 2022), where up to 60% of grain nitrogen has been found to originate from foliar remobilization (Lyu et al. 2022).

It is important to specify the biostimulant products used in this study, because biostimulants differ in composition and therefore exert distinct effects on plant physiology. In this work, the application of Codafol®, QuitoMax®, and CBFert® resulted in differential responses, reflecting the specific modes of action associated with their amino acid, chitosan, and micronutrient formulations, respectively. Recent studies also suggest that certain biostimulants can delay leaf senescence by modulating phytohormone pathways, such as those involving cytokinins, auxins and gibberellins, which may contribute to maintaining photosynthetic capacity and stress tolerance during advanced stages of crop development (Murugesan and Paramasivan 2024).

The root length values recorded for treatment T1 were slightly exceeded by those of treatment T8, by 0.2% with no significant differences. Treatments T6 and T7 exhibited intermediate behavior with significant differences compared to full fertilization. Meanwhile, treatments T2–T5 showed greater reductions, particularly T2 (Codafol®) with 16.58 cm (-21.5%), indicating that single biostimulation without sufficient nitrogen negatively impacts root development. However, combinations including QuitoMax® (T3, T5, and T7) showed improvements relative to their counterparts without this product.

The reduced root length observed in T2 (Codafol® applied alone, –21.5% relative to T1) may be associated with the exclusive presence of amino acids in the formulation, which primarily enhance metabolic activity and stress tolerance but may not be sufficient to directly stimulate root elongation. In contrast, QuitoMax®, whose active component is chitosan, has been reported to induce the expression of genes related to root initiation and development (Murugesan and Paramasivan 2024). Similarly, the micronutrients supplied by CBFert® play a key role in cell division and elongation processes. This complementarity explains why T6 (Codafol®) + (CBFert®) exhibited only a 5.5% reduction in root length, indicating

that combined biostimulant applications partially mitigate the negative effects observed with individual treatments.

The increase in plant height observed in T8 suggests a synergistic effect among the applied biostimulants, capable of matching the height growth recorded in the control with 100% mineral fertilization. This result is consistent with reports by Rouphael and Colla (2020), who indicate that combinations of amino acid-based, seaweed extract, and chitosan biostimulants promote cell elongation and vegetative growth in both aerial and root tissues. In contrast, treatments with individual applications (T2-T4) showed significant reductions, which could be attributed to insufficient nitrogen availability during critical growth stages.

This synergistic effect can be explained through two complementary mechanisms. First, the contribution of nitrogen from the biostimulants Codafol®, containing 14% N L^{-1}, and CBFert®, with 11% N L^{-1}, according to the technical specifications of the products (MINAG 2020). Foliar application may also promote rapid assimilation of this nutrient, directly supplying the nutritional requirements for vegetative growth. Second, the phytostimulant activity of chito-oligosaccharides present in QuitoMax® induces cell expansion in parenchymal phloem tissues, optimizing nutrient uptake and distribution (López-Moya et al. 2019). Additionally, chitosan stimulates cell elongation and meristematic activity, key factors contributing to increased plant height, through its interaction with phytohormone pathways involving auxins and gibberellins. Chitosan has been shown to promote the accumulation and signaling of these hormones, which are essential regulators of cell expansion and growth processes in plants (Mukarram et al. 2023). Simultaneously, chitosan enhances photosynthetic efficiency by stimulating the synthesis of chlorophyll and secondary metabolites (Murugesan and Paramasivan 2024), thereby creating a positive feedback loop between nutrition and growth. These complementary nutrient supplies and greater efficiency in uptake and assimilation appear to underpin the synergistic effect observed in the treatment with the triple combination.

Regarding the number of pods per plant, treatment T1 produced 62.26 pods (±0.16), significantly exceeding T8 (59.33 pods) by 4.7% and T7 (58.46 pods) by 6.1%. Treatments T2-T6 exhibited more pronounced reductions, ranging from 53.80 pods (-13.6%) in T2 to 56.73 pods (-8.9%) in T6. These results indicate that treatments including QuitoMax® (T3, T5, and T7) partially mitigated the reduction in pod number compared with full fertilization, whereas treatments without this biostimulant did not show a similar response.

The number of pods per plant is a crucial component that directly influences the final soybean yield. Previous research has explored the effect of chitosan, the active ingredient in QuitoMax®, in combination with biofertilizers such as Azofert-S®, whose formulation contains rhizosphere bacteria as the active component. These studies highlight that the biological activity of such products is closely linked to their specific composition, particularly the role of chitosan as a biopolymer capable of modulating plant physiological responses and enhancing interactions with beneficial microorganisms in the rhizosphere (Costales-Menéndez and Falcón-Rodríguez 2018). These studies consistently report a positive effect on the number of pods per plant in soybean. Rather than promoting the formation of additional reproductive structures, these findings suggest that the interaction between chitosan and other biostimulants likely enhances pod retention by reducing flower and young pod abortion, thereby contributing to increased soybean yield.

However, it is important to note that not all studies are in agreement. Sauvu-Jonasse et al. (2020) found no significant differences in the number of pods per plant when applying Azofert-S®, EcoMic®, and QuitoMax®, either individually or in combination, compared to NPK fertilization. Such lack of response may be explained by adequate nutrient availability and favorable growing conditions, which can reduce the expression of biostimulant effects that are often more evident under stress or nutrient-limited environments. This may indicate a dependence on the production context (climatic conditions, soil type, or soil microbiome). This divergence underscores the complexity of interactions between biostimulants, fertilization, and the plant's physiological response in pod formation, highlighting the need for more targeted research to understand the factors that modulate these interactions in soybean cultivation.

Regarding grains per plant, the control (T1) reached 134.98 grains (±0.26), a value matched by T8 (135.51 grains, +0.4%). In turn, T1 and T8 significantly exceeded T6 (Codafol® + CBFert®), which produced 128.19 grains per plant (-5.0%). Individual applications of biostimulants

(T2-T4) showed the largest reductions (111.18-113.15 grains, -17.6 to -16.1%), while the double combinations presented intermediate values (124.15-130.48 grains). Notably, T7 (Codafol® + QuitoMax®) had only 3.3% fewer grains than T1, suggesting a synergy between these biostimulants.

Examining the possible causes of the treatment effects on grains per pod, it is worth considering how each biostimulation scheme may have influenced soybean physiological processes. However, it was the triple combination in T8 that showed a significant effect on the number of grains per pod. This suggests that the coordinated action of the three types of biostimulants addressed multiple aspects of plant development more comprehensively.

Although no leaf nutrient analysis was conducted, recent studies suggest that chitosan can enhance nutrient uptake and physiological responses in plants. For example, García-García et al. (2023) demonstrate that chitosan treatments improve physiological performance and yield components in well-watered and drought-stressed plants, implying enhanced nutrient use efficiency that may include improved nitrogen assimilation under certain conditions. Similarly, the amino acids supplied by Codafol® could contribute to optimizing the synthesis of proteins and enzymes required for flowering and pod development. In addition, the micronutrients provided by CBFert® may act as essential cofactors in metabolic pathways associated with carbohydrate synthesis and the translocation of photoassimilates to developing pods (Agudelo and Rivera 2018). These mechanisms are proposed as plausible physiological explanations for the observed responses.

The individual or combined application of the biostimulants CBFert®, Codafol®, and QuitoMax® elicited a differential response in soybean yield compared to the treatment that included full mineral fertilization (Figure 3).

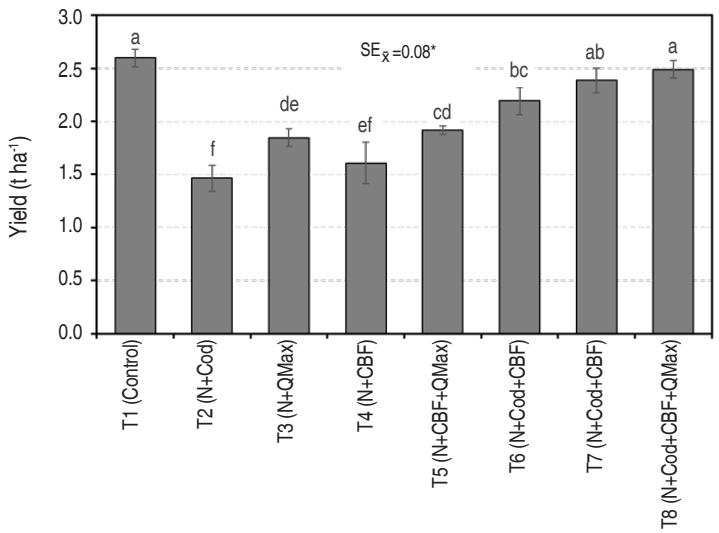


Figure 3. Soybean plant yield subjected to individual and combined application of Codafol®, QuitoMax® and CBFert®. Bars with different letters differ significantly according to Tukey's multiple range test at $P<0.05$, n=3

In this study, significant differences ($P<0.05$) were observed among the evaluated treatments. Treatment T1, recorded the highest average yield (2.60 t ha^{-1}), with no statistical differences compared to treatment T8 (2.49 t ha^{-1}), which included the complete combination of the three biostimulants. Similarly, treatment T7, combining Codafol® and QuitoMax®, reached an average yield of 2.39 t ha^{-1}, ranking among the highest-performing treatments. These findings suggest that an integrated biostimulation strategy or specific combinations of biostimulants can maintain yield levels comparable to conventional mineral fertilization.

Treatments T1, T7, and T8 showed substantial yield increases compared to treatments T2, T3, T4, and T5, which involved individual or partial combinations of biostimulants. On average, yields in T1, T7, and T8 exceeded those of the less effective treatments by 0.99, 0.66, and

0.88 t ha^{-1}, respectively. These differences correspond to relative increases of 50.78% for T1, 33.89% for T7, and 45.22% for T8, compared to the average yield of T2, T3, T4, and T5 (mean = 1.71 t ha^{-1}). Notably, T7 did not differ statistically from T6, indicating intermediate performance. Overall, these results highlight that both complete mineral fertilization and synergistic biostimulant combinations can significantly increase soybean yield compared to strategies based on partial or individual biostimulant applications. Compared with the control treatment, soybean yield under reduced mineral fertilization increased by 10 to 60%, depending on the biostimulant strategy applied. These results demonstrate that a 70% reduction in nitrogen fertilization can be achieved without compromising yield. The observed variability among treatments reflects differences in biostimulant combinations as well as the influence of edaphoclimatic conditions and cultivar-specific responses.

Moreover, a trend toward increased yield was observed in treatments involving double combinations compared to individual applications of Codafol®, QuitoMax®, and CBFert®. This reinforces the hypothesis of a synergistic effect among the evaluated biostimulants, as each formulation contributes through distinct physiological modes of action. Amino acid–based biostimulants such as Codafol® have been shown to enhance nutrient uptake efficiency, metabolic activity, and stress resilience in crops (Sun et al. 2024). Chitosan-based products like QuitoMax®, whose active ingredient is chitosan, can modulate phytohormone signaling pathways associated with cell elongation, stress responses, and delayed senescence (Murugesan and Paramasivan 2024).

In turn, micronutrient formulations such as CBFert® supply essential cofactors required for enzymatic processes involved in carbohydrate metabolism and assimilate translocation (Agudelo and Rivera 2018). The complementarity of these mechanisms aligns with the current conceptual framework of plant biostimulants (Rouphael and Colla 2020) and supports the observed benefits of integrated biostimulant applications on overall soybean performance.

Their interaction could enhance key physiological mechanisms crucial for final crop yield, as suggested by the international literature on QuitoMax® and its active ingredient, chitosan. Positive effects of chitosan on the growth and development of soybean crops have been reported (Costales-Menéndez and Falcón-Rodríguez 2018), cucumber (*Cucumis sativus* L.) (Salgado-Valle et al. 2020), and potato (*Solanum tuberosum* L.) (Ruiz et al. 2024). These studies attribute to chitosan the ability to enhance plant energy activity, stress response, and reproductive development. In combination with Codafol® and CBFert®, this may explain the similarity in yield to full nitrogen fertilization by addressing multiple aspects of soybean growth comprehensively.

These findings indicate that the integrated use of biostimulants can serve as an effective alternative to reduce synthetic nitrogen inputs under the conditions evaluated. Future studies should examine the stability of this response across environments and cultivars and include economic assessments of its practical adoption.

CONCLUSION

The integrated application of the three biostimulants Codafol® (amino acid–based), QuitoMax® (chitosan-based), and CBFert® (micronutrient-based) results in a synergistic response, achieving grain yield comparable to conventional nitrogen fertilization. Significant increases are observed in chlorophyll index, root length, and the number of pods and grains per plant. In contrast, individual biostimulant applications produce lower responses compared with the combined treatment.

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CONFLICT OF INTERESTS

The corresponding authors declare that they have no conflict of interest.

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