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Original article

Biostimulant effect of spirulina (*Arthrospira platensis*) on the initial vegetative development of rice seedlings (*Oryza sativa*)

Efecto bioestimulante de la espirulina (*Arthrospira platensis*) sobre el desarrollo inicial vegetativo de plántulas de arroz (*Oryza sativa*)

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ABSTRACT: The study evaluated the biostimulant effect of spirulina (*Arthrospira platensis*) on the early development of rice seedlings (*Oryza sativa*), under a completely randomized design with five treatments: T1 (100 %), T2 (75 %), T3 (50 %), T4 (25 %), and T5 (control, 0 %). The seeds were sown in pots to break dormancy and received applications of spirulina on days 0, 5, 10, and 15 after sowing. Germination rate, root length, height, vigor index IV, and fresh and dry biomass were evaluated. The results showed that spirulina significantly favored germination (≥93 %), with the maximum in T1 (97 %). Root development reached 16 cm in T1 compared to 9 cm in the control. Height was greater in T1 and T2 (20 and 19 cm respectively), as was vigor index IV, with T1 standing out (3492). In terms of biomass, T1 had the highest values (fresh: 6.57 g; dry: 2.7 g), exceeding the control (3.85 g and 1.42 g). Dry matter remained stable in treatments with spirulina (≈41 %), higher than the control (36.9 %). Concluding that *A. platensis* is an effective biostimulant, with dose-dependent effects, its application is recommended in early stages of development to establish the optimal threshold for its use.

Key words: biomass, cyanobacteria, development, germination.

RESUMEN: El presente estudio evaluó el efecto bioestimulante de la espirulina (*Arthrospira platensis*) en el desarrollo temprano de plántulas de arroz (*Oryza sativa*), bajo un diseño completamente al azar con cinco tratamientos: T1 (100 %), T2 (75 %), T3 (50 %), T4 (25 %) y T5 (control, 0 %). Las semillas fueron sembradas en macetas para que rompan su dormancia, recibiendo aplicaciones de espirulina en los días 0, 5, 10 y 15 días posterior a la siembra. Se evaluó la tasa de germinación, longitud radicular, altura, índice de vigor IV, biomasa fresca y seca. Los resultados mostraron que la espirulina favoreció significativamente la germinación (≥93 %), con el máximo en T1 (97 %). El desarrollo radicular alcanzó 16 cm en T1 frente a 9 cm en el control. La altura fue mayor en T1 y T2 (20 y 19 cm respectivamente), al igual que el índice de vigor IV, destacando T1 (3492,00). En biomasa, T1 presentó los valores más altos (fresca: 6,57 g; seca: 2,70 g), superando al control (3,85 g y 1,42 g). La materia seca se mantuvo estable en tratamientos con espirulina (≈41 %), superior al control (36,90 %). Concluyendo que *A. platensis* es un bioestimulante eficaz, con efectos dosis-dependientes, recomendada su aplicación en etapas tempranos de desarrollo para establecer el umbral óptimo de su uso.

Palabras clave: biomasa, cianobacteria, desarrollo, germinación.

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INTRODUCTION

Rice (*Oryza sativa*) production is fundamental to ensuring global food security, as it constitutes one of the main staple crops in the diet of millions of people worldwide (1). However, increasing pressure from environmental factors, such as agricultural soil degradation, climate change, and the excessive use of chemical fertilizers, has generated the need to seek sustainable alternatives that promote crop growth and development in an environmentally friendly manner (2).

In this context, biostimulants have emerged as an innovative and promising tool to improve the physiological performance of plants. Biostimulants are substances or microorganisms that, when applied to plants, stimulate natural processes, enhancing nutrient use efficiency, tolerance to abiotic stress, and crop quality (3). Within this category, spirulina (*Arthrospira platensis*), a cyanobacterium widely recognized for its high nutrient content, has attracted attention for its biostimulant potential in agriculture (4).

Spirulina is rich in proteins, essential amino acids, vitamins, minerals, and bioactive compounds such as phycocyanin and essential fatty acids, which may favor plant growth (5). According to recent research, the application of spirulina in agricultural crops has shown positive effects, such as increased biomass (6), improved root development, and enhanced resistance to abiotic stress (7). These effects are related to its ability to stimulate fundamental metabolic pathways in plants (8).

During the initial development of the crop, rice depends on adequate root and leaf growth to ensure efficient water and nutrient absorption, as well as proper photosynthesis (9). Therefore, exploring the use of spirulina as a biostimulant represents an opportunity to optimize the initial growth of rice, reducing dependence on chemical products and fostering sustainable agricultural practices. The present study focuses on evaluating the biostimulant effect of spirulina on the vegetative phase of rice cultivation, seedling stage (18 days), one of the critical phenological stages for crop establishment.

MATERIALS AND METHODS

Location and duration of the trial

The study was conducted outdoors at the Biotechnology Laboratory of the Faculty of Sciences and Engineering, Universidad Estatal de Milagro (UNEMI), located at Km 1.5 on the Milagro-Virgen de Fátima road, Milagro canton, Ecuador, within the coordinates 2°08'26.1"S 79°35'36.4"W. The trial lasted 18 days.

Trial Management

The experiment was carried out using hybrid rice seeds of the cultivar SFL-11, lot 387711, AGRIPAC® brand, whose characteristics are described in Table 1. The seeds underwent a pre-germination process by immersion in water for 48 hours to break dormancy and ensure uniform germination, and were subsequently sown at a depth of 2.50 cm in individual plastic pots (25 × 35 cm) with a capacity of 2 kg. The pots were filled with planting soil free of leaf litter and pre-moistened with water. Liquid spirulina produced at UNEMI laboratories was used at a concentration of 1 g L. Treatment solutions were applied at 0, 5, 10, and 15 days after sowing (DAS), and the variables were evaluated up to 18 DAS.

Table 1. Properties of the hybrid rice cultivar SFL-11 AGRIPAC®

Yield under irrigation	7 (t ha)
Yield under rainfed conditions	6 (t ha)
Vigor	Moderately high
Tillering	High
Vegetative cycle	120 (days)
Grain type	7.5 mm
Milling index	69 %
Shattering	Intermediate
Amylose content	30,5 %
Seed dormancy	6 weeks
Tolerant	Blast (*Pyricularia oryzae*); Grain discoloration; *Sarocladium oryzae*; White leaf disease; *Rhizoctonia solani*
Moderately susceptible	Lodging. However, under recommended management conditions, no lodging problems are observed.

Experimental Design

The study was based on a completely randomized design (CRD). Five treatments were established corresponding to different concentrations of *A. platensis* v/v water: T1 (100 %), T2 (75 %), T3 (50 %), T4 (25 %), and T5 control (water only). Each treatment included 10 replicates, establishing a total of 50 experimental units. In each tray, three rice seeds were sown in the same hole, considered as one experimental unit.

Evaluated Variables

On day 18 after sowing, the following variables were evaluated, taking into account the suggestions of some authors (10, 11):

Germination percentage (%)

This variable quantified the germination efficiency of the seeds under different treatments. It was determined using the following expression:

$$\%\,Germination = \left(\frac{No.\ of\ Germinated\ Seeds}{Total\ Seeds\ Sown\ (per\ E.U.)\ .}\right) \times 100$$

Root length (cm)

Measured with a graduated metal ruler, from the beginning of the mesocotyl to the distal end of the main root. To evaluate this parameter, the plant was carefully extracted without breaking the roots, the roots were gently washed with water to remove the substrate, and blotting paper was used to absorb excess moisture.

Seedling height (cm)

Measured from the base of the stem to the apex of the most developed leaf, using a graduated metal ruler.

Vigor index (VI)

Obtained once the average seedling length per treatment was calculated; the resulting value was multiplied by the corresponding germination percentage using the formula:

$$IV = Germination(\%) \times Seedling\ length(cm)$$

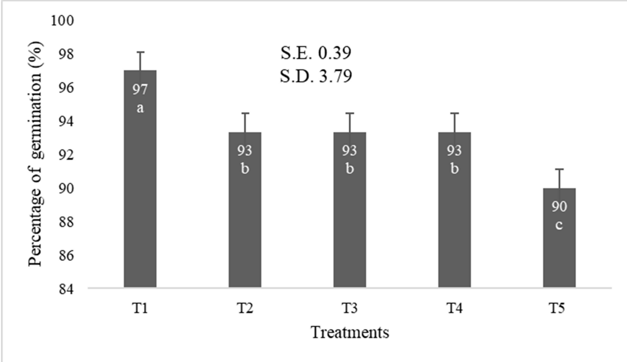
Fresh biomass, dry biomass, and total biomass

Fresh biomass was obtained by weighing the complete seedlings corresponding to each treatment using a Bektron BK-JNB100001 precision analytical balance. Subsequently, the samples were dried in a muffle furnace at 70 °C for 8 hours at constant temperature to eliminate their water content, thereby determining the dry biomass. The percentage of dry matter was calculated to determine the solid content, according to the following relation:

$$\%\ Dry\ mass = \left(\frac{Dry\ biomass}{Fresh\ biomass}\right) \times 100$$

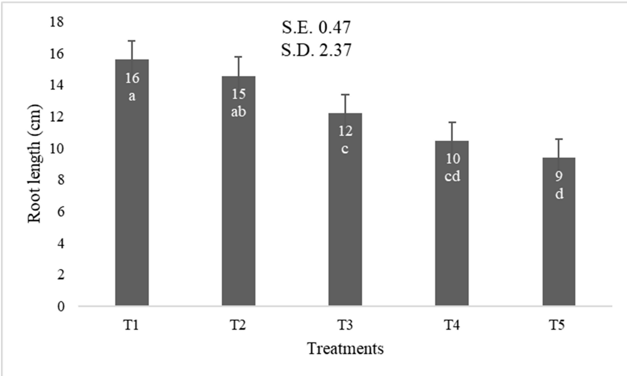
Statistical analysis

For data analysis, a one-way analysis of variance (ANOVA) was used to determine the existence of significant differences among treatments. For variables where differences were detected, Duncan's test was applied with a significance level of $p \leq 0.05$. Statistical analysis was performed using InfoStat software, version 2020.



Concentration of *A. platensis* v/v: T1 (100%), T2 (75%), T3 (50%), T4 (25%), and T5 control (water); S.E. = standard error; S.D. = standard deviation; a, b, c, d = different letters indicate statistically significant differences

Figure 1. Germination percentage of rice seeds



Concentration of *A. platensis* v/v: T1 (100%), T2 (75%), T3 (50%), T4 (25%), and T5 control (water); S.E. = standard error; S.D. = standard deviation; a, b, c, d = different letters indicate statistically significant differences

Figure 2. Results of the root length variable

RESULTS

Germination Percentage

Figure 1 presents the results of the germination percentage of rice seeds under five treatments with different concentrations of *A. platensis* to evaluate its biostimulant effect (T1, T2, T3, T4), and one control (T5, water only).

The treatment (T1) with the highest concentration of *A. platensis* (100 % v/v) reached the highest germination value (97 %). These results indicate that the effect of the cyanobacterium is dose-dependent, favoring seed viability and germination vigor at higher concentrations compared to the other treatments, and confirmed by the control treatment.

Root Length

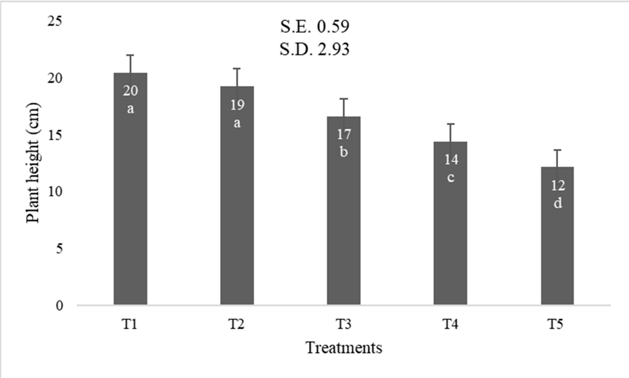
Figure 2 presents the results of the average seedling root length of rice (cm) under four treatments with different concentrations of *A. platensis* as a biostimulant (T1, T2, T3, T4) and one control (T5, water only), evaluated at 18 days after sowing.

The treatment T1 (100 % spirulina) recorded the highest average root length (16 cm). In contrast, treatments with lower doses (T4: 25 %) and the control (T5: water) showed significantly lower lengths (10 and 9 cm, respectively). Statistical analysis using Duncan's test confirmed significant differences ($p<0.05$) between treatments with higher concentrations of spirulina compared to the control, supporting the hypothesis of the biostimulant effect of the extract on root growth.

Plant Height

Figure 3 presents the results of the average seedling height of rice (cm) under five treatments with different concentrations of *A. platensis* v/v as a biostimulant (T1, T2, T3, T4) and T5 (control), evaluated at 18 days after sowing (DAS).

Treatments with higher concentrations of *A. platensis* (T1 and T2) achieved the greatest average seedling heights, with 20 and 19 cm, respectively, forming a statistically distinct group in Duncan's test compared to the control treatment. On the other hand, treatments with lower concentrations



Concentration of *A. platensis* v/v: T1 (100%), T2 (75%), T3 (50%), T4 (25%), and T5 control (water); S.E. = standard error; S.D. = standard deviation; a, b, c, d = different letters indicate statistically significant differences

Figure 3. Results of the seedling height variable

(T3 and T4) showed intermediate heights, while the control group (T5) obtained the lowest average height (12 cm), confirming the reduced growth capacity of rice in the absence of *A. platensis*.

Vigor Index (VI)

Table 2 presents the results of the VI of rice seedlings under five treatments with different concentrations of *A. platensis* v/v as a biostimulant (T1, T2, T3, T4) and control (T5).

Table 2. Results obtained for the vigor index (VI) variable

Treatment	Vigor index (IV)
T1	3492.00
T2	3152.80
T3	2687.10
T4	2317.50
T5	1920.60

Concentration of *A. platensis* v/v: T1 (100%), T2 (75%), T3 (50%), T4 (25%), and T5 control (water)

The T1 treatment (100 %) presented the highest vigor index (VI) value (3492.00), suggesting that the full application of the biostimulant allowed for faster and more vigorous growth of rice seedlings.

Fresh biomass, dry biomass, and dry matter

Table 3 presents the results of the variables fresh biomass in grams (g), dry biomass (g), and dry matter (%) of rice seedlings under five treatments with different concentrations of *A. platensis* v/v as a biostimulant (T1, T2, T3, T4) and one control (T5).

It was observed that the treatment with the highest concentration of spirulina (T1, 100 %) presented the highest fresh biomass (6.57 g). Regarding dry biomass, a similar trend to fresh biomass was observed. Treatment T1 reached the highest value (2.70 g), followed by T2 (2.60 g) and T3 (2.22 g). In terms of dry matter percentage, treatments T1, T2, and T3 maintained relatively constant values (41.10-41.30 %).

Table 3. Results obtained for fresh biomass, dry biomass, and dry matter

Treatment	Fresh biomass (g)	Dry biomass (g)	Dry matter (%)
T1	6,57	2,70	41,10
T2	6,29	2,60	41,30
T3	5,39	2,22	41,20
T4	4,82	1,84	38,20
T5	3,85	1,42	36,90

Concentration of *A. platensis* v/v: T1 (100 %), T2 (75 %), T3 (50 %), T4 (25 %), and T5 control (water)

DISCUSSION

Some authors suggested that environmental or intrinsic factors may influence the germination capacity of rice seeds (12). The observed increase in germination can be attributed to the presence of bioactive compounds in the biomass of *A. platensis*; similar results reported a significant improvement in the germination of crops such as wheat and tomato after the application of spirulina extracts, attributing this to the synergistic effect of these compounds on enzymatic activation and protein synthesis during the imbibition phase (13, 14).

The increase in root length in response to higher concentrations of spirulina v/v (100, 75 %, and T3) is consistent with published findings, where this effect is attributed to the presence of bioactive compounds in microalgae, which promote cell elongation and tissue differentiation in roots (15).

Studies support the benefits of spirulina due to its high content of metabolic compounds, reporting significant increases in shoot and root biomass in crops treated with extracts of this microalga (16). Similarly, the application of microalgae-derived bioproducts has been observed to enhance photosynthetic metabolism and nutrient assimilation, resulting in more vigorous plant growth (7).

Previous studies in tomato cultivation have demonstrated that the presence of microalgae as biofertilizers can improve germination rate, increase enzymatic activity, and optimize the absorption of essential nutrients, thereby favoring greater vigor in the early stages of crop development (17).

In addition, *A. platensis* not only increases water retention in tissues but also stimulates the accumulation of structural matter. This effect may be related to the microalga's ability to induce the synthesis of proteins, carbohydrates, and secondary metabolites associated with growth (18). Reports have shown that the application of microalgae extracts in pea crops significantly increased biomass production and photosynthetic efficiency by enhancing enzymatic activity and nutrient uptake (19). Likewise, some authors reported improved maize seedling development in the early stage with the use of spirulina (20), due to its high content of bioactive compounds that make it beneficial in the biotechnological field for agricultural use (21).

Another author indicated that *A. platensis* enhances rice seed germination due to its high concentration of proteins, phenols, and flavonoids, which significantly influence the physiological state of the seeds (22).

The increase in crop yield and productivity can be enhanced through the use of cyanobacteria of the genus *Arthrospira*, whose biological activities in physiological processes such as germination, root system development, and yield improvement have been documented (23).

CONCLUSIONS

The application of *A. platensis* showed a significant biostimulant effect on the germination and early growth of rice (*Oryza sativa*) seedlings of the SFL-11 cultivar. All spirulina treatments exceeded 93 % germination, with treatment T1 (100 %) standing out as the most efficient across all evaluated variables.

These results suggest that the bioactive compounds of spirulina (phytohormones, amino acids, and pigments) promote seedling development, consolidating its potential as a sustainable natural biostimulant to optimize rice germination and productivity, while reducing dependence on conventional chemical inputs.

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